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The 2013 Fungal Conference logo was created by Xiaoping Li and Mona Pokharel, New Mexico State University.
The 27th Fungal Genetics Conference gratefully acknowledges the following companies and organizations for their generous contributions in support of travel awards to the meeting.
Please note: The program book from the 27th Fungal Genetics Conference is published as a supplement to the Fungal Genetics Reports. Abstracts will be available on the FGSC website and may be cited as follows: Fungal Genetics Reports 60(Suppl): Abstract #
SCHEDULE OF EVENTS

Tuesday, March 12
2:00 pm - 10:00 pm  Registration  Chapel
6:00 pm - 7:00 pm  Dinner  Crocker Hall
7:30 pm - 10:30 pm  Opening Mixer  Merrill Hall

Wednesday, March 13
7:30 am - 8:30 am  Breakfast  Crocker Hall
8:00 am – 5:00 pm  Registration  Chapel
8:30 am - 12:00 noon  Plenary Session I  Merrill Hall and Chapel

Metabolic Pathways: Cell Growth, Pathogenesis and Bioenergy  Chair: Louise Glass

- Robert A. Cramer  Hypoxia and Mechanisms of Human Fungal Pathogenesis: To Air or Not to Air?
- Audrey P. Gasch  Tackling biofuel bottlenecks through genome wide association studies in  *Saccharomyces cerevisiae*
- Kim E. Hammond-Kosack
- Bernard Henrissat
- Adrian Tsang  Exploring the metabolome of cereal infecting *fusaria*  Carbohydrate-active enzymes in fungal genomes  Genome-wide approaches to identify and characterize lignocellulolytic enzymes

12:00 noon - 1:00 pm  Lunch – Crocker Hall
Box lunches will be available on a first come, first served basis for meeting attendees on the deck outside of the Administration Building.

Following lunch, the morning speakers will be available on the benches outside the administration building to meet with students. Please allow time for students. In the event of rain, please go inside the Administration Building.

Ad hoc Workshops  – Box lunches will be available for those attending the session.
12:15 pm - 1:30 pm  Fungal Genome Tools  Chapel
1:30 pm - 1:45 pm  One Name = One Fungus  Chapel

3:00 pm - 6:00 pm  Concurrent Sessions I

Cell Signaling Involved in Fungal Development and Pathogenesis  Naweed Naqvi and Stefanie Pöggeler
Genetics and Genomics of Interactions with Bacteria, Insects and Plants  Nemat Keyhani and Christian Hertweck
Membrane Trafficking and Molecular Organization  Vicky Sophianopoulou and Gero Steinberg
Genome Defense, Epigenetics and RNAi  Patrick Shiu and Sven Saupe
Genomics and Mycorrhizae  Anders Tunlid and Tom Bruns
Regulation and Comparative Genomics of Carbon and Nitrogen Metabolism  Richard Wilson and Ronald de Vries
Education, Outreach, and Professional Development  Steven Denison and Mimi Zolan

6:00 pm - 7:00 pm  Dinner  Crocker Hall
7:30 pm - 10:30 pm  Poster Session I and Exhibits  Fireside
ODD numbered posters from 7:30 – 8:30 and EVEN numbered posters 8:30 – 9:30.

<table>
<thead>
<tr>
<th>Poster Number</th>
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<tr>
<td>1-68</td>
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<td>Cell Biology and Development</td>
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<tr>
<td>351-353</td>
<td>Education and Professional Development</td>
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</tbody>
</table>
Thursday, March 14
7:30 am - 8:30 am  Breakfast  Crocker Hall
8:30 am – 5:00 pm  Registration  Surf and Sand

8:30 am - 12:00 noon  Plenary Session II  Merrill Hall and Chapel

Organismic Molecular Interactions  Chair: Nick Talbot

Yong-Hwan Lee  Large-scale Biology for Fungal Pathogenicity in *Magnaporthe oryzae*
Antonio Di Pietro  MAPK-mediated control of infectious growth in *Fusarium oxysporum*
Peter Dodds  Analysis of effector proteins from flax rust and wheat stem rust
Francine Govers  Dissecting *Phytophthora* blight; making sense out of signalling, effectors and host targets
Alexandra C. Brand  Understanding directional growth in fungi

12:00 noon - 1:00 pm  Lunch – Crocker Hall
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12:15 pm - 1:15 pm  GSA Careers Luncheon  Crocker Hall

Ad hoc Workshops  Box lunches will be available for those attending the sessions.

12:15 pm - 1:30 pm  Neurospora Business Meeting  Chapel
12:30 pm - 2:00 pm  JGI Sequencing and Analysis Tools and Initiatives  Merrill Hall

3:00 pm - 6:00 pm  Concurrent Sessions II

Cool Tools for Fungal Biology  Miguel Penalva and Kevin McCluskey  Merrill Hall
Fungi and Evolutionary Theory  Hanna Johannesson and Duur Aanen  Chapel
Cytoskeleton, Motors, and Intracellular Transport  Samara Reck-Petterson and Ping Wang  Heather
Nucleic Acid-Protein Interactions that Impact Transcription and Translation  Michael Freitag and Mark Caddick  Fred Farr
Interactions between Fungi and Animals  Neil Gow and Clarissa Nobile  Kiln
Fungal Volatiles and Organic Compounds as Signaling Agents  Joan Bennett and Richard Splivallo  Nautilus
Genomics and Biochemistry of Degradation of Complex Molecules in the Environment  Jonathan Walton and Dan Cullen  Scripps

6:00 pm - 7:00 pm  Dinner  Crocker Hall

7:30 pm - 10:30 pm  Poster Session II and Exhibits  Fireside
ODD numbered posters from 7:30 – 8:30 and EVEN numbered posters from 8:30 – 9:30  Pavilion

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<td>221-350</td>
<td>Comparative and Functional Genomics</td>
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<tr>
<td>354-475</td>
<td>Gene Regulation</td>
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</tbody>
</table>
Friday, March 15
7:30 am - 8:30 am  Breakfast  Crocker Hall
8:30 am – 5:00 pm  Registration  Surf and Sand

8:30 am - 12:00 noon  Plenary Session III  Merrill Hall and Chapel

**Sensing, Cell Biology and Development**  *Chair: Michelle Momany*

- Meritxell Riquelme  *The illuminated Spitzenkörper of Neurospora crassa: tracking and tracing secretory vesicles*
- Joseph Heitman  *Evolution of sexual reproduction: A view from the Fungal Kingdom*
- Michael Brunner  *Metabolic compensation of the Neurospora clock by a glucose-dependent feedback of the circadian repressor CSP1 on the core oscillator*
- Stephen Osmani  *Integration of the fungal cell cycle with growth and development*
- Gregory Jedd  *A Neurospora cell-free system reconstitutes peroxisome membrane protein synthesis and organelle-specific targeting*

12:00 noon - 1:00 pm  Lunch – Crocker Hall
Box lunches will be available on a first come, first served basis for meeting attendees on the deck outside of the Administration Building.

Following lunch, the morning speakers will be available on the benches outside the administration building to meet with students. Please allow time for students. In the event of rain, please go inside the Administration Building.

**Ad hoc Workshops**  Box lunches will be available for those attending the sessions.
12:15 pm - 1:30 pm  FungiDB  Kiln
12:15 pm - 1:30 pm  Magnaporthe Comparative Genomics  Chapel

3:00 pm - 6:00 pm  Concurrent Sessions III

- **Pathogenic Signaling via Effector Proteins**  Brett Tyler and Sebastien Dupressis  Merrill Hall
- **Cell Wall, Polarity and Hyphal Tip Growth**  Stephan Seiler and Ernestina Castro-Longoria  Chapel
- **Sexual Regulation and Evolution in the Fungi**  Frances Trail and Nicolas Corradi  Heather
- **Oxidative Stress, ROS Signaling and Adaptation to Hypoxia**  Geraldine Butler and Barry Scott  Fred Farr
- **Phylogenomics**  Jason Stajich and Joey Spatafora  Forum
- **Synthetic Biology**  Nancy Keller and Peter Punt  Nautilus
- **Fungicides and Antifungal Compounds**  Daniele Debieu and Paul Verweij  Scripps

6:00 pm - 7:00 pm  Dinner  Crocker Hall
7:00 pm - 8:00 pm  GSA Education Special Interest Group Mixer  Surf and Sand

7:30 pm - 10:30 pm  **Poster Session III and Exhibits**  Fireside
ODD numbered posters 7:30 – 8:30 and EVEN numbered posters 8:30 – 9:30.  Pavilion

**Poster Number**  **Topic**
476 – 639  Pathogenic and Mutualistic Interactions
640 - 688  Population and Evolutionary Genetics
714 - 741  Other Topics
Saturday, March 16

7:30 am - 8:30 am Breakfast Crocker Hall
9:00 am – 12:00 noon Registration Surf and Sand

8:30 am - 12:00 noon Plenary Session IV Merrill Hall and Chapel

Functional Ecology and Fungal Communities Chair: Jim Anderson

Tatiana Giraud
Mechanisms allowing the formation of new fungal pathogenic species on novel hosts, causing emerging diseases

B. D. Lindahl
The decisive role of mycorrhizal fungi as regulators of carbon sequestration in boreal forest ecosystems

Edward J. Louis
Population Genomics of Saccharomyces Yeasts: Ecology and Adaptation

Marc-André Selosse
The mycorrhizal symbiosis as a network linking plants

Eva H. Stukenbrock
Unraveling speciation and specialization processes in plant pathogenic fungi using comparative population genomics

12:00 noon - 1:00 pm Lunch – Crocker Hall
Box lunches will be available on a first come, first served basis for meeting attendees on the deck outside of the Administration Building.

Following lunch, the morning speakers will be available on the benches outside the administration building to meet with students. Please allow time for students. In the event of rain, please go inside the Administration Building.

2:00 pm - 5:00 pm Concurrent Session IV

Parallels between Fungal Pathogens of Plants and Animals
Barbara Howlett and Axel Brakhage Merrill Hall

Secondary Metabolism
Gillian Turgeon and Bettina Tudzynski Chapel

Light Sensing and Circadian Rhythms
Luis Larrondo and Reinhard Fischer Fred Farr

Fungal Evo-Devo
Steve Harris and Brian Shaw Forum

Environmental Metagenomics
Chris Schadt and Betsy Arnold Kiln

Dimorphic Transitions
Anne Dranginis and Alex Nautilus

Andrianopoulos

Tropic Growth and Fusion
Andre Fleissner and Nick Read Scripps

5:30 pm - 5:45 pm Poster Awards Merrill Hall and Chapel

5:45 pm - 6:30 pm Perkins/Metzenberg Lecture:
Regine Kahmann, Max Planck Institute for Terrestrial Microbiology Merrill Hall and Chapel

6:30 pm - 8:30 pm Closing Banquet Crocker Hall

8:30 pm - 12:00 am Closing Party featuring The Amplified DNA Band Merrill Hall

Sunday, March 17

7:30 am - 8:30 am Breakfast Crocker Hall
Registration and Information Desk
The Conference registration desk will be open according to the following schedule:

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<thead>
<tr>
<th>Date</th>
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<tr>
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<td>Surf and Sand</td>
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<tr>
<td>Wednesday, March 13</td>
<td>8:00 am – 5:00 pm</td>
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<td>Thursday, March 14</td>
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<td>Saturday, March 16</td>
<td>9:00 am – 12:00 noon</td>
<td>Surf and Sand</td>
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Instructions for Speakers
Please arrive 45 minutes before the beginning of your session with your USB flash drive to load your presentation on the laptop computer. Label your presentation with your last name and presentation number, i.e. Chen12. You do not need to bring your laptop to the meeting room. However there will be connections for presenters to use their own laptops. Speakers using their own laptops must have a VGA HD 15pin female output.

Poster Sessions
All posters will be displayed in the garage under the Fred Farr Forum. Please set up your poster immediately after lunch the day of your poster session. Two authors will share a 4 x 8 poster board. All abstracts will be up for one day. Authors of ODD numbered posters should be at their poster from 7:30 pm – 8:30 pm and authors of even numbered posters should be at the posters from 8:30 pm – 9:30 pm. Authors will present according to the following schedule:

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<tr>
<th>Topic</th>
<th># Range</th>
<th>Wednesday, Posters I</th>
<th>Thursday, Posters II</th>
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<td>689 - 713</td>
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<td>714 - 741</td>
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</table>

All presenters should remove their abstracts at the end of their poster session. After that time, remaining posters will be removed and may be lost or thrown away. The meeting does not take responsibility for posters that are not removed on time.

Internet Access
Complimentary internet access is available in the Fireside Pavilion. Instructions to connect to wifi:
1. Locate - Network name is "Asilomar Conference"
2. Enter Network Passcode -conference - all lower case.
3. Once connected open web browser and you will be redirected to Asilomar Conference login page.
4. Enter 8690, 8691 or 8692 for the username and password
EXHIBITS

The following companies have contributed to the support of this meeting. Registrants are encouraged to visit the exhibits.

**Bayer SAS – Bayer CropScience**
14 Impasse Pierre Baizet
Lyon, France  69009
Tel: 33 472 85 23 43
Email: marco.busch@bayer.com

Bayer CropScience, the subgroup of Bayer AG responsible for the agricultural business, has annual sales of EUR 7.255 billion (2011) and is one of the world’s leading innovative crop science companies in the areas of seeds, crop protection and non-agricultural pest control. The company offers an outstanding range of products including high value seeds, innovative crop protection solutions based on chemical and biological modes of action as well as an extensive service backup for modern, sustainable agriculture. In the area of non-agricultural applications, Bayer CropScience has a broad portfolio of products and services to control pests from home and garden to forestry applications. The company has a global workforce of 21,000 and is represented in more than 120 countries.

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Elsevier is a world-leading provider of scientific, technical and medical information products and services. The company works in partnership with the global science and health communities to publish more than 2,000 journals, including Fungal Genetics and Biology, Fungal Biology and Fungal Ecology. All articles are available online through ScienceDirect.

**Union Biometrica, Inc.**
Tel: 508-893-3115
E-mail: dstrack@unionbio.com
Website: www.unionbio.com

Union Biometrica provides flow cytometry for objects that are too large for traditional cytometers, such as fungal pellets, and offers an alternative to manual sorting. These instruments analyze and dispense objects based on size and fluorescent parameters. Automating this process offers increased speed, sensitivity, quantification, and repeatability of experiments.

**MO BIO Laboratories**
2746 Loker Avenue
Carlsbad, CA 92010
Tel: 760-929-9911
Email: customercare@mobio.com
Website: www.mobio.com

MO BIO Laboratories, Inc. is a global leader in solutions for nucleic acid purification, offering innovative tools for research in plant biology. Our patented Inhibitor Removal Technology® ensures isolation of high quality, inhibitor-free nucleic acids from even the toughest plant samples, removing phenolics, polysaccharides and other PCR inhibiting substances.
Wednesday, March 13   3:00 PM–6:00 PM
Merrill Hall

Cell Signaling Involved in Fungal Development and Pathogenesis
Co-chairs: Naweed Naqvi and Stefanie Pöggeler

Abstracts for this session begin on page 29.

3:00 - 3:20
Alexander V. Michkov
Stability of a G protein alpha subunit in genetic backgrounds lacking the G beta subunit or a cytosolic guanine nucleotide exchange factor.

3:20 - 3:40
Jae-Hyuk Yu
The Putative Guanine Nucleotide Exchange Factor RicA Mediates Upstream Signaling for Growth and Development in Aspergillus.

3:40 - 4:00
Oezguer Bayram
The Aspergillus nidulans MAPK module AnSte11-Ste50-Ste7-Fus3 controls development and secondary metabolism.

4:00 - 4:20
Ines Teichert
The developmental PRO40/SOFT protein participates in signaling via the MIK1/MEK1/MAK1 module in Sordaria macrospora.

4:20 - 4:40 Break

4:40 - 5:00
Linqi Wang
A Fungal Adhesin Guides Community Behaviors by Autoinduction and Paracrinal Signaling.

5:00 - 5:20
JinRong Xu
Surface recognition and appressorium morphogenesis in Magnaporthe oryzae.

5:20 - 5:40
Marie Nishimura
Plant cues promote stealth infection in fungal plant pathogens.

5:40 - 6:00
Andrea Herrmann
Unravelling the GTPase polarity complex in Claviceps purpurea.

Wednesday, March 13   3:00 PM–6:00 PM
Chapel

Genetics and Genomics of Interactions with Bacteria, Insects and Plants
Co-chairs: Nemat Keyhani and Christian Hertweck

Abstracts for this session begin on page 32.

3:00 - 3:20
M. J. Bidochka
Endophytic insect parasitic fungi feed insect-derived nitrogen to plants.

3:20 - 3:40
Rusty J. Rodriguez
Genotype-Environment Interactions and the Interplay Between Climate Change and Plant-Fungal Symbioses.

3:40 - 4:00
Kirstin Scherlach
Chemical mediators of pathogenic and mutualistic bacterial-fungal interactions.

4:00 - 4:20
Chengshu Wang
Comparative genomic analysis of entomopathogenic fungi.

4:20 - 4:40 Break

4:40 - 5:00
Morten Schiøtt
Synergistic interactions between leaf-cutting ants and their fungal symbiont facilitate degradation of plant substrate.

5:00 - 5:20
Charissa de Bekker
Unraveling the metabolome: how zombie ant fungi heterogeneously control ant brains.

5:20 - 5:40
Artemio Mendoza
Trichoderma rhizosphere’s competency, endophytism and plant communication: A molecular approach.

5:40 - 6:00
Markus Künzler
Effector proteins in fungal defense against fungivorous nematodes: Targets and functional significance.
Wednesday, March 13  3:00 PM–6:00 PM

**Heather**

**Membrane Trafficking and Molecular Organization**
Co-chairs: Vicky Sophianopoulou and Gero Steinberg

Abstracts for this session begin on page 35.

3:00 - 3:20
Barbara Valent
**Distinct secretion systems operate during biotrophic invasion by the rice blast fungus, Magnaporthe oryzae.**

3:20 - 3:40
Yujiro Higuchi
**The cellular role of early endosome motility in Ustilago maydis.**

3:40 - 4:00
George Diallinas
**The arrestin-like protein ArtA is essential for ubiquitylation and endocytosis of the UapA transporter in response to both broad-range and specific signals.**

4:00 - 4:20
Guido Grossmann
**Escaping the hustle - zones of differential protein turnover in the yeast plasma membrane.**

4:20 - 4:40 Break

5:00 - 5:20
Samara Reck-Peterson
**Whole-genome sequencing identifies novel alleles of genes required for organelle distribution and motility in Aspergillus nidulans.**

5:20 - 5:40
Rosa R. Mouriño-Pérez
**Dynamics of exocytic markers and cell wall alterations in an endocytosis mutant of Neurospora crassa.**

5:40 - 6:00
Barry J. Bowman
**“The vacuole” of Neurospora crassa may be composed of multiple compartments with different structures and functions.**

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Wednesday, March 13  3:00 PM–6:00 PM

**Fred Farr Forum**

**Genome Defense, Epigenetics and RNAi**
Co-chairs: Patrick Shiu and Sven Saupe

Abstracts for this session begin on page 37.

3:00 - 3:20
Patrick K. T. Shiu
**Meiotic silencing by unpaired DNA in Neurospora.**

3:20 - 3:40
Zhenyu Zhang
**Mechanism of quelling, a small RNA-mediated gene silencing pathway.**

3:40 - 4:00
Xuying Wang
**SIS, a sex genome defense mechanism operating in Cryptococcus neoformans.**

4:00 - 4:20
Asen Daskalov
**Fungi use prion folds for signal transduction processes involving STAND proteins.**

4:20 - 4:40 Break

4:40 - 5:00
Haoping Liu
**Regulation of white and opaque cell-type formation in Candida albicans by H3K56 acetylation and nucleosome assembly factors CAF-1 and HIR.**

5:00 - 5:20
Matthew Z. Anderson
**Epigenetic Regulation of Subtelomeric Gene Noise in Candida albicans.**

5:20 - 5:40
Zachary A. Lewis
**Chromatin regulation of genome stability.**

5:40 - 6:00
Shinji Honda
**Opposing activities of the HHC and DMM complexes maintain proper DNA methylation in Neurospora crassa.**
Wednesday, March 13  3:00 PM–6:00 PM  
**Kiln**

**Genomics and Mycorrhizae**  
Co-chairs:

Abstracts for this session begin on page 39.

3:00 - 3:20
A. Kohler  
The mycorrhizal genome initiative (MGI): Identification of symbiosis-regulated genes by using RNA-Seq.

3:20 - 3:40
Jaqueline Hess  
Transposable element dynamics in the Amanita: insights on the evolution of genome architecture accompanying the transition from saprotrophic to ectomycorrhizal ecologies.

3:40 - 4:00
Alga Zuccaro  
Broad compatibility in the root endophyte Piriformospora indica is associated with host-adapted colonization strategies.

4:00 - 4:20
Anders P. V. Tunlid  
Examining the saprotrophic ability of ectomycorrhizal fungi using genomics, transcriptomics and spectroscopy.

4:20 - 4:40  **Break**

4:40 - 5:00
Nils O. Högberg  
Interaction between the saprotrophic fungus Serpula lacrymans and living pine roots.

5:00 - 5:20
Stephen J. Mondo  
Uncovering the evolutionary pressures shaping the Glomeromycota-Glomeribacter endosymbiosis.

5:20 - 5:40
Alija Mujic  
A draft genome of the ectomycorrhizal fungus Rhizopogon vesiculosus: Characterization of mating system and heterozygosity within the dikaryon.

5:40 - 6:00
H. L. Liao  

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Wednesday, March 13  3:00 PM–6:00 PM  
**Nautilus**

**Regulation and Comparative Genomics of Carbon and Nitrogen Metabolism**  
Co-chairs: Richard Wilson and Ronald de Vries

Abstracts for this session begin on page 42.

3:00 - 3:20
Carl R. Fellbaum  
The role of carbon in fungal nutrient uptake and transport: implications for resource exchange in the arbuscular mycorrhiza.

3:20 - 3:40
Jessie Fernandez  
Mechanisms of adaptation to host rice cells by the blast fungus.

3:40 - 4:00
Sylvia Klaubauf  
Similar is not the same: Differences in the function of the (hemi-)cellulolytic regulator XlnR (Xlr1/Xyr1) in filamentous fungi.

4:00 - 4:20
Richard B. Todd  
Regulating the *Aspergillus nidulans* global nitrogen transcription factor AreA.

4:20 - 4:40  **Break**

4:40 - 5:00
Mia R. Mäkelä  
Transcriptional analysis of oxalate degradation in the white rot basidiomycete *Dichomitus squalens*.

5:00 - 5:20
Gesabel Y. Navarro Velasco  
TOR-mediated control of virulence functions in the trans-kingdom pathogen *Fusarium oxysporum*.

5:20 - 5:40
Firoz Shah  
Transcriptional regulation of peptidases and nitrogen transporters during the assimilation of organic nitrogen by the ectomycorrhizal fungi *Paxillus involutus*.

5:40 - 6:00
Michael Hynes  
Regulation of glycolysis and gluconeogenesis by antisense transcription in *Aspergillus nidulans*?
Wednesday, March 13   3:00 PM–6:00 PM
Scripps

**Education, Outreach, and Professional Development**
Co-chairs: Steven Denison and Mimi Zolan

Abstracts for this session begin on page 45.

3:00 - 3:20
Michael Koonce
Centrosome-Nuclear Disconnect Creates Mitotic Chaos in a Closed Mitosis System.

3:20 - 3:40
Claire Burns
Using Fungal Barcoding to Introduce Non-science Majors to Scientific Research.

3:40 - 4:00
Andrea Gargas
ComGen Authentic Research Experiences (C-ARE): Fungal genetic analysis.

4:00 - 4:20
Patricia J. Pukkila
Wearing two hats: Tips for combining commitments to research and to university-wide initiatives in education.

4:20 - 4:40 Break

4:40 - 5:00
Virginia K. Hench
Facilitating an Interdisciplinary Learning Community Amongst Undergraduate Research Fellows By Emphasizing Scientific Inquiry as the Unifying Thread.

5:00 - 5:20
Relly Brandman
MOOCs: Education for Everyone.

5:20 - 5:40
Break into groups to discuss promises and pitfalls of online courses.

5:40 - 6:00
Panelists Relly Brandman and Heather Hallen-Adams respond to questions and comments from the working groups.

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Thursday, March 14   3:00 PM–6:00 PM
Merrill Hall

**Cool Tools for Fungal Biology**
Co-chairs: Miguel Penalva and Kevin McCluskey

Abstracts for this session begin on page 47.

3:00 - 3:20
S. E. Baker
The Environmental Molecular Sciences Laboratory molecular analysis capabilities for fungal biology.

3:20 - 3:40
Aric E. West
Development and utilization of arrayed mutant sets for yeasts and filamentous fungi.

3:40 - 4:00
Minou Nowrousian
Sequencing-based solutions to identify and characterize fungal developmental genes.

4:00 - 4:20
Susan Kaminskyj
*Aspergillus nidulans* as an experimental system to identify novel cell wall growth and maintenance genes through identification of anti-fungal drug resistance mutations.

4:20 - 4:40 Break

4:40 - 5:00
David L. Joly
Illumina-based genetic linkage map for wheat leaf rust.

5:00 - 5:20
Miguel Penalva
Peering into the secret-ory life of *Aspergillus nidulans* with a little help from classical genetics.

5:20 - 5:40
Patricia J. Pukkila
Domains of meiotic DNA recombination and gene conversion in *Coprinopsis cinerea* (*Coprinus cinereus*).

5:40 - 6:00
Xin Xiang
A Hook protein is critical for dynein-mediated early endosome movement in *Aspergillus nidulans*. 
THURSDAY, MARCH 14  3:00 PM–6:00 PM

CHAPEL

Fungi and Evolutionary Theory
Co-chairs: Hanna Johannesson and Duur Aanen

Abstracts for this session begin on page 49.

3:00 - 3:20
Anne Pringle
Reaching the wind: the fluid mechanics of spore discharge, and potential for dispersal mechanisms to shape the evolution of sporocarp and spore morphologies.

3:20 - 3:40
Jennifer L. Anderson
Neurospora tetrasperma mating-type chromosomes: Testing hypotheses on the effects of degeneration and introgression on performance.

3:40 - 4:00
Bart Nieuwenhuis
Nuclear arms races: sexual selection for masculine mushrooms.

4:00 - 4:20
James B. Anderson
Genome-wide mutation dynamic within a long-lived individual of Armillaria.

4:20 - 4:40 Break

4:40 - 5:00
C. Angelard
Rapid genetic change and plasticity in arbuscular mycorrhizal fungi is caused by a host shift and enhanced by segregation.

5:00 - 5:20
Pierre Grognét

5:20 - 5:40
Pierre Gladieux
Cryptic population subdivision, sympatric coexistence and the genetic basis of local adaptation in Neurospora discreta.

5:40 - 6:00
Georgiana May
Ecological context in symbioses: when is your enemy also your friend?

THURSDAY, MARCH 14  3:00 PM–6:00 PM

HEATHER

Cytoskeleton, Motors, and Intracellular Transport
Co-chairs: Samara Reck-Peterson and Ping Wang

Abstracts for this session begin on page 51.

3:00 - 3:20
Gero Steinberg
The molecular basis of extended dynein run-length.

3:20 - 3:40
Martin Egan
The role of microtubule-based motors in the spatiotemporal control of autophagy.

3:40 - 4:00
Sebastian Baumann
Microtubule-dependent co-transport of mRNPs and endosomes.

4:00 - 4:20
Flora Banuett
Role of tea1 and tea4 homologs in cell morphogenesis in Ustilago maydis.

4:20 - 4:40 Break

4:40 - 5:00
Yainitza Hernandez-Rodriguez
Aspergillus nidulans septin interactions and post-translational modifications.

5:00 - 5:20
Connie B. Nichols
Altered Ras1 trafficking impairs the pathogenicity of Cryptococcus neoformans.

5:20 - 5:40
Karen Stephenson
Quantification of the thigmotropic response of Neurospora crassa to microfabricated slides with ridges of defined height and topography.

5:40 - 6:00
P. Philippsen
Dynein drives oscillatory nuclear movements in the phytopathogenic fungus Ashbya gossypii and prevents nuclear clustering.
Fred Farr Forum

Nucleic Acid-Protein Interactions that Impact Transcription and Translation
Co-chairs: Michael Freitag and Mark Caddick

Abstracts for this session begin on page 53.

3:00 - 3:20
Koon Ho Wong
ChIP-seq: an inexpensive and powerful method for studying genome-wide chromatin remodeling and transcription regulation in fungi.

3:20 - 3:40
Jay C. Dunlap
Regulatory Networks Governing Global Responses to Changes in Light and Time.

3:40 - 4:00
L. F. Larrondo
Protein Binding Microarrays and high-throughput real-time reporters studies: Building a four-dimensional understanding of transcriptional networks in Neurospora crassa.

4:00 - 4:20
Ane Sesma
Ending messages: alternative polyadenylation in filamentous fungi.

4:20 - 4:40 Break

4:40 - 5:00
Amanda L. Misener Bloom
Post-transcriptional gene regulation contributes to host temperature adaptation and virulence in Cryptococcus neoformans.

5:00 - 5:20
Johannes Freitag
Dual targeting of glycolytic enzymes by alternative splicing and translational read-through.

5:20 - 5:40
Mian Zhou
Non-optimal codon usage determines the expression level, structure and function of the circadian clock protein FREQUENCY.

5:40 - 6:00
Michael Feldbrugge
A transcriptome-wide view on microtubule-dependent mRNA transport.

Kiln

Interactions between Fungi and Animals
Co-chairs: Neil Gow and Clarissa Nobile

Abstracts for this session begin on page 55.

3:00 - 3:20
Elaine M. Bignell
Elicitation of host damage occurs in a temporally programmed manner during Aspergillus fumigatus infections.

3:20 - 3:40
Stuart Levitz
Exploiting innate recognition of fungi for vaccine development.

3:40 - 4:00
Jose C. Perez
Regulatory circuits governing Candida albicans proliferation in a mammalian host.

4:00 - 4:20
Judith Berman
Dramatic ploidy change as an adaptive strategy in Candida albicans...

4:20 - 4:40 Break

4:40 - 5:00
Yen-Ping Hsueh
Nematode-trapping fungi eavesdrop on nematode pheromones.

5:00 - 5:20
Xiaorong Lin
A morphogenesis regulator controls cryptococcal neurotropism.

5:20 - 5:40
M. Brock
Sit and wait: Special features of Aspergillus terreus in macrophage interactions and virulence.

5:40 - 6:00
Dawn Thompson
The mutational landscape of gradual acquisition of drug resistance in clinical isolates of Candida albicans.
CONCURRENT SESSIONS SCHEDULES

Thursday, March 14   3:00 PM–6:00 PM
Nautilus

Fungal Volatiles and Organic Compounds as Signaling Agents
Co-chairs: Joan Bennett and Richard Splivallo

Abstracts for this session begin on page 58.

3:00 - 3:20
Birgit Piechulla
Fungi reacting to rhizobacterial volatiles.

3:20 - 3:40
Seogchan Kang

3:40 - 4:00
Jessica C. Hargarten
The Role of Quorum-sensing Molecules in Interactions between Candida albicans and its Host.

4:00 - 4:20
Vong shian Simon Ip Cho
Innate Immunity in Fusarium graminearum.

4:20 - 4:40 Break

4:40 - 5:00
Lea Atanasova
The Trichoderma reesei polyketide synthase gene pks1 is necessary for yellow-green pigmentation of conidia and is involved in the establishment of environmental fitness.

5:00 - 5:20
Richard Hung
Semiochemicals and signaling: plant responses to Trichoderma volatile organic compounds.

5:20 - 5:40
El Ghalid Mennat
Identification of chemoattractant compounds from tomato root exudate that trigger chemotropism in Fusarium oxysporum.

5:40 - 6:00
Richard Splivallo
The mixed fungal and bacterial origin of truffle aroma.

Thursday, March 14   3:00 PM–6:00 PM
Scripps

Genomics and Biochemistry of Degradation of Complex Molecules in the Environment
Co-chairs: Jonathan Walton and Dan Cullen

Abstracts for this session begin on page 61.

3:00 - 3:20
K. Igarashi
Fungal transcriptome as database for proteome and refinement tool of gene annotation.

3:20 - 3:40
Irina S. Druzhinina
Developmental regulation and cellulase gene expression in Trichoderma reesei.

3:40 - 4:00
D. Floudas
Parallel losses of genes associated with saprotrophy in ectomycorrhizal Agaricomycotina lineages.

4:00 - 4:20
Emma Master
Co-expression analysis of Phanerochaete carnosa during growth on hardwood and softwood species to predict proteins with unknown function relevant to biomass conversion.

4:20 - 4:40 Break

4:40 - 5:00
Yitzhak Hadar
Functional Analysis of the Pleurotus ostreatus Manganese-Peroxidase Gene Family.

5:00 - 5:20
Monika Schmoll
Carbon source and light dependent regulation of gene clusters in Trichoderma reesei (Hypocrea jecorina).

5:20 - 5:40
Chiaki Hori
Genome-wide analysis of eleven white- and brown-rot Polyporales provides insight into mechanisms of wood decay.

5:40 - 6:00
Alex Lichius
Transcription factor shuttling during cellulase induction in Trichoderma reesei.
**CONCURRENT SESSIONS SCHEDULES**

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<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Location</th>
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<tbody>
<tr>
<td>3:00 PM–6:00 PM</td>
<td><strong>Pathogenic Signaling via Effector Proteins</strong>&lt;br&gt;Co-chairs: Brett Tyler and Sebastien Duplessis</td>
<td>Merrill Hall</td>
</tr>
<tr>
<td>3:00-3:20</td>
<td>Marie-Cécile Caillaud&lt;br&gt;Dissecting nuclear immunity using Arabidopsis downy mildew effector as probes.</td>
<td></td>
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<tr>
<td>3:20-3:40</td>
<td>Claire Veneault-Fourrey&lt;br&gt;The mutualistic fungus <em>Laccaria bicolor</em> uses the effector protein MisSP7 to alter host jasmonate signaling and establish symbiosis.</td>
<td></td>
</tr>
<tr>
<td>3:40-4:00</td>
<td>Shiv D. Kale&lt;br&gt;Identification and characterization of an RXLR-like effector family from medically relevant fungi.</td>
<td></td>
</tr>
<tr>
<td>4:00-4:20</td>
<td>Yuanchao Wang&lt;br&gt;Identification and functional assay of Phytophthora sojae avirulence effectors.</td>
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<tr>
<td>4:20-4:40 Break</td>
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<td></td>
</tr>
<tr>
<td>4:40-5:00</td>
<td>Gregory J. Fischer&lt;br&gt;Fungal lipoxygenases: a novel instigator of asthma?</td>
<td></td>
</tr>
<tr>
<td>5:00-5:20</td>
<td>Martha C. Giraldo&lt;br&gt;<em>Magnaporthe oryzae</em> has evolved two distinct mechanisms of effector secretion for biotrophic invasion of rice.</td>
<td></td>
</tr>
<tr>
<td>5:20-5:40</td>
<td>Anupama Ghosh&lt;br&gt;Domains for plant uptake of <em>Ustilago maydis</em> secreted effectors.</td>
<td></td>
</tr>
<tr>
<td>5:40-6:00</td>
<td>Edouard Evangelisti&lt;br&gt;Penetration-specific effectors from <em>Phytophthora parasitica</em> favour plant infection.</td>
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<tr>
<th>Time</th>
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<tbody>
<tr>
<td>3:00 PM–6:00 PM</td>
<td><strong>Cell Wall, Polarity and Hyphal Tip Growth</strong>&lt;br&gt;Co-chairs: Stephan Seiler and Ernestina Castro-Longoria</td>
<td>Chapel</td>
</tr>
<tr>
<td>3:00-3:20</td>
<td>Michael Bölkner&lt;br&gt;The function of Rho type small GTPases for cell polarity in <em>Ustilago maydis</em>.</td>
<td></td>
</tr>
<tr>
<td>3:20-3:40</td>
<td>Peter Sudbery&lt;br&gt;A quantitative model of hyphal tip growth based on the spatial distribution of exocyst subunits in the human fungal pathogen <em>Candida albicans</em>.</td>
<td></td>
</tr>
<tr>
<td>3:40-4:00</td>
<td>Johannes Wagener&lt;br&gt;Cell wall integrity signaling in <em>Aspergillus fumigatus</em>.</td>
<td></td>
</tr>
<tr>
<td>4:00-4:20</td>
<td>Roland Wedlich-Soldner&lt;br&gt;Optimization of polarity establishment through coupling of multiple feedback loops.</td>
<td></td>
</tr>
<tr>
<td>4:20-4:40 Break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4:40-5:00</td>
<td>Vincent Bulone&lt;br&gt;Cell wall structure and biosynthesis in oomycetes and true fungi: a comparative analysis.</td>
<td></td>
</tr>
<tr>
<td>5:00-5:20</td>
<td>Lakshmi Preethi Yerra&lt;br&gt;Cellular morphogenesis of <em>Aspergillus nidulans</em> conidiophores: a systematic survey of protein kinase and phosphatase function.</td>
<td></td>
</tr>
<tr>
<td>5:20-5:40</td>
<td>Diego Delgado-Álvarez&lt;br&gt;Septum formation starts with the establishment of a septal actin tangle (SAT) at future septation sites.</td>
<td></td>
</tr>
<tr>
<td>5:40-6:00</td>
<td>Norio Takeshita&lt;br&gt;Visualization of apical membrane domains in <em>Aspergillus nidulans</em> by Phot activated Localization Microscopy (PALM).</td>
<td></td>
</tr>
</tbody>
</table>
### Friday, March 15  3:00 PM–6:00 PM

#### Heather

**Sexual Regulation and Evolution in the Fungi**  
Co-chairs: Frances Trail and Nicolas Corradi

Abstracts for this session begin on page 69.

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<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
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<tbody>
<tr>
<td>3:00</td>
<td>Ignazio Carbone</td>
<td>Clonality and sex impact aflatoxigenicity in <em>Aspergillus</em> populations.</td>
</tr>
<tr>
<td>3:20</td>
<td>Nicolas Corradi</td>
<td>Toolkit for sexual reproduction in the genome of Glomus spp; a supposedly ancient asexual lineage.</td>
</tr>
<tr>
<td>3:40</td>
<td>Frances Trail</td>
<td>Comparative transcriptomics identifies new genes for perithecium development.</td>
</tr>
<tr>
<td>4:00</td>
<td>Hanna Johannesson</td>
<td>Rapid evolution of female-biased genes: a novel example from the eukaryotic model organism <em>Neurospora crassa</em>.</td>
</tr>
<tr>
<td>4:20</td>
<td>Katherine A. Borkovich</td>
<td>Self-attraction can not bypass the requirement for two mating type genes during sexual reproduction in <em>Neurospora crassa</em>.</td>
</tr>
<tr>
<td>4:40</td>
<td>Céline M. O’Gorman</td>
<td>Fertility in <em>Aspergillus fumigatus</em> and the identification of an additional ‘supermater’ pair.</td>
</tr>
<tr>
<td>5:00</td>
<td>Julia Böhm</td>
<td>Sexual reproduction and mating type function in the penicillin producing fungus <em>Penicillium chrysogenum</em>.</td>
</tr>
<tr>
<td>5:20</td>
<td>Patrik Inderbitzin</td>
<td>The <em>Sclerotinia sclerotiorum</em> mating type locus (MAT) contains a 3.6-kb region that is inverted in every generation.</td>
</tr>
</tbody>
</table>

#### Break

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
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</thead>
<tbody>
<tr>
<td>3:00</td>
<td>A. Nantel</td>
<td>Transcriptional regulatory networks controlling the early hypoxic response in <em>Candida albicans</em>.</td>
</tr>
<tr>
<td>3:20</td>
<td>Olaf Kniemeyer</td>
<td>Proteomic analysis of the hypoxic response of the human-pathogenic fungus <em>Aspergillus fumigatus</em>.</td>
</tr>
<tr>
<td>3:40</td>
<td>N. Ponts</td>
<td>Fgap1-mediated response to oxidative stress in trichothecene-producing <em>Fusarium graminearum</em>.</td>
</tr>
<tr>
<td>4:00</td>
<td>Nallely Cano-Dominguez</td>
<td>The role of NADPH oxidases in <em>Neurospora crassa</em> cell fusion.</td>
</tr>
<tr>
<td>4:20</td>
<td>Elizabeth A. Veal</td>
<td>Peroxiredoxins in ROS responses -Why evolve peroxidases that are inactivated by peroxides?</td>
</tr>
<tr>
<td>4:40</td>
<td>Lauren S. Ryder</td>
<td>NADPH oxidases regulate septin-mediated cytoskeletal re-modeling during plant infection by the rice blast fungus <em>Magnaporthe oryzae</em>.</td>
</tr>
<tr>
<td>5:00</td>
<td>Gemma M. Cartwright</td>
<td>Redox regulation of an AP-1-like transcription factor, YapA, in the fungal symbiont <em>Epichloë festucae</em>.</td>
</tr>
<tr>
<td>5:20</td>
<td>Benjamin A. Horwitz</td>
<td>Interaction between phenolic and oxidant signaling in <em>Cochliobolus heterostrophus</em>.</td>
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**CONCURRENT SESSIONS SCHEDULES**

Friday, March 15  3:00 PM–6:00 PM  
**Kiln**

**Phylogenomics**  
Co-chairs: Jason Stajich and Joey Spatafora

Abstracts for this session begin on page 75.

3:00 - 3:20  
Dannie Durand  
*Characterizing Gene Tree Incongruence on a Genome Scale.*

3:20 - 3:40  
Mary L. Berbee  
*Early fungi and their carbohydrate active enzymes.*

3:40 - 4:00  
Jason Slot  
*Better evolution through gene clustering.*

4:00 - 4:20  
C. Alisha Owensby  
*Phylogenomics unveils secondary metabolites specific to mycoparasitic lineages in Hypocreales.*

4:20 - 4:40  
Break

4:40 - 5:00  
Robert Riley  
*Comparative analysis of 35 basidiomycete genomes reveals diversity and uniqueness of the phylum.*

5:00 - 5:20  
Helene Chiapello  
*Genome evolution of fungal pathogens from the Magnaporthe oryzae/grisea clade.*

5:20 - 5:40  
Jonathan Grandaubert  
*Leptosphaeria maculans 'brassicae': "Transposable Elements changed my life, I feel different now".*

5:40 - 6:00  
Emily A. Whiston  
*Comparing comparative "omics" in Cocciidiodes spp.*

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Friday, March 15  3:00 PM–6:00 PM  
**Nautilus**

**Synthetic Biology**  
Co-chairs: Nancy Keller and Peter Punt

Abstracts for this session begin on page 78.

3:00 - 3:20  
Debbie S. Yaver  
*Engineering Aspergillus oryzae for high level production of L-malic acid.*

3:20 - 3:40  
Pascale Daran-Lapujade  
*When synthetic biology meets metabolic engineering: in vivo pathway assembly in Saccharomyces cerevisiae.*

3:40 - 4:00  
Levente Karaffa  
*Analysis of the intracellular galactoglycom of Trichoderma reesei grown on lactose.*

4:00 - 4:20  
Peter J. Punt  
*Novel transcriptomics approaches for metabolic pathway engineering target identification in Aspergillus.*

4:20 - 4:40  
Break

4:40 - 5:00  
Y. Huang  
*A new method for gene mining and enzyme discovery.*

5:00 - 5:20  
Koichi Tamano  
*Increased production of fatty acids and triglycerides in Aspergillus oryzae by modifying fatty acid metabolism.*

5:20 - 5:40  
Ana Rems  
*Molecular biological basis for statin resistance in naturally statin-producing organisms.*

5:40 - 6:00  
Hong Luo  
*Engineering Cyclic Peptide Biosynthesis in Poisonous Mushrooms.*
Friday, March 15  3:00 PM–6:00 PM
Scripps

**Fungicides and Antifungal Compounds**
Co-chairs: Daniele Debieu and Paul Verweij

Abstracts for this session begin on page 81.

3:00 - 3:20
D. A. Macdonald
Chemically Induced Haploinsufficiency Screens to Identify Drug Mechanism of Action in Aspergillus Fumigatus.

3:20 - 3:40
Branka Korosec
Inhibition of benzoate 4-monoxygenase (CYP53A15) from Cohliobolus lunatus by cinnamic acid derivatives.

3:40 - 4:00
Marcelo HS Ramada
Secretome analysis of Trichoderma harzianum cultivated in the presence of Fusarium solani cell wall or glucose.

4:00 - 4:20
Carol E. Davis
Metabolic adaptation of the oomycete Phytophthora infestans during colonization of plants and tubers.

4:20 - 4:40 Break

4:40 - 5:00
Paul E. Verweij
The fungi strike back: multidrug resistance in Aspergillus fumigatus and agricultural use of fungicides.

5:00 - 5:20
D. Sanglard
Effect of antifungal resistance on virulence of Candida spp.

5:20 - 5:40
Sabine Fillinger
From enzyme to fungal development or how sdhB mutations impact respiration, fungicide resistance and fitness in the grey mold agent Botrytis cinerea.

5:40 - 6:00
Gabriel Scalliet
Deciphering fungicide resistance mechanisms in phytopathogenic fungi, towards an assessment of resistance risk in new active ingredient research.

Saturday, March 16  2:00 PM–5:00 PM
Merrill Hall

**Parallels between Fungal Pathogens of Plants and Animals**
Co-chairs: Barbara Howlett and Axel Brakhage

Abstracts for this session begin on page 84.

2:00 - 2:20
Sarah J. Gurr
Emerging fungal (and Oomycete) threats to plant and ecosystem health.

2:20 - 2:40
Axel A. Brakhage
Melanin as virulence determinant of human and plant pathogenic fungi.

2:40 - 3:00
Joanna Potrykus
Nutrient immunity and systemic readjustment of metal homeostasis modulate fungal iron availability during the development of renal infections.

3:00 - 3:20
A. Sharon
Common strategies in plant and human "necrotrophic" pathogens: role of PCD.

3:20 - 3:40 Break

3:40 - 4:00
Nick J. Talbot
Septin-mediated plant tissue invasion by the rice blast fungus Magnaporthe oryzae.

4:00 - 4:20
Katja Schaefer
Components of the urease complex govern virulence of Fusarium oxysporum on plant and animal hosts.

4:20 - 4:40
Anja Kombrink
The role of LysM effectors in fungal fitness.

4:40 - 5:00
Harshini C. Weerasinghe
Genes important for in vivo survival of the human pathogen Penicillium marneffei.
Saturday, March 16  2:00 PM–5:00 PM

Chapel

Secondary Metabolism
Co-chairs: Gillian Turgeon and Bettina Tudzynski

Abstracts for this session begin on page 87.

2:00 - 2:20
B. Condon
Genomic profiles of secondary metabolism genes in Cochliobolus pathogens.

2:20 - 2:40
Candace Elliott
A biosynthetic gene cluster for the antifungal metabolite phomenoic acid in the plant pathogenic fungus, Leptosphaeria maculans.

2:40 - 3:00
Eva-Maria Niehaus
Fusarin C biosynthesis in Fusarium fujikuroi: the fusarin C gene cluster, their function and regulation.

3:00 - 3:20
H. Corby Kistler
Cellular development integrating primary and induced secondary metabolism in the filamentous fungus Fusarium graminearum.

3:20 - 3:40 Break

3:40 - 4:00
Nancy Keller
LaeA sleuthing reveals cryptic gene clusters in pathogenic Aspergilli.

4:00 - 4:20
Kristina M. Smith
The KMT6 Histone H3 K27 Methyltransferase Regulates Expression of Secondary Metabolites and Development in Fusarium graminearum.

4:20 - 4:40
M. Viaud
Secondary metabolism in Botrytis cinerea: the grey and pink sides of a pathogen.

4:40 - 5:00
Frank Kempken
Is fungal secondary metabolism regulated by competing insects?

Saturday, March 16  2:00 PM–5:00 PM

Heather

Light Sensing and Circadian Rhythms
Co-chairs: Luis Larrondo and Reinhard Fischer

Abstracts for this session begin on page 90.

2:00 - 2:20
Martha W. Merrow
Circadian rhythms in gene expression in Aspergillus nidulans.

2:20 - 2:40
C. Hong
Circadian clock-gated cell division cycles in Neurospora crassa.

2:40 - 3:00
Kevin K. Fuller
Light regulates growth, stress resistance and metabolism in the fungal pathogen Aspergillus fumigatus.

3:00 - 3:20
Paulo Canessa
Shedding light on Botrytis biology: characterization of the WC1 photoreceptor and FRQ homologues in the necrotrophic plant pathogen Botrytis cinerea.

3:20 - 3:40 Break

3:40 - 4:00
Carmen Ruger-Herreros
The transcription factor FL is phosphorylated and interacts with a trehalose related protein in Neurospora crassa.

4:00 - 4:20
Alfredo H. Herrera-Estrella
Regulation of gene expression in response to light in Trichoderma atroviride.

4:20 - 4:40
Victoriano Garre
Genome-wide analysis of light responses in Mucor circinelloides.

4:40 - 5:00
Phillipp Wiemann
Shedding light on secondary metabolite cluster gene expression, sporulation, UV-damage repair and carotenogenesis in the rice pathogen Fusarium fujikuroi.
## CONCURRENT SESSIONS SCHEDULES

### Saturday, March 16  2:00 PM–5:00 PM  **Fred Farr Forum**

**Fungal Evo-Devo**  
Co-chairs: Steve Harris and Brian Shaw

Abstracts for this session begin on page 93.

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<tr>
<th>Time</th>
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</table>
| 2:00-2:20 | Antonis Rokas  
The Molecular Foundations of the Fungal Lifestyle.                                    |
| 2:20-2:40 | Daniel J. Ebbole  
Gene expression and regulation during conidial morphogenesis in *Neurospora crassa*.       |
| 2:40-3:00 | David S. Hibbett  
Comparative developmental morphology in lentinoid mushrooms: toward a new fungal evo-devo? |
| 3:00-3:20 | Steven D. Harris  
The Cdc42 GTPase module and the evolution of conidiophore architecture in *Aspergillus*. |
| 3:20-3:40 | Break                                                                                             |
| 3:40-4:00 | Audrey M. V. Ah-Fong  
Cdc14 association with basal bodies in the oomycete *Phytophthora infestans* indicates potential new role for this protein phosphatase. |
| 4:00-4:20 | Jurgen W. Wendland  
Molecular Determinants of Sporulation in *Ashbya gossypii*.                                   |
| 4:20-4:40 | Heesoo Park  
THE velvet regulators in *Aspergilli*.                                                          |
| 4:40-5:00 | R. Debuchy  
A network of HMG-box transcription factors regulates sexual cycle in the fungus *Podospora anserina*. |

### Saturday, March 16  2:00 PM–5:00 PM  **Kiln**

**Environmental Metagenomics**  
Co-chairs: Chris Schadt and Betsy Arnold

Abstracts for this session begin on page 95.

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<tr>
<th>Time</th>
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| 2:00-2:20 | Donald R. Zak  
Microbial Responses to a Changing Climate: Implications for the Future Functioning of Terrestrial Ecosystems. |
| 2:20-2:40 | Mizue Naito  
The Interaction of Mycoplasma-related Endobacteria with their Arbuscular Mycorrhizal Fungal Host. |
| 2:40-3:00 | Ning Zhang  
Metagenomic analysis reveals hidden fungal diversity in grass rhizosphere and tree foliage.    |
| 3:00-3:20 | Weiguo Fang  
Host-to-pathogen gene transfer facilitated infection of insects by a pathogenic fungus.      |
| 3:20-3:40 | Break                                                                                             |
| 3:40-4:00 | Kabir Peay  
Structure and function of soil fungal communities across North American pine forests.       |
| 4:00-4:20 | Gregory Bonito  
Genomic analysis of Mortierella elongata and its endosymbiotic bacterium.         |
| 4:20-4:40 | Richard C. Hamelin  
Integrative genomics of poplar-fungal pathogen interactions.                              |
| 4:40-5:00 | M.-S. Benitez  
Fungal pathogen and endophyte genetics within the context of forest community dynamics.   |
CONCURRENT SESSIONS SCHEDULES

Saturday, March 16   2:00 PM–5:00 PM

Nautilus

**Dimorphic Transitions**  
Co-chairs: Anne Dranginis and Alex Andrianopoulos

Abstracts for this session begin on page 97.

2:00 - 2:20  
Richard Bennett  
**Epigenetic Switching Regulates the Yeast-Hyphal Transition in Candida albicans.**

2:20 - 2:40  
Linqi Wang  
**Extracellular and intracellular signaling orchestrates morphotype-transition and virulence in human pathogen Cryptococcus neoformans.**

2:40 - 3:00  
Chad A. Rappleye  
**Histoplasma strain variations and differences in pathogenic-phase transcriptomes.**

3:00 - 3:20  
Hayley E. Bugeja  
**The C_{2}H_{2} transcription factor HgrA promotes hyphal growth in the dimorphic pathogen Penicillium marneffei.**

3:20 - 3:40 Break

3:40 - 4:00  
Joerg T. Kaemper  
**A conserved splicing factor is required for vesicle transport in Ustilago maydis.**

4:00 - 4:20  
Sarah A. Gilmore  
**N-acetylglucosamine (GlcNAc) Triggers a Morphogenetic Program in Systemic Dimorphic Fungi.**

4:20 - 4:40  
Gregory M. Gauthier  
**A GATA transcription factor encoded by SREB functions as a global regulator of transcription in Blastomyces dermatitidis.**

4:40 - 5:00  
Bridget M. Barker  
**Functional Analysis of Genes in Regions of Introgression in Coccidioides.**

Saturday, March 16   2:00 PM–5:00 PM

Scripps

**Tropic Growth and Fusion**  
Co-chairs: Andre Fleissner and Nick Read

Abstracts for this session begin on page 100.

2:00 - 2:20  
Carla J. Eaton  
**Role of the cell fusion gene idcA in fungal mutualism.**

2:20 - 2:40  
Pablo S. Aguilar  
**Role of extracellular calcium in budding yeast cell fusion.**

2:40 - 3:00  
Chia-Chen Chang  
**The role of calcium and calmodulin during cell fusion and colony initiation in Neurospora crassa.**

3:00 - 3:20  
Javier Palma-Guerrero  
**LFD-1 is a component of the membrane merger machinery during cell-cell fusion in Neurospora crassa.**

3:20 - 3:40 Break

3:40 - 4:00  
Martin Weichert  
**Specific Structural Features of Sterols Affect Cell-Cell Signaling and Fusion in Neurospora crassa.**

4:00 - 4:20  
David Turra  
**Co-option of a sex pheromone receptor and MAPK signalling pathway for chemotropism of Fusarium oxysporum towards plant host compounds.**

4:20 - 4:40  
Britta Herzog  
**Characterization of new STRIPAK complex interaction partners in the filamentous ascomycete Sordaria macrospora.**

4:40 - 5:00  
Darren Thomson  
**Characterisation of contact-dependant tip re-orientation in Candida albicans hyphae.**
Hypoxia and Mechanisms of Human Fungal Pathogenesis: To Air or Not to Air? Robert A. Cramer, Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH.

Human disease caused by fungi is increasing in frequency and clinical outcomes remain unacceptably poor due in part to a limited spectrum of treatment options. New insights into fungal pathogenesis mechanisms has great potential to uncover new therapeutic options for these devastating diseases. Our laboratory is investigating how the most common causal agent of human airborne fungal infections, Aspergillus fumigatus, adapts and grows in vivo microenvironments generated during the fungal-host interaction. We have observed that significant oxygen depletion, hypoxia, occurs at sites of A. fumigatus infection in the lung. The ramifications of hypoxia on obligate aerobic fungal metabolism, both from the perspective of in vivo fungal growth and the production of fungal metabolites that influence the innate immune response, are largely unknown. Using a combination of genomics, fungal molecular genetics, animal models of fungal disease, and immunology we have begun to unravel the impact of hypoxia on outcomes of invasive pulmonary aspergillosis. We have observed that fungal metabolic responses to hypoxia are largely interconnected with an increased demand for iron uptake that directly influences metabolic pathways requiring both oxygen and iron as co-factors such as ergosterol, heme, and cell wall biosynthesis. These responses are regulated in part by 2 transcription factors with sequence similarity to the sterol regulatory element binding protein family (SREBPs). In turn, these metabolic pathways are not only essential for fungal growth under hypoxia, but also for the production of pathogen associated molecular patterns (PAMPs) that influence the innate immune response to the invading fungus. Our data suggest that hypoxia alters the composition of the fungal cell wall resulting in increased production of pro-inflammatory cytokines from host effector cells that may cause host tissue damage. Taken together, our data suggest that manipulation of in vivo oxygen levels may be a promising strategy to augment existing antifungal drug treatment through manipulation of fungal metabolism and improve patient outcomes from these increasingly common human diseases.

Tackling biofuel bottlenecks through genome wide association studies in Saccharomyces cerevisiae. Dana J. Wohlbach1,2, Trey Sato1, Audrey P. Gasch1,2. 1) Great Lakes Bioenergy Research Center, Univ Wisconsin, Madison, Madison, WI; 2) Laboratory of Genetics, Univ Wisconsin, Madison, Madison, WI.

Generating biofuels from cellulosic plant material is a major goal in bioenergy research. However, a critical bottleneck is the inhibition of microbial fermentation by toxic compounds in the hydrolyzed plant biomass, generated during chemical pretreatment. We are exploiting natural variation in yeast hydrolysate tolerance to implicate genes and processes for targeted strain engineering. We phenotyped growth rates of 65 diverse wild and industrial S. cerevisiae strains grown in several different plant hydrolysates, and then performed a genome-wide association study (GWAS) to identify loci that correlated with hydrolysate tolerance. This identified nearly 70 loci, encompassing over 100 genes that fell into specific pathways and signaling networks. We also identified pervasive epistasis across loci, suggesting the importance of gene-gene interactions in phenotypic variation. The results provide a platform for synthetic engineering of hydrolysate tolerance in diverse yeast strains.

Exploring the metabolome of cereal infecting fusaria. Kim E. Hammond-Kosack1, Rohan G.T. Lowe1, Gail Canning1, Martin Urban1, Michael H. Beale2, Jane L. Ward1. 1) Dept of Plant Biology and Crop Sciences, Rothamsted Research, Harpenden, Herts, ALS 2JQ, UK; 2) National Centre for Plant and Microbial Metabolomics, Rothamsted Research, Harpenden, Herts, ALS 2JQ, UK.

The Ascomycete fungus Fusarium graminearum (Gibberella zeae) causes plant disease on many cereal crop species including wheat, barley and maize and is also becoming a problem pathogen in several non-cereal crop species, including soybean and sugar beet. In cereal crops, floral infections at anthesis go on to reduce grain yield and quality and contaminate the crop with secondary metabolites harmful to plants, animals and humans. These metabolites include trichotheceene mycotoxins such as deoxynivalenol (DON) and their acetylated derivatives. We have already reported on the use of metabolomic analysis to understand the basal metabolism in four Fusarium spp., F. graminearum, F. culmorum, F. pseudograminearum and F. venenatum under DON and non-DON inducing conditions [Lowe et al., 2010, MPMI, 23, 1605-1618]. Three additional studies have now been completed using a ‘triple-fingerprinting’ of analytical techniques including 1H-NMR and electrospray mass-spectroscopy (+/− ESI-MS). First, we have investigated the global metabolic changes occurring during a time course of DON mycotoxin induction in vitro using the F. graminearum wild-type strain PH-1 for which the complete genomic sequence is available (http://www.broad.edu; www.phytopathdb.org). Second, we have characterised many single gene deletion mutants affected in mycotoxin biosynthesis, cell signalling and plant pathogenicity. Interestingly, most mutants show significant and highly specific metabolome changes compared to the parental wild-type strain(s). Third, we have explored the metabolome of several reduced virulence mutants arising from a random plasmid insertion, forward genetic screen to determine whether the triple fingerprinting technique can be used predictively. Metabolic network analysis of the data sets is now being used to link the co-occurrence of known and unknown metabolites to DON mycotoxin production and to aid the future characterisation of the many unknown metabolites present in F. graminearum. This research was supported by a metabolomics special initiative grant from the Biotechnology and Biological Sciences Research Council.
Carbohydrate-active enzymes in fungal genomes. **Bernard Henrissat.** AFMB, CNRS and Aix-Marseille University, Marseille, France.

We term carbohydrate-active enzymes (CAZymes) the enzymes that assemble and breakdown complex carbohydrates and carbohydrate polymers. As such carbohydrates are crucial for fungi as carbon sources but also for cell wall synthesis/remodelling, host pathogen interactions, energy storage etc. Unlike many other classes of enzymes which carry limited informative power, the peculiarities of CAZymes and of their substrates turn these enzymes into extremely powerful probes to examine genomes and explain the lifestyle of living organisms and fungi in particular. Over the last few years we have explored the CAZyme content of over 200 fungal genomes and we will review how evolution shapes the CAZyme profiles of fungi.

Suggested reading:
- O’Connell et al. (2012) Life-style transitions in plant pathogenic Colletotrichum fungi defined by genome and transcriptome analyses. Nature Genetics 44, 1060-1065

Genome-wide approaches to identify and characterize lignocellulolytic enzymes. **Adrian Tsang.** Biol, Concordia Univ, Montreal, Canada.

Lignocellulosic material is both the most abundant source of biomass on the planet and an enormous storehouse of sugars. Yet the sugars in cellulosic material are remarkably recalcitrant. The ability to detect new enzymes, to produce them in large quantities, and to understand how they work will lay the groundwork for the development of more efficient and economical processes for lignocellulosic biomass. We are particularly interested in harnessing the lignocellulolytic ability of thermophilic fungi as they are potential reservoirs of thermostable enzymes for industrial applications. So far, fewer than 50 fungal species have been described as thermophiles. We have sequenced over 20 species of thermophilic fungi, see www.fungalgenomics.ca. Most of these thermophiles belong to the orders Sordariales and Eurotiales, three species belong to the Mucorales and one to Onygenales. We have developed computational tools to improve the identification genes in fungal genomes in general, and genes encoding extracellular proteins in particular because biomass-degrading enzymes are predominantly extracellular proteins. In addition to using informatics tools to identify orthologues of lignocellulolytic enzymes, we have analyzed the transcriptomes and exo-proteomes of the thermophilic fungi when cultured in a variety of agricultural straws to reveal the strategies used by different fungi in the decomposition of lignocellulose as well as identifying novel extracellular proteins that may play a role in biomass decomposition. Over 2000 genes encoding potential lignocellulolytic proteins have been identified. The Sordariales possess a larger repertoire of lignocellulolytic enzymes than the thermophiles from other orders. The genes predicted to encode lignocellulolytic proteins have been cloned and transformed into Aspergillus niger for the production of recombinant enzymes. Biochemical characterization of the recombinant enzymes show that in addition to producing enzymes that are thermostable, the thermophiles also produce enzymes that have temperature optimum in the 40-50°C range.
PLENARY SESSION ABSTRACTS

Thursday, March 14 8:30 AM–12:00 NOON
Merrill Hall and Chapel

Plenary Session II: Organismic Molecular Interactions
Chair: Nick Talbot

Large-scale Biology for Fungal Pathogenicity in Magnaporthe oryzae, Yong-Hwan Lee1,2. 1) Department of Agricultural Biotechnology, Seoul National Univ, Seoul, 151-921, Korea; 2) Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea.

Rice blast is a compelling model system for studying host-parasite interactions due to its socioeconomic impact and the availability of both the rice and fungal genomic sequences. In an attempt to understand the molecular mechanisms of rice blast, we have been taking both forward and reverse genetics approaches. Our researches using reverse genetics approach focus on identifying and characterizing the genes involved in signal transduction pathways leading to appressorium formation, genes encoding transcription factors, and genes that are required for post-penetration stages. For forward genetics studies, we carried out a large-scale insertional mutagenesis of the Magnaporthe oryzae strain K201 via Agrobacterium tumefaciens-mediated transformation, generating over 25,000 mutants. We also developed high throughput phenotype screening system that enables rapid and robust assay of mutant phenotypes. In addition to our endeavor to functional and comparative genomics, we built a cyber-infrastructure for storage of heterogeneous data and analysis of such data in multiple contexts. The whole genome sequence information of M. oryzae as well as most of the results from experimental biology is housed in our customized database. Our comprehensive and integrative approaches coupled with a web-based Laboratory Information Management System would provide a novel platform for systems biology initiatives for fungal pathogenesis.


In fungal pathogens, contact with the host triggers a developmental and metabolic transition towards infectious growth. What exactly defines infectious growth and how it is controlled by environmental and host-derived stimuli is not fully understood. We study infectious growth in Fusarium oxysporum, a soilborne fungus that causes vascular wilt disease on a wide range of plant species and opportunistic infections in immunocompromised humans. One of the key players in pathogenicity is Fmk1, a conserved mitogen-activated protein kinase (MAPK) that is essential for infection-related processes such as chemotropism, host adhesion, penetration and invasive growth in the plant tissue. Most Fmk1-dependent virulence functions require the homeodomain transcription factor Ste12, and are repressed in the presence of the preferred nitrogen source ammonium through a mechanism that requires the transporter MepB and the bZIP factor MeaB. Recent data suggest that ammonium repression is mediated by a shift in extracellular pH, which results in rapid changes in the phosphorylation pattern of different MAPKs. Our current research addresses the mechanisms through which pH controls invasive growth of F. oxysporum by reprogramming the activation status of cellular MAPK signalling cascades.

Analysis of effector proteins from flax rust and wheat stem rust, Peter Dodds1, narayana Upadhyaya1, Ann-Maree Catanzariti1, Markus Koeck1, Adnane nemri1, Rohit Mago1, Simon Williams1, Thomas Ve1, Maryam Rafiq1, Wenjie Wu2, Adrienne Hardham2, David Jones2, Jeff Ellis2, Bostjan Kobe1, Robert Park1. 1) Plant Industry, CSIRO, Canberra, ACT, Australia; 2) Australian National University, Research School of Biology,.; 3) University of Queensland, School of Chemistry and Molecular Biosciences; 4) Justus Liebig University, Giessen, Germany; 5) University of Sydney, Camden.

Rust fungi cause economically important diseases of cereal crops worldwide, with stem rust caused by the fungus Puccinia graminis tritici one of the most serious diseases in wheat. Because of the ability of the fungus to evolve increased virulence towards previously resistant varieties, continuous breeding and the identification of new sources of resistance is necessary to keep apace of the threat of rust epidemics. We have been studying how the plant immune system can recognise and respond to rust pathogens using the flax rust model system. Rusts are obligate parasites of plants, and produce a specialised infection structure called the haustorium which directly penetrates an infected cell and is the main site of nutrient extraction for the fungus. A suite of disease effector proteins are secreted from haustoria and enter the host cells where they may allow the rust to commandeer host cell biology. It is these translocated effector proteins that are recognised by host immune receptors, known as resistance (R) proteins. We have been exploring the structure and function of host-translocated effectors from flax rust and also searching for effector candidates from stem rust that are recognised by known wheat R genes. Using genome and transcriptome sequencing we have predicted, carefully curated and analysed the transcription of 400 candidate effector genes from the Australian stem rust strain 21-0. To screen for effectors recognized by wheat R genes, we have developed a bacterial Type III Secretion System (TTSS)-based delivery assay from the non-pathogen Pseudomonas fluorescens strain Pf0. We are screening candidate effectors on a set of 18 wheat cultivars carrying 22 different R genes. Thus far we have identified on effector protein induces a rapid cell death response specifically on a wheat genotype carrying Sr22. We are also analyzing sequence variation in effector candidates between clonal field isolates that have mutated to overcome the resistance genes that have been deployed in agriculture.
Dissecting Phytophthora blight; making sense out of signalling, effectors and host targets. Francine Govers, Lab. of Phytopathology, Wageningen University, Wageningen, Netherlands.

The plant pathogen *Phytophthora infestans* causes late blight, the disease that was responsible for the Irish potato famine in the mid-nineteenth century. This oomycete has a hemibiotrophic life style, a narrow host range and a large genome of ~ 240 Mb. Comparative genomics revealed features illuminating its success as a pathogen, such as rapid turnover and massive expansion of families encoding secreted proteins, and peculiar gene innovations resulting in proteins with oomycete-specific domain combinations. An example of a novel protein family is the GPCR-PIPK family. Its twelve members all have a N-terminal 7-transmembrane domain typical for G-protein coupled receptors (GPCRs) combined with a phosphatidylinositol phosphate kinase (PIPK) domain at the C-terminus. This domain structure suggests that GPCR-PIPKs use GPCRs to directly feed extracellular signals into phospholipid signalling pathways. Their differential expression and localization point to distinct roles in various cellular processes. For one GPCR-PIPK we could demonstrate a role in asexual development, including spore germination, hyphal elongation and sporangia cleavage, whereas inactivation of another GPCR-PIPK disturbs sexual development. For successful infection *Phytophthora* secretes a variety of proteins including a large number of effectors that share the host-cell targeting motif RXLR. Inside host cells these RXLR effectors promote virulence by manipulating the cell machinery via interaction with host targets thereby suppressing host defence. However, in plants carrying matching resistance genes RXLR effectors trigger defence and thus act as avirulence factors. Here I will focus on an RXLR effector that interacts with an exocyst component and show how the interplay between this effector and its host target influences the host-pathogen interaction.

Understanding directional growth in fungi. Alexandra C. Brand, Aberdeen Fungal Group, Univ Aberdeen, Scotland, United Kingdom.

Fungal hyphae are programmed to explore their surroundings in search of nutrients and, for pathogens, success can depend on locating and identifying suitable host penetration sites. Fungi have therefore evolved mechanisms that link the sensing of environmental cues with an appropriate growth response. The intracellular components involved in polarised growth in fungi are generally well-conserved and have been studied in model organisms such as *Saccharomyces cerevisiae*, *Neurospora crassa* and *Aspergillus* spp. However, how environmental signals interact with the molecular machinery of hyphal tip growth is less well-understood. *Candida albicans* is an opportunistic pathogen that exhibits pre-programmed, or tropic, growth responses to specific stimuli. This makes it a useful model for dissection of the regulatory pathways that control hyphal tip behaviour. A variety of external stimuli, including electric fields, surface modification and nanofabrication techniques, have been used to examine the physical properties of apical growth, such as directional memory, asymmetric tip organisation and hyphal tip force. In addition, these methods have been coupled with reverse genetics, fluorescence protein-tagging and live-cell imaging to identify cell-polarity components that can enhance, or even reverse, the direction of hyphal growth. The evidence to date suggests that the direction of hyphal growth reflects the net output from overlapping positional determinants. In addition, there is a strong association between proper hyphal tip regulation and the ability of a fungus to invade and damage host tissue.
A universally important aspect of growth and development is the integration of mitosis with cell division. This helps ensure that cells maintain their normal size, shape and nuclear number, which in the fungi can vary considerably. For example, the highly polarized mode of growth of the filamentous fungi is subject to complex developmental regulation yielding diverse cell types containing from one to dozens of nuclei. How fungi integrate the regulation of the developmental axis involving mitosis, cytokinesis, and morphogenesis to maintain their defined cellular shapes, with distinctive numbers...
of nuclei, remains a mystery. However, recent studies of the mitotic NIMA kinase indicates it plays additional non-mitotic cytoplasmic functions in *Aspergillus nidulans* that impinge on fungal development. These insights were derived initially from defining the interphase subcellular locations of NIMA which revealed it locates to both forming and mature septa and additionally locates to tips of growing interphase cells. Subsequent studies revealed that septal pores are subject to cell cycle regulation which prevents cytoplasmic movement between mitotic cell compartments and their adjacent interphase partners. We further find that NIMA markedly affects the regulation of cell tip dominance and morphology via a mechanism involving NIMA location at microtubule +ends and the modulation of interphase cytoskeletal functions. Collectively the findings indicate that the mitotic NIMA kinase has roles to regulate communication between adjacent hyphal cells as well as cytoskeletal functions important for normal tip cell growth. Thus NIMA has the potential to help integrate nuclear division with cell division and morphogenesis.

**A Neurospora cell-free system reconstitutes peroxisome membrane protein synthesis and organelle-specific targeting.** [Gregory Jedd](#). Temasek Life Sciences Laboratory, Singapore, Singapore.

A central problem faced by eukaryotic cells is how to ensure that membrane proteins are localized to the appropriate organelle. Peroxisomes are ubiquitous eukaryotic organelles that proliferate through growth and division, and can also arise de novo from endoplasmic reticulum (ER)-derived precursors. Two distinct views for the biogenesis of peroxisome membrane proteins (PMPs) are currently entertained. In the direct targeting model, PEX19 recognizes PMPs in the cytosol and ferries them to the peroxisome where interaction with PEX3 prompts PMP release and membrane integration. In the second model, nascent PMPs are integrated to the ER membrane first, and then traffic to the peroxisome membrane. In this case, PEX19 functions as a sorting receptor to package PMPs into ER-derived vesicles. My talk will focus on development of a cell-free system that reconstitutes PMP synthesis and targeting to the peroxisome membrane. I will discuss how distinct chaperones and sequences associated with transmembrane domains distinguish direct ER and peroxisome targeting pathways.
Mechanisms allowing the formation of new fungal pathogenic species on novel hosts, causing emerging diseases. Tatiana Giraud, ESE, Univ Paris 11, Orsay, France.

We have studied different mechanisms allowing the formation of novel fungal pathogenic species on novel hosts. A theoretical model combined with a literature survey have confirmed that mating within hosts in pathogenic ascomycetes may allow the rapid formation of new species by host adaptation alone, without requiring the evolution of mate choice. We also present studies on mechanisms of speciation in Microbotryum a fungal complex causing the sterilizing anther-smut disease in many species of Caryophyllaceae. Multiple gene phylogenies and measures of hybrid inviability and sterility have revealed the existence of multiple cryptic species, each being specific to one or a few plant host. We investigated the evolution of reproductive isolation in the species complex, and showed that hybrid inviability and sterility increased with the genetic distance between the species. We show that selfing is the main barrier to gene flow between close species and that hybrid sterility arise because of karyotypic rearrangements. Cophylogeny analyses showed that Microbotryum species have evolved through frequent host shifts to moderately distant hosts. Current geographic distribution of host species seemed to be of little relevance for understanding the putative historical host shifts, because most fungal clades had overlapping ranges. We did detect some ecological similarities between host species that were diseased by closely related anther smut species, including pollinator or habitat similarities. However, genetics underlying the host-parasite interactions appeared to be the most important factor influencing host-shifts and specialization: multi-host species parasitized closely related plant species and related species in the Microbotryum phylogeny were associated with members of the same host clade. We performed a cross-inoculation experiment and showed that both host and pathogen phylogenies were indeed significant predictors of host range, with at least partly independent effects. We investigated whether some Microbotryum species have arisen via hybridization. We also detected hybrids in nature and underwent a population genomic study to unravel the genomic architecture of introgression. Anther smut fungi appear as excellent models to unravel the mechanisms of formation of new fungal species onto novel hosts.


Boreal forest soils represent a significant global sink for carbon, but poor knowledge about the mechanisms that regulate the dynamics of soil carbon pools hampers the development of predictive ecosystem models. Such models are urgently needed to guide proper management of forest land, in order to mitigate increasing atmospheric CO2 levels. By analysing the natural abundance of rare isotopes (14C, 13C and 15N), we found that the mycelium of mycorrhizal fungi represents a major source of soil carbon. Up to 50-70% of stored carbon was estimated to enter the soil via plant roots. Ratios between fungal biochemical markers (ergosterol and chitin) indicate that a rapid turnover of fungal mycelium minimizes carbon storage and favours efficient nitrogen recycling, whereas slow mycelial turnover favours carbon sequestration and immobilisation of nitrogen. In order to relate taxonomic and functional diversity to carbon dynamics, we used 454-sequencing of ITS2 amplicons to analyse fungal communities in environmental samples. In forest soils with low nitrogen availability we found high abundance of ectomycorrhizal genera with differentiated extra-radical mycelium (cord formers). Member of this group also correlated with high activities of classII peroxidases, known to facilitate break down of complex organic matter. Further evidence that these fungi act as “mycorrhizal white rotters” were obtained by amplification of peroxidase mRNA from soil extracts. The mRNA could be connected to mycorrhizal species by sequence homology. We propose that conditions of low nutrient availability favour the establishment of mycorrhizal species that are adapted to minimize immobilisation of nitrogen in stable organic pools. As their own mycelium represents a major sink for soil-derived nutrients, these species have to re-cycle their own biomass rapidly, in order to enable efficient delivery of nutrients to their host plants. They also possess potent oxidative enzymes that may be used to increase mobility of organic nutrients. As a side-effect of their highly efficient nutrient recycling, presence of these fungi also minimizes long term carbon sequestration in soils.


The budding yeast, Saccharomyces cerevisiae, along with its close relatives, have only recently become reasonable models for the study of population genetics and evolution. This has been due to the lack of understanding of their natural history and ecology. Now that some understanding of budding yeast in nature is in hand, we can apply the powerful genetic and molecular tools available to questions of evolution through adaptation to ecological niches. The combination of population genomics and quantitative trait analysis has led to some understanding of the genetic architecture underlying traits which may be relevant to adaptation to particular environments in different yeast populations/species.
PLENARY SESSION ABSTRACTS

The mycorrhizal symbiosis as a network linking plants. Marc-André Selosse. Centre d’Ecologie Fonctionnelle et Evolutive, Montpellier, France.

Most plants form mycorrhizal symbioses with soil fungi, which turn out to form networks between plants. Indeed, fungal individuals are large enough to colonize several root systems, and most mycorrhizal fungi are host-generalists that can link different plant species. The most dramatic evidence for such networks is the repeated emergence of mycoheterotrophy (MH) in plant evolution. MH plants are achlorophyllous and receive carbon (C) from surrounding green plants by way of shared mycorrhizal fungi. They recently made strong achievements due to two tools: molecular barcoding identified the fungi, and natural isotopic abundances ($^{13}$C, $^{15}$N) confirmed which fungal mycorrhizal guild provides C. Temperate MHs belong to orchids and Monotropoidea (an Ericaceae subfamily) associate often with high specificity to basidiomycetes that form themselves ectomycorrhiza with surrounding trees. Intermediate evolutionary steps exist, in green orchids and Montropoidea that use C from mycorrhizal fungi in addition to their photosynthesis. This so-called mixotrophic nutrition depends on ectomycorrhizal fungi, and thus also on mycorrhizal networks. Phylogenies support that mixotrophy predisposed to evolution of MHs. In some green mixotrophic orchids, the survival of rare achlorophyllous variants further supports MH abilities, but the low fitness of these variants suggests that mixotrophy can be evolutionarily metastable. By contrast, tropical MH plants belong to diverse families, display lower specificities, and often associate to arbuscular mycorrhizal (AM) fungi. The isotopic fractionation for $^{15}$N and $^{13}$C along the [green plant-AM fungi-MH plants] continuum shows differences as compared to the same continuum for temperate MHs, which associate with ectomycorrhizal basidiomycetes. This supports different exchange mechanisms. Moreover, MHs associated to basidiomycetes have higher total N and $^{15}$N content than autotrophic plants, while AM-associated MHs do not. I hypothesize that AM-associated MHs evolved mainly to support C nutrition, under selection of shaded, N-rich tropical forests. Conversely, basidiomycetes-associated MHs may have been first selected for N acquisition in N-limited, but less shaded, temperate forests. Thus, the convergent exploitation of mycorrhizal networks may result from different evolutionary pathways that depend on the biome.

Unraveling speciation and specialization processes in plant pathogenic fungi using comparative population genomics. Eva H Stukenbrock1, Freddy B Christiansen2, Julien Y Dutheil3, Bruce A McDonald4, Thomas Bataillon4, Mikkel H. Schierup5. 1) Fungal Biodiversity, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany; 2) Bioinformatics Research Center, Aarhus University, C.F. Moellers Alle 1110, 8000 Aarhus, Denmark; 3) Plant Pathology, ETH Zurich, LFW B16, 8092 Zurich, Switzerland.

The emergence of new fungal pathogens in managed ecosystems is an urgent matter of consideration. Studies have documented that natural ecosystems serve as reservoirs and sources of new crop infecting pathogens. We know, however, very little about the ecology and diversity of fungal pathogens in natural ecosystems. A goal of our research is to infer diversification and speciation processes of plant pathogens in natural and managed ecosystems. We study a species complex of plant pathogenic fungi including the wheat pathogen Zymoseptoria tritici (synonym Mycosphaerella graminicola). Speciation of Z. tritici was associated with wheat domestication and dates back to 10-12000 ya. Several closely related species of Z. tritici exist in natural grasslands in Iran. These wild grass pathogens co-exist and have over-lapping host ranges. In spite of their close relatedness, ecological or spatial factors have allowed speciation to occur. We have taken a comparative population genomics approach to study the underlying evolutionary processes that drive Zymoseptoria diversification. Our study includes full genome sequences from 52 fungal isolates representing four Zymoseptoria species. We perform population genomics analyses and document recent speciation times in the Zymoseptoria complex and present day small effective population sizes. Using within and between species rates of non-synonymous and synonymous variation we show a strong impact of natural selection in genome evolution of Zymoseptoria spp. This is at odds with the small effective population sizes estimated and suggests that population sizes were historically large but unstable. A significant finding is that speciation of Z. tritici did not entail an apparent loss of variation in spite of the homogenous agro-ecosystem where it has evolved. In contrast, we observe a dramatic loss of variation in the closest wild relative, Z. pseudotritici. The mosaic genome patterns in Z. pseudotrictici are consistent with a very recent hybrid speciation event resulting from a cross between two divergent haploid individuals. We estimate that the hybridization occurred “500 sexual generations ago between closely related, but isolated species. Based on the comparative population genomic analyses we reveal rapid evolution and distinct patterns of species evolution in natural and managed ecosystems.
CONCURRENT SESSION ABSTRACTS

Wednesday, March 13  3:00 PM–6:00 PM
Merrill Hall

Cell Signaling Involved in Fungal Development and Pathogenesis
Co-chairs: Naweed Naqvi and Stefanie Pöggeler

Stability of a G protein alpha subunit in genetic backgrounds lacking the G beta subunit or a cytosolic guanine nucleotide exchange factor. Alexander V. Michkov and Katherine A. Borkovich. Plant Pathology and Microbiology, University of California, Riverside, CA.

Heterotrimeric G proteins consist of alpha, beta and gamma subunits. Regulation is accomplished through the alternation between binding of GDP (inactive form) and GTP (active form) by the alpha subunit and dissociation of the alpha subunit and beta-gamma dimer. GDP/GTP exchange is facilitated by both cell surface G protein coupled receptors and cytosolic guanine nucleotide exchange factors (GEFs), such as RIC8. *Neurospora crassa* has three G alpha subunits (GNA-1, GNA-2 and GNA-3), one G beta (GNB-1), and one G gamma (GNG-1). Interestingly, mutants lacking gnb-1 or the cytosolic GEF ric8 exhibit some defects in common with the gna-1 deletion mutant, which may be explained by the reduced GNA-1 protein levels observed in these mutants. Previous studies in our laboratory showed that levels of gna-1 mRNA are similar in wild type and mutants lacking gnb-1 or ric8, consistent with a post-transcriptional mechanism. Using genetic and biochemical approaches, this study investigated the mechanism underlying regulation of GNA-1 stability in regards to GTP/GDP bound state and amount of protein (normal or overexpressed). The results demonstrate that levels of GNA-1 protein are not visibly reduced over 36 hours in a wild-type background after halting translation using cycloheximide, suggesting GNA-1 is very stable in wild type. To check stability of GDP or GTP bound GNA-1 in different backgrounds, we transformed mutants lacking the gna-1 gene and gnb-1 or ric8 with a wild type (gna-1WT) or constitutively active, GTPase-deficient gna-1 allele (gna-1DGNB). Overexpressing gna-1WT (GDP bound) in a wild-type background increased the level of GNA-1 protein ~3 fold, while overexpression in a gnb-1 mutant gave a nominal increase (~1.6x). Overexpressing gna-1DGNB (GTP bound) in the Dgnb-1 or Dric8 backgrounds led to ~2 fold higher levels of GNA-1 compared to wild type. In summary, GNA-1 is very stable in wild type, but stability decreases dramatically in gnb-1 and ric8 deletion mutants. The GTP-bound G alpha protein is more stable in a gnb-1 mutant background than GDP-bound GNA-1 protein.

The Putative Guanine Nucleotide Exchange Factor RicA Mediates Upstream Signaling for Growth and Development in *Aspergillus*. Nak-Jung Kwon1, Hee Soo Park2, Seunho Jung3, Sun Chang Kim4, Jae-Hyuk Yu5. 1) Dept Bacteriology, University of Wisconsin, Madison, WI, USA; 2) Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, WI, USA; 3) Department of Bioscience and Biotechnology, and Center for Biotechnology Research in UBITA, Konkuk University, Seoul, Republic of Korea; 4) Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Dae-Jon, Republic of Korea.

Heterotrimeric G proteins (G proteins) govern growth, development, and secondary metabolism in various fungi. Here, we characterized ricA, which encodes a putative GDP/GTP exchange factor for G proteins in the model fungus *Aspergillus nidulans* and the opportunistic human pathogen *Aspergillus fumigatus*. In both species, ricA mRNA accumulates during vegetative growth and early developmental phases, but it is not present in spores. The deletion of ricA results in severely impaired colony growth and the total (for *A. nidulans*) or near (for *A. fumigatus*) absence of asexual sporulation (conidiation). The overexpression (OE) of the *A. fumigatus* ricA gene (*AfricA*) restores growth and conidiation in the DricA mutant to some extent, indicating partial conservation of RicA function in *Aspergillus*. A series of double mutant analyses revealed that the removal of RgsA (an RGS protein of the GanB G protein family), but not sfgA, fibA, rgsB, or rgsC, restored vegetative growth and conidiation in AnricA. Furthermore, we found that RicA can physically interact with GanB in yeast and in vitro. Moreover, the presence of two copies or OE of pkaA suppresses the profound defects caused by DAnricA, indicating that RicA-mediated growth and developmental signaling is primarily through GanB and PkaA in *A. nidulans*. Despite the lack of conidiation, brlA and voxA mRNAs accumulated to normal levels in the ricA mutant. In addition, mutants overexpressing fluG or brlA (OEfluG or OEbrlA) failed to restore development in the AnricA mutant. These findings suggest that the commencement of asexual development requires unknown RicA-mediated signaling input in *A. nidulans*.

The *Aspergillus nidulans* MAPK module AnSte11-Ste50-Ste7-Fus3 controls development and secondary metabolism. Oezguer Bayram1, Oezlem Sarikaya Bayram2, Yasar Luqman Ahmed3, Jun-Ichi Maruyama4, Oliver Valerius5, Silvio Rizzoli6, Ralf Ficner7, Gerhard Braus8. 1) Institute of Microbiology & Genetics, Department of Molecular Microbiology and Genetics, Georg-August-Universität, Grisebachstr. 8, D 37077 Goettingen, Germany; 2) Department of Molecular Structural Biology, Institute for Microbiology and Genetics, Georg-August-Universität, Goettingen, Germany; 3) European Neuroscience Institute, Deutsche Forschungsgemeinschaft Center for Molecular Physiology of the Brain/Excellence Cluster 171, 37077 Göttingen; 4) Department of Biotechnology, The University of Tokyo, Tokyo, Japan.

The sexual Fus3 MAP kinase module of yeast is highly conserved in eukaryotes and transmits external signals from the plasma membrane to the nucleus. We show here that the module of the filamentous fungus *Aspergillus nidulans* (An) consists of the AnFus3 MAP kinase, the upstream kinases AnSte7 and AnSte11, and the AnSte50 adaptor. The fungal MAPK module controls the coordination of fungal development and secondary metabolite production. It lacks the membrane docking yeast Ste5 scaffold homolog but similar to yeast the entire MAPK module interacts with each other at the plasma membrane. AnFus3 is the only subunit with the potential to enter the nucleus from the nuclear envelope. AnFus3 interacts with the conserved nuclear transcription factor AnSte12 to initiate sexual development and phosphorylates VeA which is a major regulatory protein required for sexual development and coordinated secondary metabolite production. Our data suggest that not only Fus3 but even the entire MAPK module complex of four physically interacting proteins can migrate from plasma membrane to nuclear envelope.
The developmental PRO40/SOFV2 protein participates in signaling via the MIK1/MEK1/MAK1 module in *Sordaria macrospora*. Ines Teichert¹, Eva Steffen¹, Nicole Schnabl¹, Benjamin Fränzel², Christoph Krisp², Dirk A. Wolters², Ulrich Kück³. 1) General & Molecular Botany, Ruhr University Bochum, Bochum, Germany; 2) Analytical Chemistry, Ruhr University Bochum, Bochum, Germany.

Filamentous fungi are able to differentiate multicellular structures like conidiophores and fruiting bodies. Using the homothallic ascomycete *Sordaria macrospora* as a model system, we have identified a number of developmental proteins essential for perithecium formation. One is PRO40 [1], the homolog of *Neurospora crassa* SOFT, and this protein was employed for protein-protein interaction studies to gain insights into its molecular function. Data from yeast two hybrid experiments with PRO40 as bait show an interaction of PRO40 with the MAP kinase kinase (MAPKK) MEK1. MEK1 is a member of the cell wall integrity (CWI) pathway, one of three MAP kinase modules present in *S. macrospora*. The *S. macrospora* CWI pathway consists of MAP kinase kinase (MAPKKK) MIK1, MAPKK MEK1 and MAP kinase (MAPK) MAK1, with additional upstream components, protein kinase C (PKC1) and RHO GTPase RHO1. Data from tandem affinity purification - MS experiments with PRO40 and MEK1 as bait indicate that PRO40 forms a complex with components of the CWI pathway. Analysis of single and double knockout mutants shows that PRO40, MIK1, MEK1 and MAK1 are involved in the transition from protoperithecia to perithecia, hyphal fusion, vegetative growth, and cell wall stress response. Differential phosphorylation of MAPKs in a pro40 knockout strain was detected by Western analysis. We propose that PRO40 modulates signaling through the CWI module in a development-dependent manner. Further interaction studies and complementation analyses with PRO40 derivatives provide mechanistic insight into the function of PRO40 domains during fungal development. [1] Engh et al. (2007) Eukaryot Cell 6:831-843.

A Fungal Adhesin Guides Community Behaviors by Autoinduction and Paracrine Signaling. Linqi Wang, Xunyun Tian, Rachana Gyawali, Xiaorong Lin. Biology, Texas A&M University, College Station, TX.

Microbes live mostly in a social community rather than in a planktonic state. Such communities have complex spatiotemporal patterns that require intercellular communication to coordinate gene expression. Here, we demonstrate that *Cryptococcus neoformans*, a model eukaryotic pathogen, responds to an extracellular signal in constructing its colony morphology. The signal that directs this community behavior is not a molecule of low molecular weight like pheromones or quorum sensing molecules, but a secreted protein. We successfully identified this protein as the conserved adhesin/matrix protein interaction studies to gain insights into its molecular function.

Surface recognition and appressorium morphogenesis in Magnaporthe oryzae. JinRong Xu. Dept Botany & Plant Pathology, Purdue Univ, West Lafayette, IN 47906.

Appressorium formation and penetration play critical roles in plant infection in the rice Blast fungus and other foliar pathogens. In Magnaporthe oryzae, the cAMP signaling and Pmk1 MAP kinase pathways are known to regulate surface recognition, appressorium formation, penetration, and invasive growth. Like other filamentous ascomycetes, *M. oryzae* contains two genes encoding catalytic subunits of PKA. Whereas the cPkA mutant was delayed in appressorium formation and reduced in virulence, the cPk2 mutant had no detectable phenotypes except a slight reduction in conidiation. However, the cPkA cpk2 double mutant recently identified in our lab had distinct defects in growth, conidiation, appressorium formation, and cell wall stress response. The cPkA cpk2 double mutant recently identified in our lab had distinct defects in growth, conidiation, appressorium formation, and cell wall stress response. Differential phosphorylation of MAPKs in a pro40 knockout strain was detected by Western analysis. We propose that PRO40 modulates signaling through the CWI module in a development-dependent manner. Further interaction studies and complementation analyses with PRO40 derivatives provide mechanistic insight into the function of PRO40 domains during fungal development. [1] Engh et al. (2007) Eukaryot Cell 6:831-843.


Fungal cell wall, mainly composed of polysaccharides, is a major source of microbe-associated molecular patterns (MAMPs) which are recognized by host innate immune receptors. Although recognition of the fungal cell wall MAMPs, such as chitin oligomers, activates defense responses in plants, fungal plant pathogens invade the hosts likely by evading the host innate immunity. We have found that the ascomycete rice pathogen *Magnaporthe oryzae* accumulates a1,3-glucan on the cell wall during infection. The accumulation of a1,3-glucan was dependent on the cell wall integrity MAP kinase (Mps1) pathway, which was activated by a plant wax component. a1,3-glucan was not essential for formation of infectious structures but was required for the successful infection by promoting the cells from plants' antifungal enzymes and by delaying the host defense responses. Furthermore, histocytochemical observation have revealed that the ascomycete *Cochlioborus miyabeanus* and the basidiomycete *Rhizoctonia solani* have also accumulate a1,3-glucan on the cell walls specifically during plant invasion. Thus, plant cues appear to trigger surface accumulation of a1,3-glucan in these fungi. In addition, rice plants secreting bacterial a1,3-glucanase rapidly induced defense responses against these pathogens and showed multiple fungal disease resistance. Considering that a1,3-glucan is non-degradable in many plants, our study suggested that masking cell wall surfaces with a1,3-glucan is a stealth infection strategy commonly used by fungal plant pathogens. Our study also indicated that recognition of plant cues play an important role in promoting stealth infection in fungal plant pathogens.
Unravelling the GTPase polarity complex in *Claviceps purpurea*. Andrea Herrmann¹, Janine Schürmann¹, Britta Tillmann², Michael Bölker², Paul Tudzynski¹.

¹) IBBP, WWU Muenster, Schlossplatz 8, 48143 Muenster, Germany; ²) Philipps-Universität, Karl-von-Frisch-Strasse 8, 35032 Marburg, Germany.

*Claviceps purpurea* is a plant pathogen infamous for its production of toxic alkaloids on infected host plants like barley. Consumption of infected grains leads to severe symptoms up to the death of the patient. Infection patterns are complex and the topic of intensive research. One interesting aspect is the strict polarity of the hyphal growth during the first infection stage which seems to be crucial for the non-recognition of *C. purpurea* as a pathogen by the host. To address the question of the importance of polarity the structure and dynamics of the polarity complex are the focus of this work. The guanine nucleotide exchange factors (GEFs) Cdc24 and Dock180 belong to different families, Cdc24 being a member of the Dbl GEF family and Dock180 a CZH GEF. Cdc24-GFP localises cytosolically and to hyphal tips whereas Dock180-GFP is present in small vesicles in the hypha, though concentrated at the tip region, too. Cdc24 DHPH domains are able to activate the small GTPases Rac and Cdc42 of *C. purpurea* and *U. maydis in vitro*, whereas the catalytic domain of Dock180 only activates Rac in both organisms. Despite the proven activation Cdc24 does not interact with any GTPase in yeast two hybrid assays. Dock180 shows a weak interaction with Rac and the two p21-activated kinases (PAKs) Ste20 and Cla4. Thus, both GEFs do not share many characteristics apart from their GEF activity. The PAKs Ste20 and Cla4 and the scaffold protein Bem1 are involved in the polarity complex, too. Ste20 localises to hyphal tips and interacts with Cdc42 in a loading status dependent manner, whereas Cla4 is the main partner of Rac. Other interactions of Ste20 with Dock180 and Cla4 could also be shown. Bem1 is present in the cytosol - concentrated at the hyphal tip - and links most of the proteins of the polarity complex as interactions with Cdc24, Cla4, Ste20 and Dock180 have been detected. Taken together we postulate at least two different polarity complexes, the Rac complex and the Cdc42 complex. Both are gathered by Bem1, but Cla4 is the main partner of Rac, whereas Ste20 plays a similar role for Cdc42. Dock180 is mainly linked to Rac, Cdc24 can be active in both complexes. We are interested in the spatial and temporal formation and regulation of these complexes and its influence on polarity and virulence which will be the subject of further studies.
CONCURRENT SESSION ABSTRACTS

Wednesday, March 13  3:00 PM–6:00 PM
Chapel

Genetics and Genomics of Interactions with Bacteria, Insects and Plants
Co-chairs: Nemat Keyhani and Christian Hertweck

Endophytic insect parasitic fungi feed insect-derived nitrogen to plants. M.J. Bidocha, S.W. Behie. Brock University, St. Catharines, ON, Canada.

Metarhizium is a fungus with a bifunctional lifestyle: it is a common plant endophyte and is also a pathogen to a large number of insects, which are a source of nitrogen. It is possible that the endophytic capability and insect pathogenicity of Metarhizium are coupled to provide an active method of nitrogen transfer to plant hosts via fungal mycelia. We used soil microcosms to show the ability of M. robertsi to translocate insect-derived nitrogen to plants. Insects were injected with 15N-labeled nitrogen, and we tracked the incorporation of 15N into amino acids in two plant species. We also investigated the exchange of plant carbon for insect-derived nitrogen in this symbiosis. Unlike mycorrhizal fungi, Metarhizium, is not a fastidious fungus and is easily genetically manipulated. We also performed gene knockout experiments on carbon and nitrogen transporters to test if the exchange of carbon and nitrogen is reciprocal in this fungus-plant symbiosis.

Genotype-Environment Interactions and the Interplay Between Climate Change and Plant-Fungal Symbioses. Rusty J. Rodriguez1,2,3, Yong Ok Kim4, Claire Woodward2, Leesa Wright2, Regina Redman1,2. 1) Symbiogenics, Seattle, WA; 2) Adaptive Symbiotic Technologies, Seattle, WA; 3) University of Washington, Biology, Seattle, WA.

Symbiotic associations span a continuum from parasitism to mutualism and the outcome of specific associations is context driven based on intergenomic interactions and environmental factors. These factors will determine the ability plants and animals to adapt to a changing climate. For example, plants in natural ecosystems adapt to abiotic stress by forming symbiotic associations with fungal endophytes that confer stress tolerance. Without the endophytes, the plants are not stress tolerant and do not survive in the habitats to which they are adapted. Symbiotically conferred stress tolerance typically occurs in a habitat-specific manner, a phenomenon we designate Habitat Adapted Symbioses (HAS). Although several biochemical processes have been correlated to plant stress tolerance, few processes correlate with symbiotically conferred stress tolerance. Symbiotically conferred stress tolerance involves altered plant gene regulation, increased metabolic efficiency, and an increased ability to manage reactive oxygen species. I will describe how fungal endophytes adapt plants across environmental gradients and present a working model for symbiotically conferred stress tolerance.

Chemical mediators of pathogenic and mutualistic bacterial-fungal interactions. Kirstin Scherlach. Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology, HKI Beutenbergstr. 11a 07745 Jena, Germany.

Natural products play a key role in symbiotic interactions between microorganisms and higher organisms. Their function may range from signaling compounds in mutualism to virulence factors and toxins in pathogenic relationships. In many cases the chemical basis of such interactions is still unknown. By combining genome mining, bioinformatic analyses and chemical analytical techniques we uncovered the biosynthesis of a number of virulence factors and toxins from bacterial-fungal associations that impact agriculture, medicine and biotechnology. We elucidated an unprecedented case of symbiotic cooperation where a fungus tailors a pathogenicity factor provided by its endosymbiotic bacteria to increase its phytotoxic potency. This toxin (rhizoxin) causes rice seedling blight, a plant disease accounting for severe losses in agriculture. Another bacterium, Janthinobacterium agaricidamnosum, initiates soft rot of cultured mushrooms. With the help of imaging mass spectrometry we discovered a peptide toxin that not only contributes to pathogenicity but also displays potent antifungal activity against major human pathogens. Furthermore, we identified the molecular basis for the biosynthesis of a highly toxic polyketide produced by Burkholderia gladioli, a common contaminant of the food fermentation fungus Rhizopus oligosporus. Bongkrekic acid is a respiratory toxin that efficiently inhibits adenine nucleotide translocase. Through sequencing of the bacterial genome and functional analyses of the biosynthetic genes new insights into the process of polyketide assembly were gained. The discovery of these secondary metabolites as mediators of bacterial-fungal interaction and their biogenetic origins not only facilitates the understanding of complex ecological processes but also opens avenues to the development of new drug candidates and potential bio-control agents against crop diseases.

Comparative genomic analysis of entomopathogenic fungi. Guohua Xiao, Qiang Gao, Peng Zheng, Xiao Hu, Chengshu Wang. Institute of Plant Physiology and Eclogy, Shanghai Institutes for Biological Sciences, CAS, Shanghai, China.

There are about one thousand of fungal species capable of infecting and killing insects, spiders and mites. The species like Metarhizium anisopliae, M. robertsii, M. acridum and Beauveria bassiana has been developed as environmentally friendly biological control agents against different insect pests. The caterpillar pupae specific fungus Cordyceps militaris, however, has been used a traditional Chinese Medicine for hundreds of years. We sequenced the genome of model entomopathogenic fungi Metarhizium spp., B. bassiana and C. militaris and conducted comparative genomic studies. We found that the insect pathogens have a strikingly larger proportion of genes encoding secreted proteins, particularly proteases, than other sequenced fungi. A phylogenetic analysis confirmed that fungal entomopathogenicity has evolved multiple times so similar expansion of families of proteases and chitinases reflects a convergent evolution. Like that of C. militaris which can readily performs a sexual life, a single mating type locus identified in clonally reproductive species Metarhizium and Beauveria indicated that the later are sexually heterothalic. High throughput transcriptome analysis indicated that the pathogen could regulate transcriptional responses with fine-tuned gene sets for host recognition, development and adaptation to different niches. The information from our studies advanced the understanding of the evolution of fungal entomopathogenicity and will benefit future molecular studies of fungus-host interactions and thereby facilitate the development of cost-effective mycoinsecticides.
Synergistic interactions between leaf-cutting ants and their fungal symbiont facilitate degradation of plant substrate. Morten Schiatt¹, Henrik H. de Fine Licht², Adelina Rogowska-Wrzesinska³, Pepijn Kooij⁴, Peter Roepestorf⁵, Jacobus J. Boomsma⁶. 1) Department of Biology, University of Copenhagen, Copenhagen, Denmark; 2) Department of Mycology, Lund University, Lund, Sweden; 3) Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark.

About 50 million years ago a single ancestor of today’s more than 230 species of fungus-growing ants committed herself irreversibly to farming fungi for food instead of being a hunter-gatherer as most other ants. However, the white-rot litter decomposing Leucocorinpori (Agaricales) that were domesticated remained mostly uncommitted to the symbiosis until a single lineage became an obligate symbiont of a derived clade of these ants - the so called higher attines. Coevolution of ants and fungi has subsequently produced specific adaptations in both partners, including the development of special hyphal tips (gongylidia) of the fungus on which the ants feed. Recent work has shown that many fungal enzymes pass through the ant digestive system unharmed to be mixed (as fecal fluid) with the fresh leaf pulp that the ants deposit on top of their gardens. To understand the function of this form of fungal enzyme transfer, we have used state of the art proteomics and high-throughput genome sequencing to identify the proteins found in the ant fecal fluid. Fecal proteins of Acromyrmex leafcutter ants were separated with SDS-PAGE followed by tandem mass spectrometry, and the resulting peptide tags were used as queries to Blast-search a low coverage genome sequence of the fungal symbiont. Using this strategy we identified 34 protein sequences encoded by the fungal genome. Enzyme assays for selected fecal proteins showed that they functionally disappeared from the fecal droplets when the ants were deprived of their fungal symbiont. We further used qPCR to establish that many of these proteins are more highly expressed in gongylidia than in mycelium, suggesting that they have been actively selected to be ingested by the ants. A substantial fraction of the fecal proteins are enzymes that are widely used by plant-pathogens to break down cell walls to access the easily degradable nutrients inside living cells. Of special interest is the finding of a polyphenol-oxidizing laccase enzyme that shows signs of positive selection in the higher attine ant symbionts, and may be an important prerequisite for the ability to cope with the polyphenols present in plant tissues. The results indicate that the leafcutter ants and their fungal symbionts have evolved traits-syndromes that are partially convergent with those found in plant-pathogenic fungi.

Unraveling the metabolome: how zombie ant fungi heterogeneously control ant brains. Charissa de Bekker, David Hughes. Biology and Entomology, Center for Infectious Disease Dynamics, Pennsylvania State University, State College, PA.

Fungal entomopathogens rely on cellular heterogeneity during the different stages of insect host infection. Their pathogenicity is exhibited through the secretion of secondary metabolites. Infection strategies of this group of environmentally important fungi can thus be studied by analyzing their metabolome. Next to generalists such as Beauveria bassiana and Metarhizium anisopliae, specialist species exist that are able to control host behavior. One of the most dramatic examples is the death grip of ants infected by Ophiocordyceps unilateralis, where ants are being used as a vehicle and finally bite into vegetation before dying, aiding fungal spore dispersal after death. To establish this the fungus must not only overcome the immune system of the host, but also manipulate the brain and atrophy the muscles. To date, most work on manipulation of host behavior has described the ant’s behavior, leaving the molecular processes from the fungal point of view unresolved. To start unraveling the mechanisms underlying this phenomenon we are combining metabolite profiling with an ex vivo insect tissue culturing system that allows us to study fungal metabolites secreted in different parts within the host. Using this technique we established that B. bassiana and M. anisopliae, and O. unilateralis heterogeneously react to brain and muscle tissue by secreting a significantly different array of metabolites. The combination of these approaches with a concrete understanding of the host-parasite interaction in nature is allowing us to understand both the diversity of secondary metabolites as well as make discoveries regarding the temporal dynamics these fungi employ when releasing metabolites that affect the host. This project is financed by the Marie Curie International Outgoing Fellowships and Penn State University.

Trichoderma rhizophere's competency, endophytism and plant communication: A molecular approach. Artemio Mendónça¹, Johanna Steyaert², Natalia Guazzone³, Maria Fernanda Nieto-Jacobo³, Mark Braithwaite¹, Robert Lawry¹, Damian Bienkowski¹, Christopher Brown¹, Kirstin MacLean¹, Robert Hill¹, Alison Stewart¹. 1) Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand; 2) Biochemistry Department and Genetics Otago, University of Otago, New Zealand.

Establishment of root symbiosis is one the key drivers of biocontrol success for members of the fungal genus Trichoderma. This root symbiosis is described as a two-step process, whereby Trichoderma species colonize the soil surrounding the root (rhizosphere) and then penetrate the root tissue and establish an endophytic relationship. The ability to colonise and then proliferate over time within the rhizosphere is termed rhizosphere competence (RC). There have been numerous reports of Trichoderma biocontrol strains which persist within the rhizosphere for the growing season of the crop plant. Our results strongly suggest that RC is widespread among members of the genus Trichoderma and that RC interactions are strain and host plant specific. For endophytes and their host plants to maintain a mutualistic relationship requires a constant molecular dialogue between the organisms involved. For example, the fungal-derived phytohormone, indole acetic acid (IAA), plays an important role in signalling between Trichoderma and the model plant Arabidopsis thaliana. There are however, additional, currently unknown, chemical signals which may be even more important for a positive interaction between Trichoderma and plants. By using a soil-maize-Trichoderma as a model system in in situ sterile conditions we are currently analysing the RC and endophytism transcriptomes of two Trichoderma species: T. virens and T. atroviride. Using a combination of bioinformatics, quantitative RT-PCR (for stage specific genetic markers from Trichoderma) and fluoro-labelled Trichoderma strains we are currently identifying and analysing promising Trichoderma candidates involved in endophytism and RC. A comprehensive panorama of the Trichoderma-soil-plant interaction will be discussed in this conference.
**Concurrent Session Abstracts**

**Effector proteins in fungal defense against fungivorous nematodes: Targets and functional significance.** Therese Wohlschlager, Stefanie Schmieder, Alex Butschi, Paola Grassi, Alexander Titz, Stuart Haslam, Michael Hengartner, Markus Aebi, Markus Künzler. 1) Institute of Microbiology, ETH Zürich, Switzerland; 2) Institute of Molecular Life Sciences, University of Zürich, Switzerland; 3) Division of Molecular Biosciences, Imperial College, London, United Kingdom; 4) Department of Chemistry, University of Konstanz, Germany.

The defense of fungi against fungivores is largely based on the production of intracellular toxins. A significant proportion of these toxins are peptides and proteins that are synthesized by the ribosome and stored in the cytoplasm. Protein toxins include lectins that target specific glycoepitopes in the intestine of the fungivore upon ingestion and kill the fungivore by a yet unknown mechanism. In our laboratory, we focus on the functional characterization of fungal protein toxins that are directed against nematodes. We use the model nematode *Caenorhabditis elegans* to identify the targets and to study the toxicity mechanism of these fungal defense effector proteins in the nematode. In addition, we employ the fungivorous nematodes *Aphelenchus avenae* and *Bursaphelenchus willibaldi* to study the diversity, the functional significance and the transcriptional regulation of these proteins in the fungus.

Recently, we identified a nematotoxic lectin from the mushroom *Laccaria bicolor* that is homologous to animal lectins involved in innate immunity against bacteria. We found that the nematotoxicity of the lectin is based on its specific binding to methylated fucose residues on nematode N-glycans. Among animals, this epitope is only present in worms and molluscs but not in insects or vertebrates. We performed affinity chromatography of *C. elegans* whole worm protein extracts using the *L. bicolor* lectin and other nematotoxic fungal lectins recognizing protein-bound glycans. The results of this analysis suggest that these lectins target the same set of glycoproteins in the nematode intestine and may confer toxicity by a common mechanism. In order to address the functional significance of these proteins for fungal defense against fungivorous nematodes, we expressed some of the fungal proteins displaying toxicity towards *C. elegans*, in the filamentous ascomycete *Ashbya gossypii*. These transformants were fed to *A. avenae* and the propagation of the fungivorous nematode on the various transformants was determined. Expression of some effector proteins significantly inhibited propagation of the nematode suggesting that these proteins have a role in fungal defense against these organisms. Experiments addressing the relative fitness of the various *A. gossypii* transformants upon selective pressure of feeding by *A. avenae* are under way.
Membrane Trafficking and Molecular Organization
Co-chairs: Vicky Sophianopoulos and Gero Steinberg

Distinct secretion systems operate during biotrophic invasion by the rice blast fungus, *Magnaporthe oryzae*. Barbara Valent1, Martha Giraldo2, Chang Hyun Khang3,4, Yasin Dagdas3, Yogesh Gupta2, Thomas Mentlak2,3, Mihwa Yi1, Melinda Dalby2, Hiromasa Saitoh5, Ryoei Terauchi5, Nicholas Talbot1. 1) Dept Plant Pathology, Kansas State Univ, Manhattan, KS; 2) School of Biosciences, Univ of Exeter, Exeter, U.K; 3) Iwate Biotechnology Research Center, Kitakami, Iwate, Japan; 4) Current Address: Dept of Plant Biology, Univ of Georgia, Athens, GA; 5) Current Address: Cambridge Consultants Ltd, Cambridge, U.K.

During biotrophic invasion, *Magnaporthe oryzae* secretes cytoplasmic effectors, which preferentially accumulate in biotrophic interfacial complexes (BICs) and are translocated into the cytoplasm of the rice cells, and apoplastic effectors, which remain in the extracellular space between the fungal cell wall and the rice plasma membrane. BICs localize in front of the tips of filamentous hyphae that enter rice cells, and remain subapically beside the first bulbous invasive hyphal cells after hyphal differentiation. In contrast, secreted apoplastic effectors uniformly outline the entire bulbous invasive hypha.

We have determined that cytoplasmic effector genes were highly up-regulated in the BIC-associated cells at early invasion stages, and that effector promoters played the major role in determining preferential BIC localization of cytoplasmic effectors. Subapical BIC-associated hyphal cells continued to express protein secretion machinery components while invasive hyphae grew elsewhere in the host cell, suggesting that these subapical invasive hyphal cells are involved in active secretion. Disruption of the conventional ER-Golgi secretion pathway by Brefeldin A treatment blocked secretion of apoplastic effectors, but not secretion of cytoplasmic effectors. Pathogen mutants that failed to express exocyst complex components or a t-SNARE were defective in secretion of cytoplasmic effectors, as well as defective in pathogenicity. In contrast, secretion of apoplastic effectors was not impaired in these mutants. Our data suggest that *M. oryzae* possesses distinct secretory mechanisms for targeting cytoplasmic and apoplastic effectors during rice invasion.

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Many organelles are transported long distances along microtubules in eukaryotic organisms by dynein and kinesin motors. To identify novel alleles and genes required for microtubule-based transport, we performed a genetic screen in the filamentous fungus, Aspergillus nidulans. We fluorescently-labeled three different organelle populations known to be cargo of dynein and kinesin in Aspergillus: nuclei, endosomes, and peroxisomes. We then used a fluorescence microscopy-based screen to identify mutants with defects in the distribution or motility of these organelles. Using whole-genome sequencing, we found a number of single nucleotide polymorphisms (SNPs) that resulted in misdistribution of peroxisomes, endosomes, or nuclei. Some of these SNPs were novel alleles of cytoplasmic dynein/ nudA, Arp1/ nudK (dynactin), Lis1/ nudF, and kinesin-1/ kinA. Here, we characterize the in vivo transport defects in these novel mutants and analyze the single molecule in vitro motility properties of purified mutant motor proteins. We also describe our methods for using whole genome sequencing as a tool in mutagenesis studies in A. nidulans.


Morphogenesis in filamentous fungi depends principally on the establishment and maintenance of polarized growth. This is accomplished by the orderly migration and discharge of exocytic vesicles carrying cell wall components. We have been searching for evidence that endocytosis, an opposite process, could also play a role in morphogenesis. Previously, we found that coronin deletion (Neurospora crassa mutant, Dcrn-1) causes a decrease in endocytosis (measured by the rate of uptake of FM4-64) together with marked alterations in normal hyphal growth and morphogenesis accompanied by irregularities in cell wall thickness. The absence of coronin destabilizes the cytoskeleton and leads to interspersed periods of polarized and isotropic growth of the hyphae. We used CRIB fused to GFP as an exocytic reporter of activated Cdc-42 and Rac-1. By confocal microscopy, we found that CRIB-GFP was present in wild-type hyphae as a thin hemispherical cap under the apical dome, i.e. when growing in a polarized fashion and with regular hyphoid morphology. In the Dcrn-1 mutant, the location of CRIB-GFP shifted between the periods of polarized and isotropic growth, it migrated to the subapical region and appeared as localized patches. Significantly, cell growth occurred in the places where the CRIB-GFP reporter accumulated, thus the erratic location of the reporter in the Dcrn-1 mutant correlated with the morphological irregularity of the hyphae. We found that the Dcrn-1 mutant had a higher proportion of chitin than the WT strain (14.1% and 9.1% respectively). We also compared the relative cell wall area (TEM images) and we found a difference in wall/cytoplasm between the Dcrn-1 mutant and the WT strain. In conclusion, we have found that the mutant affected in endocytosis has an altered pattern of exocytosis as evidenced by its distorted morphology and displaced exocytic markers. A direct cause-effect relationship between endocytosis and exocytosis remains to be established.

“The vacuole” of Neurospora crassa may be composed of multiple compartments with different structures and functions. Barry J. Bowman, Emma Jean Bowman, Robert Schnittker, Michael Plamann. 1) MCD Biology, University of California, Santa Cruz, CA; 2) Department of Biology, University of Missouri, Kansas City, KA.

The structure of the “vacuole” in Neurospora crassa and other filamentous fungi is highly variable with cell type and position in the hypha. Large spherical vacuoles are typically observed in older hyphal compartments, but approximately 100 microns behind the hyphal tip, vacuolar markers are seen in a dynamic network of thin tubules. At the edge of this network nearest the tip, a few distinct round organelles of relatively uniform size (2-3 microns) have been observed (Bowman et al. Eukaryotic Cell 10:654). The function of these round organelles is unknown, although the vacuolar ATPase and a vacuolar calcium transporter are strongly localized there. To help identify organelles we have tagged SNARE proteins and Rab GTPases with GFP and RFP. Several of these tagged proteins (sec-22, rab-7, rab-8) appear in the tubular vacuolar network and in the membrane of the round organelles. A unique aspect of the round organelles is their association with dynein and dynactin (Sivagurunathan et al. Cytoskeleton, 69:613). In strains with mutations in the tail domain of the dynein heavy chain the dynein is often seen in clumps. This aggregated dynein appears to be tightly associated with (and possibly inside) the round organelles, but not in the tubular vacuolar network. Further analysis of the location of SNARE and Rab proteins may help to identify the function of the round organelles.
CONCURRENT SESSION ABSTRACTS

Wednesday, March 13  3:00 PM–6:00 PM  
Fred Farr Forum

**Genome Defense, Epigenetics and RNAi**  
Co-chairs: Patrick Shiou and Sven Saupe

**Meiotic silencing by unpaired DNA in Neurospora.**  
1) School of Biological Sciences, Illinois State University, Normal, IL; 2) Division of Biological Sciences, University of Missouri, Columbia, MO; 3) Department of Biology, University of North Carolina, Chapel Hill, NC.

*Neurospora crassa* has a cytoplasm that is shared by the entire hyphal network, making it particularly vulnerable to attack by repetitive elements. Accordingly, several surveillance mechanisms are in place to protect the genome integrity of the fungus. For example, if a gene is lacking a partner during homolog pairing in meiosis, all copies of this gene are silenced by a process known as meiotic silencing by unpaired DNA (MSUD). MSUD requires common RNAi proteins (e.g., RNA-directed RNA polymerase, Dicer, and Argonaute) and may work as follows: an unpaired gene triggers the production of an aberrant RNA molecule, which is made double-stranded (by SAD-1) and processed into siRNAs (by DCL-1). These siRNAs are subsequently used to destroy complementary mRNAs (by SMS-2). The aforementioned proteins colocalize in the perinuclear region, possibly forming a silencing complex that inspects and processes RNA molecules as they exit the nucleus. We will discuss the recent advances in our understanding of this unique silencing pathway.

**Mechanism of quelling, a small RNA-mediated gene silencing pathway.**  
Zhenyu Zhang, Shwu-Shin Chang, Yi Liu. Dept Physiology, Univ Texas SW Med Ctr, Dallas, TX.

RNAi is a conserved gene silencing mechanism from fungi to mammals. Quelling is an RNAi-related phenomenon that post-transcriptionally silences repetitive DNA and transposon in Neurospora. We previously identified a type of DNA damage-induced small RNA called qiRNAs that originate from ribosomal DNA. To understand how small RNAs are generated from repetitive DNA, we carried out a genetic screen to identify genes required for qiRNA biogenesis. Factors directly involved in homologous recombination (HR) and chromatin remodeling factors required for HR are essential for qiRNA production. HR is also required for quelling, and quelling is also the result of DNA damage, indicating that quelling and qiRNA production share a common mechanism. Together, our results suggest that DNA damage triggered HR-based recombination allows the RNAi pathway to recognize repetitive DNA to produce small RNA.

**SIS, a sex genome defense mechanism operating in Cryptococcus neoformans.**  
Xuying Wang, Sabrina Darwiche, Joseph Heitman. Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

*Cryptococcus neoformans* is a human fungal pathogen that undergoes a morphic transition from yeast to hyphae during a-a opposite-sex mating and a-a unisexual reproduction (same-sex mating). Infectious spores are generated during both processes. We previously identified a sex induced silencing (SIS) pathway in the *C. neoformans* serotype A var. *grubii* lineage, in which tandem transgene arrays trigger RNAi-dependent gene silencing at a high frequency during a-a opposite-sex mating, but at an ~250-fold lower frequency during asexual mitotic vegetative growth. Here we report that SIS also operates during a-a unisexual reproduction. A self-fertile strain containing either SKI2a-URAS or NEO-URA5 transgene arrays exhibited an elevated silencing frequency during solo and unisexual mating compared with mitotic vegetative growth. We also found that SIS operates at a similar efficiency on transgene arrays of the same copy number during either a-a unisexual reproduction or a-a opposite-sex mating. URAS-derived small RNAs were detected in the silenced progeny of a-a unisexual reproduction and RNAi core components were required, providing evidence that SIS induced by same-sex mating is also mediated by RNAi via sequence-specific small RNAs. This study, together with our previous finding of SIS in a-a opposite-sex mating of the *C. neoformans* serotype A var. *grubii* lineage, demonstrates that SIS is a conserved process between the divergent *C. neoformans* serotype A and serotype D sibling species. In each case, our data show that the SIS RNAi pathway operates to defend the genome via silencing transposon activity during the sexual cycles. Thus, our discovery of SIS brings a fresh perspective to meiotic silencing involving the upregulation of RNAi pathways as a strategy to guard genomic integrity during sex. More importantly, the presence of SIS in both a-a unisexual reproduction and a-a opposite-sex mating indicate that SIS may be triggered by the shared pheromone sensing Cpk1 MAPK signal transduction cascade. Ongoing studies focus on defining at a mechanistic level how the SIS RNAi pathway is initiated, including identifying new components involved in SIS.

**Fungi use prion folds for signal transduction processes involving STAND proteins.**  
Asen Daskalov, Khalid Salamat, Sven J. Saupe. CNRS, IBGC UMR5095, BORDEAUX, AQUITAINE, France.

Prions are proteins embedding genetic information into their structural state. Generally, those proteins exist in a soluble state and sporadically as infectious amyloid aggregates. Podospora anserina’s [Het-s] is one of the best characterized fungal prions with a remarkably high prevalence in wild populations. [Het-s] functions in vegetative incompatibility - a biological process occurring during anastomosis between two genetically incompatible strains. The [Het-s] protein exists in a soluble state - [Het-s*] - or can switch to an aggregated amyloid state - [Het-s] - the prion form. When an [Het-s] prion infected strain fuses with a strain expressing the alternative allelic variant of the [Het-s] locus - [Het-s] - a cell death reaction of the heterokaryon occurs. Recent studies shed light on the mechanism of [Het-s]/[Het-s] incompatibility reaction. Differing by 13 amino acids both proteins share a two domain architecture; a globular N-terminal domain called HeLo and a C-terminal Prion Forming Domain (PFD). The latter is able to adopt a b-sheet rich conformation with a specific b-solenoid fold. It has been demonstrated that in presence of [Het-s] amyloid fibers [Het-s] turns into a pore-forming toxin: transconformation of the [Het-s] PFD by [Het-s] fibers triggers the refolding of the [Het-s] HeLo domain, inducing the cell death reaction. In an attempt to better characterize the conserved features of the [Het-s] b-solenoid fold and identify new distant homologues of [Het-s]/s, we have generated a minimal consensus sequence motif of it. Surprisingly, the second best hit in a BLAST search is in the N-terminal region (3-23) of the product encoded by nwd2, the immediately adjacent gene to het-s. NWD2 is a STAND protein. STAND proteins form signal transducing hubs through oligomerization upon ligand recognition. That in mind and several bioinformatics observations led us to propose that [Het-s] and NWD2 are functional partners in various filamentous fungal species using the amyloid fold in a signal transducing pathway. We will present experimental evidence that NWD2 is able to trigger [Het-s] toxicity in...
Regulation of white and opaque cell-type formation in Candida albicans by H3K56 acetylation and nucleosome assembly factors CAF-1 and HIR. John S. Stevenson, Haping Liu. Department of Biological Chemistry, University of California, Irvine, Irvine, CA.
CAF-1 and HIR are highly conserved histone chaperone protein complexes that function in the assembly of nucleosomes onto chromatin. CAF-1 is characterized as having replication-coupled activity, whereas the HIR complex can assemble nucleosomes independent of replication. Histone H3K56 acetylation, controlled by the acetyltransferase Rtt109 and deacetylase Hst3, also plays a significant role in nucleosome assembly. How different cell types with the same genotype are formed and heritability maintained is a fundamental question in biology. We utilized white-opaque switching in Candida albicans as a system to study mechanisms of cell-type formation and maintenance. Opaque cell specification is under the control of interlocking transcriptional feedback loops, with Wor1 being the master regulator. We showed that H3K56 acetylation plays an important role in the regulation of white-opaque switching. The rtt109D/D mutant is defective in stochastic and environmentally stimulated white-opaque switching and cannot maintain opaque cell type. Inhibition of Hst3 by nicotinamide induces opaque cell formation in Rtt109 dependent manner. The Hst3 level is down-regulated in the presence of genotoxins and ectopic expression of HST3 blocks genotoxin induced switching, providing a pathway for genotoxin induced white-opaque switching. We now show that CAF-1 and HIR modulate white-opaque switching frequencies in a H3K56 acetylation associated manner. Unique to C. albicans, the cac2D/D mutant shows increased sensitivity to the Hst3 inhibitor nicotinamide, while the rtt109D/D cac2D/D and hir1D/D cac2D/D mutants are resistant to nicotinamide. CAF-1 plays a major role in maintaining cell types as the cac2D/D mutant exhibited increased switching frequencies in both directions, and switches at a high frequency to opaque in response to nicotinamide. Like the rtt109D/D mutant, the hir1D/D cac2D/D double mutant is defective in maintaining the opaque cell fate, blocks nicotinamide induced opaque formation, and the defects are suppressed by ectopic expression of the master white-opaque regulator Wor1, suggesting an overlapping function of CAF-1 and HIR in epigenetic regulation cell fate determination in a H3K56 acetylation dependent manner.

Epigenetic Regulation of Subtelomeric Gene Noise in Candida albicans. Matthew Z. Anderson, Joshua A. Baller, Lauren J. Wigen, Judith Berman. Genetics, Cell Biology and Development, University of Minnesota, St Paul, MN.
Candida albicans grows within a wide range of fluctuating host niches, and the ability to rapidly adapt enhances its success as a commensal and a pathogen. The recently expanded telomere-associated TLO gene family consists of fourteen expressed members in C. albicans. Each TLO gene encodes a paralog of a single Mediator complex component. Thirteen expressed TLOs are located at the chromosome ends as the most telomere-proximal open reading frame. Individual TLO expression at both the transcript and protein level was extremely noisy. Noise originated from single cell variability in TLO expression due to intrinsic factors. Deletion of chromatin modifying enzymes that function in subtelomeric silencing abolished TLO noise, as did ectopically expressing a TLO from an internal locus. Conversely, transcriptional variation of a low noise gene increased significantly when ectopically expressed in the subtelomere. Interestingly, deletion of the Mediator component MED3, which inhibits Tlo from incorporating into Mediator, also drastically reduced TLO noise and supports an autoregulatory mechanism for TLO noise. These data suggest subtelomeric chromatin structure regulates TLO gene noise through the action of chromatin modifiers and Mediator. We propose that TLO noise is beneficial to C. albicans by producing heterogeneous cell populations that incorporate different Tlo proteins in Mediator, producing a range of transcriptional profiles in the population that allows some cells to survive in altered environmental conditions.

Chromatin regulation of genome stability. Zachary A. Lewis. Department of Microbiology, University of Georgia, Athens, GA.
Genome instability results from defective DNA replication or repair and is associated with human diseases such as cancer. Chromatin structure impacts virtually all DNA-templated processes in the nucleus, including replication and repair. To identify new chromatin factors that are required for genome stability, we screened the Neurospora knockout collection for strains that are sensitive to the DNA damaging agent methyl methanesulfonate (MMS). The primary screen uncovered over 500 MMS-sensitive knockout strains, including knockouts of putative regulators of chromatin structure. We are currently testing this group of knock out strains for sensitivity to other agents that induce DNA damage. We have also initiated molecular analyses of newly identified regulators of genome stability. Our current progress will be summarized.

Opposing activities of the HCHC and DMM complexes maintain proper DNA methylation in Neurospora crassa. Shinji Honda1,2, Eun Yu1, Eric Selker3. 1) University of Oregon, Institute of Molecular Biology, Eugene, OR; 2) University of Fukui, Life Science Unit, Fukui, Japan.
Proper regulation of heterochromatin and DNA methylation is critical for the normal function of cells. We show that heterochromatin and DNA methylation are faithfully controlled in Neurospora by opposing activities of the silencing complex HCHC and the anti-silencing complex DMM. The workings of these two complexes were investigated. HCHC consists of four proteins, the two chromo domain proteins HP1 and CDP-2, the histone deacetylase HDA-1 and the AT-hook motif protein CHAP. We found that histone deacetylase activity is critical for HCHC function but the H3K9me3 binding activity of the CDP-2 chromo domain is not. Instead, CDP-2 serves as an essential bridge between HP1 and HDA-1. CHAP interacts directly with HDA-1, binds in a methylation-independent way to the A:T-rich DNA that forms the cores of methylated regions and is important for stable association of HDA-1 with chromatin. HCHC is involved in the spreading of DNA methylation in dmm mutants. The DMM complex consists of a presumed histone demethylase, DMM-1, plus DMM-2, which is characterized by a fungal-specific Zn(II)Cys6 DNA-binding domain (“Zn-Cys”). We found that DMM-2 strongly binds to DNA from euchromatin/heterochromatin junctions, thereby promoting the stable association of DMM-1 at the edge of heterochromatin domains to prevent aberrant spreading of DNA methylation.
Genomics and Mycorrhizae

Co-chairs: Anders Tunlid and Tom Bruns

The mycorrhizal genome initiative (MGI): Identification of symbiosis-regulated genes by using RNA-Seq. A Kohler1, E. Tisserant2, E. Morin3, C. Veneault-Fourrey1, S. Abba1, F. Buscot1, J. Dore1, G. Gay1, M. Girlanda1, S. Herrmann1, T. Johansson1, U. Lahrmann1, E. Martin1, S. Perotto2, M. Tarrka3, A. Tunlid3, A. Zuccaro1, I. Grigoriev1, F. Martin1. 1) Lab of Excellence ARBRE, Tree-Microbes Department, INRA-Nancy, Champenoux, France; 2) Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Torino, Italy; 3) Department Soil Ecology, UFZ Centre for Environmental Research Leipzig-Halle Ltd., Halle, Germany; 4) Ecologie Microbienne UMR CNRS 5557, INRA INRA 1193, Universite Claude-Bernard LYON 1, Villeurbanne, France; 5) Microbial Ecology, Lunds University, Lund, Sweden; 6) Max-Planck Institute for Terrestrial Microbiology, Marburg, Germany; 7) DOE Joint Genome Institute, Walnut Creek, California, USA.

Genome and transcriptome analyses of Laccaria bicolor and Tuber melanosporum (Martin et al., 2008, 2010) revealed that the ectomycorrhizal symbiosis probably developed several times during evolution by generating different ‘symbiosis molecular toolkits’. In L. bicolor a large set of small-secreted proteins acts as putative effectors but not in T. melanosporum, while the up-regulation of transporter-coding genes seems to be a common feature of both interactions. To better understand the evolutionary origin of mycorrhizal symbiosis and to elucidate the molecular mechanisms involved, a large sequencing project of species from different taxa, phylogenetic clades and symbiotic lifestyles (ectomycorrhizae, ericoid and orchid mycorrhizae) was started in 2011 by the Joint Genome Institute and the mycorrhizal genome initiative. To identify and to compare symbiosis-regulated genes large scale illumina transcriptome sequencing of mycelium and mycorrhizal roots from Paxillus involutus, Piloderma croceum, Hebeloma cylindrosporum, Sebacina vermifera, Tulasnella calospora and Oidiodendron maius was performed. Small-secreted proteins, transporters, CAZymes but also many lineage specific proteins were among the highly up-regulated transcripts.


Transposable element dynamics in the Amanita: insights on the evolution of genome architecture accompanying the transition from saprotrophic to ectomycorrhizal ecologies. Jaqueline Hess1, Inger Skrede2, Anne Pringle1. 1) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; 2) Microbial Evolution Research Group, Department of Biology, University of Oslo, Oslo, Norway.

Transposable elements (TEs) form an integral structural part of the genomes of many higher Eukaryotes. Their ability to proliferate independently and into a large number of copies can lead to extensive amounts of repetitive DNA that is of no obvious benefit to the host. At first thought to be relatively underrepresented in Fungi, genome sequencing over the last decade has led to the discovery of many fungal genomes that are densely populated with TEs. Among those are the genomes of the ectomycorrhizal (ECM) fungi Laccaria bicolor (around 30% TE) and Tuber melanosporum (around 60% TE) as well as a number of fungal pathogens, including Puccinia graminis and Melampsora larici-populina (both around 45% TE). The high TE content in these species, especially when compared to saprotrophic fungal species, suggests an association between symbiotic ecology, both mutualistic and antagonistic, and the ability of TEs to invade and persist in their genomes. However, the mechanisms for this are currently not well understood. In order to assess whether high TE content is a feature of other ECM species and to get a more detailed picture of TE content changes around the transition from free-living to ECM ecology, we have sequenced the genomes of five members of the genus Amanita: three ECM species and two saprotrophs, as well as the saprotrophic outgroup Volvariella volvacea. Using the draft genome assemblies, we have developed methodology to estimate TE content from short-read data and examine changes therein within quantitative and phylogenetic frameworks. Overall, we find no direct relationship between ECM status and increased TE content in the Amanita but instead discover patterns that suggest population genetics to be a strong driver of TE content. We will discuss our findings with respect to the influence of TEs in the evolution of genome architecture around the origin of ECM symbiosis.

Broad compatibility in the root endophyte Piriformospora indica is associated with host-adapted colonization strategies. Urs Lahrmann, Yi Ding, Alka Zuccaro. Organismic Interactions, MPI Marburg, Marburg, Germany.

Their host range defines plant associated fungi as either specialists, which are adapted to one or few distinct hosts, or generalists who are able to thrive in highly variable host environments. Specialists and their hosts are in an evolutionary arms race that leads to the development of weapons perfectly tailored to the respective host. Conversely, broad-host range species must evolve adaptations to cope with a plethora of different host-associated signals and host-specific defense mechanisms. The evolutionary force, in this case, drives the expansion and diversification of the fungal arsenal and the host-adapted gene expression to better suite different plants. The mechanisms underpinning broad compatibility in root symbiosis are largely unexplored. The generalist root endophyte Piriformospora indica that stimulates growth, alleviates salt stress and induces systemic resistance to pathogens in different living to ECM in barley the symbiont undergoes a nutritional switch to saprotrophy that is associated with the production of secondary thiner hyphae (SH) in dead cortex cells. Consistent with a diversifed trophic behavior, genome-wide expression profiling revealed a strong induction of genes encoding cell wall degrading enzymes and nutrient transporters in barley but not in Arabidopsis at a late colonization stage. In particular small secreted proteins (SSPs < 300 amino acids) known as effectors have been shown to facilitate colonization by manipulating host defense and reprogramming plant metabolism during symbiosis. Expression of P. indica genes encoding SSPs was induced in both hosts at different symbiotic stage, but the majority of these SSPs were either Arabidopsis or barley responsive with the larger number expressed during biotrophy in Arabidopsis and during saprotrophy in barley. Our study reveals that broad compatibility in root endophytes requires strong phenotypic plasticity and the expression of alternative lifestyle strategies in a host-dependent way.
CONCURRENT SESSION ABSTRACTS


A large part of the nitrogen in forest soils is found in recalcitrant organic matter-protein complexes. Ectomycorrhizal fungi are thought to have a key role in the decomposition and mobilization of nitrogen from such complexes. The knowledge on the functional mechanisms of these processes, and how they are regulated by carbon from the host plant and the availability of more easily available forms of nitrogen sources are limited. We examined how the ECM fungus Paxillus involutus degrade organic litter material using spectroscopy and transcriptome profiling. The fungus partially degraded polysaccharides and modified the structure of polyphenols. The observed chemical changes and the expressed transcriptome were consistent with a hydroxyl radical attack, involving Fenton chemistry similar to that of brown-rot fungi. The set of enzymes expressed by P. involutus during the degradation of the organic matter was similar to the set of enzymes involved in the oxidative degradation of wood by brown-rot fungi. However, P. involutus lacked transcripts encoding extracellular enzymes needed for metabolizing the released carbon. Further experiments have shown that the decomposition and assimilation of nitrogen from organic litter material are triggered by adding glucose. Addition of easily available forms of nitrogen (i.e. ammonium) had minute effects on these processes. Experiments and comparative genomics demonstrate that the saprotrophic activity of P. involutus has been reduced to a radical-based biodegradation system that can efficiently disrupt the organic matter-protein complexes and thereby mobilize the entrapped nutrients.

Interaction between the saprotrophic fungus Serpula lacrymans and living pine roots. Nils OS Högberg, Anna Rosling, Annegret Kohler, Martin Francis, Stenlid Jan. 1) Department of Forest Mycology, BioCenter, SLL, Uppsala, Sweden; 2) INRA, Nancy, France.

Recently it has been shown, with a Comparative genomic perspective, that brown rot and mycorrhiza fungi have evolved from white rot ancestors. Wood is a composite material composed of lignin, cellulose and hemicellulose. White rot fungi are able to degrade all of these components with a combination of carbohydrate active and oxidative enzymes. During the course of evolution brown rot and mycorrhiza have lost most of the genes in these gene families. Nevertheless, brown rot fungi are efficient wood decomposers that degrade cellulose and hemicellulose by means of hydroxyl radical production and remaining carbohydrate active enzymes. The family Boletales includes both brown rot fungi and mycorrhiza and it is tentative to speculate that there has been a parallel evolution of these ecological strategies. Here we test the effect of infecting pine roots with the brown rot fungus Serpula lacrymans. The interaction was neutral since plant growth was not stimulated but not reduced either. The fungus formed a mantle around the pine roots but not the Hartig net that is typical for ectomycorrhiza. Fungal gene expression was compared with the wood decay transcriptome. 1250 genes were more than two-fold upregulated compared to a glucose medium control. A large proportion of the upregulated genes (62 %) are unknown. Carbohydrate active genes represent only 3% of this gene set and genes with oxidoreductase activity, including monooxygenases represent 4% of the upregulated genes. This is considerably lower compared to saprotrophic growth on wood where carbohydrate active enzymes accounted for 26% and oxidative enzymes for 19% which dominated the gene expression on wood. Gene expression for genes involved in transportation was about the same, around 10% in this experiment and under wood decomposition. Several genes that indicate an interaction with a host were also upregulated. In conclusion, gene expression was markedly different between a glucose medium, wood decomposition and growth on pine roots. This may be a signal of symbiosis, the effect on pine seedling growth was neutral. Thus we cannot conclude if the interaction is beneficial or negative to the host.

Uncovering the evolutionary pressures shaping the Glomeromycota-Glomeribacter endosymbiosis. Stephen J. Mondo, Teresa E. Pawlowska. Plant Pathology, Cornell University, Ithaca, NY.

Many eukaryotes interact with heritable endobacteria to satisfy diverse metabolic needs. Of the characterized fungal-bacterial endosymbioses, the association between Gigasporaceae (Glomeromycota) and Ca. Glomeribacter is one of the best described. Glomeribacter is a member of the Burkholderia lineage of b-proteobacteria, and was shown previously to represent one of the few cases of an ancient, long-term non-essential endosymbiont. In order to further explore what adaptations have taken place to shape this unique bacterial lifestyle, we have sequenced three Glomeribacter genomes and developed a computational pipeline to compare across bacteria engaging in different lifestyles using genome wide patterns of mutation accumulation. In order to use PAML to identify gene orthologs that exhibited both over-accumulation and under-accumulation of amino acid substitutions and then used these data to compare across taxa at the level of functional gene categories. We found that bacteria can be grouped by lifestyle using this approach. Glomeribacter, as expected, appears most similar to other potentially long-term non-essential endosymbionts. Therefore, we were able to exploit the differences in mutation accumulation patterns between these taxa to identify processes, which may be relevant within the particular interaction between Glomeribacter and its host. While several of these processes, including vitamin synthesis and amino acid transport, have been identified previously, we additionally discovered features related to lipid biosynthesis and energy metabolism to be of potential importance for this symbiosis. Interestingly, genes exhibiting an under-accumulation of nonsynonymous substitutions (indicative of purifying selection) in Glomeribacter tend to be involved in recombination, cell division, and ribosome maintenance. While these processes are typically fast evolving in endosymbiotic organisms, they may represent features that increase the stability of Glomeribacter in their fungal host population and increase their resilience to genetic drift. We speculate that these processes are unique to the Glomeribacter-Glomeromycota symbiosis and could partially explain why Glomeribacter has been successful as a non-essential endosymbiont for over 400 million years.
A draft genome of the ectomycorrhizal fungus Rhizopogon vesiculosus: Characterization of mating system and heterozygosity within the dikaryon. Alija Mujic, Joseph Spatafora. Botany and Plant Pathology, Oregon State University, Corvallis, OR. Species of Rhizopogon are EM symbionts of trees in family Pinaceae and produce basidiospores within hypogeous false truffles that are dispersed by mycophagous mammals. All known members of R. subgenus Villosuli form obligate EM relationships with Pseudotsuga spp. (Douglas Fir) and are the only members of the genus known to possess this host association. R. vesiculosus, along with its cryptic sister species R. vinicolor, possess a sympatric distribution where sampled within the range of their host tree, P. menziesii. While the sporocarp and EM morphology of these fungi may be highly similar; they possess striking life history differences with R. vesiculosus producing larger vegetative genets and displaying greater population structure at both local and landscape scales. We have sequenced the genome of R. vesiculosus using dikaryotic tissue and a whole genome shotgun sequencing approach on the Illumina HiSeq platform. De novo assembly of the genome was performed using VELVET 1.19 and gene predictions were made using AUGUSTUS with Laccaria bicolor as a training model. The draft genome assembled to a total length of 46 Mb in 6700 contigs with an N50 of 26,783, a maximum contig size of 446,818 bp, and 12,604 predicted genes. Here we characterize the mating system of R. vesiculosus, which possesses both an A-locus encoding a heterodimer transcription factor, as well as a B-locus encoding transmembrane pheromone receptors and pheromone precursor genes. We present comparisons of the mating system of R. vinicolor and its similarities to other members of Boletales (e.g., Serpula) and differences with Agaricales (e.g., Laccaria). Due to the dikaryotic nature of the genome sequence produced for R. vesiculosus, single nucleotide polymorphisms (SNPs) can be observed and used to characterize allelic variation. SNPs observed in protein coding regions of both MAT loci indicate that R. vesiculosus is likely heterothallic. We have also characterized heterozygosity across the whole genome in order to identify hypervariable regions. This genome will allow for comparative analysis of gene content, mating type system with other Basidiomycota and, ultimately, for population/species-level genomic studies within Rhizopogon.


A highly diverse community of ectomycorrhizal (ECM) fungi are known to associate with members of the genus Pinus. Less is known about how diverse fungal communities affect functional diversity within ECM roots. Here we present an optimized method for metatranscriptomic analysis of the ECM-pine root interaction in a natural system. RNA was purified using a CTAB method from individual ECM root clusters collected at varying spatial scales across the distribution range of P. taeda, and sequenced using Illumina HiSeq technology. About 35 million qualified reads were obtained. Sequences were initially assembled using reference based mapping (Bowtie) to sort the reads that represent rRNA from fungal and bacterial species. Reads from divergent regions (D1-D2) of fungal LSU rRNA were used to identify dominant ECM and other fungal community members. Subsequently, P. taeda genes and functional genes of dominant fungal species were sorted using public cDNA databases. The Trinity package was used for de novo assembly of un-mapped reads (mostly fungal genes). Blastx and Go packages were used for gene annotation. A typical ECM root cluster was found 45% P. taeda genes, 3% fungal rRNA, 0.05% bacterial 16S rRNA, 30% fungal functional genes, 10% unknown sequences, and 12% unassembled reads. Analysis of D1-D2 LSU sequences confirmed that a single ECM fungal species usually dominates individual root clusters. De novo assemblies of fungal genes yielded 120 thousand contigs from 10 million reads representing 90 thousand unique genes with highly similarity to known ECM fungal. Functional analysis revealed that most of the transcripts recovered were involved with translation, protein degradation, heat shock, superoxide metabolism, electron transfer, signaling, and C/N metabolism. Highly expressed transcripts recovered from Piloderma, which was abundant in our samples, included genes encoding a wide array of metabolic enzymes: chitosanase, phosphatase, glutamine synthetase, terpene synthases, b-glucanase; transporters for P+ and oligopeptides; cell signaling: calmodulin, cAMP-regulated phosphoprotein (Igo1); C/N related genes: lectin, cross-pathway control (cpc1); as well as several genes with unknown function. Future studies will seek to address how ECM metatranscriptomes change in response to different Pinus hosts and across different spatial scales.
Regulation and Comparative Genomics of Carbon and Nitrogen Metabolism
Co-chairs: Richard Wilson and Ronald de Vries


Arbuscular mycorrhizal (AM) fungi can substantially contribute to host plant nitrogen (N) nutrition in exchange for carbon (C). We studied the effect of C supply on fungal N uptake and transport in the AM symbiosis via 15N labeling, enzymatic assays and qPCR analysis of fungal genes putatively involved in N metabolism. We found that an increase in C supply stimulated 15N transport and increased the enzymatic activity of arginase and urease in the intraradical mycelium (IRM). The fungus responded to an increase in the C supply with an upregulation of genes involved in N assimilation and arginine biosynthesis, but with a downregulation of a fungal urease in the extraradical mycelium (ERM). The effect on fungal gene expression in the IRM was relatively small, but genes involved in arginine biosynthesis were downregulated by an increase in C availability. The results indicate that C from the host triggers N uptake by the AM fungus, the conversion of N into arginine in the ERM, the transport of arginine to the IRM and subsequent breakdown of arginine via the catabolic arm of the urea cycle. When the fungus had access to a C supply independent from the host 15N transport was reduced and a change in the gene expression pattern indicated that the fungus changed its nutrient allocation strategy when the fungus was less dependent on the host for its C supply. In a common mycelial network, the AM fungus Glomus aggregatum transported more N to a more photosynthetically active plant when given the choice between a shaded versus a non-shaded plant host. The results indicate that AM fungi are able to distinguish between hosts differing in their carbon supply and that carbon is an important trigger for fungal nitrogen uptake and transport in the AM symbiosis.

Mechanisms of adaptation to host rice cells by the blast fungus. Jessie Fernandez, Richard A. Wilson. Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68516, USA.

To infect rice, the devastating blast fungus Magnaporthe oryzae has distinct morphogenetic stages that allow it to breach the surface of the host leaf and invade the fungus monitors the transition from the nutrient-free surface to the nutrient-rich interior of the leaf, what controls the genetic reprogramming necessary to produce infectious hyphae, and how it acquires nutrient during biotrophic in planta growth is poorly understood. M. oryzae’s trehalase-6-phosphate synthase 1 (Tps1) enzyme integrates carbon and nitrogen metabolism in the fungal cell to regulate virulence via a novel NADPH-dependent genetic switch. Loss of Tps1 function results in D. vries1* strain that can form functional appressoria and penetrate the rice surface but fail to grow beyond the first infected cell. Impaired invasive growth of Dtps1 strains is due to loss of glucose sensing, inactivation of the NADPH-dependent genetic switch, and altered carbon assimilation. Moreover, NADPH-requiring antioxidant defenses are shut down in Dtps1 strains, rendering them hypersensitive to oxidative stress. Taken together, we discuss here how, using classical and high-throughput reverse genetics, we are exploring the dynamics of this critical NADPH-dependent genetic switch to understand how M. oryzae controls infectious hyphal development during biotrophy, how it responds to and acquires nutrient from the host, and how these processes are integrated to allow successful colonization of rice cells.

Similar is not the same: Differences in the function of the (hemi-)cellulolytic regulator XlnR (Xlr1/Xyr1) in filamentous fungi. Sylvia Klaubauf1, Hari Mander Narang1, Evy Battaglia1, Tetsuo Kobayashi1, Kurt Brunner1, Astrid R. Mach Aigner1, Robert L. Mach1, Ronald P. de Vries2*; 1) Fungal Physiology, CBC-NKAW Fungal Biodiversity Centre, Utrecht, Netherlands; 2) Microbiology, Utrecht University, Utrecht, Netherlands; 3) Department of Biological Mechanisms and Functions, Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya-shi, Aichi, Japan; 4) Institute of Chemical Engineering, Research Area Biotechnology and Microbiology, Working Group Gene Technology, Vienna, Austria

The (hemi-)cellulolytic transcriptional activator XlnR (Xlr1/Xyr1) is a major regulator in fungal xylan and cellulose degradation as well as in the utilization of D-xylene via the pentose catabolic pathway. XlnR homologs are commonly found in filamentous ascomycetes and often assumed to have the same function in different fungi. However, a comparison of the saprobe Aspergillus niger and the plant pathogen Magnaporthe oryzae showed different phenotypes for deletion strains of XlnR. In this study wild type and xlnR/xlr1/xyr1 mutants of six fungi were compared: Fusarium graminearum, M. oryzae, Trichoderma reesei, A. niger, Aspergillus nidulans and Aspergillus oryzae. The comparison included growth profiling on relevant substrates and detailed analysis of protein profiles of extracellular enzymes as well as extracellular enzyme activities. The resulting data demonstrated significant differences in the influence of XlnR and its orthologs on plant polysaccharide degradation by these fungi. For example, in A. niger cellulolytic enzymes, such as cellobiohydrolase and b-glucosidase are strongly down-regulated in the mutant strain, whereas this is not the case for the other two Aspergillus species. Moreover, in A. oryzae the L-arabinose releasing enzyme a-arabinofuranosidase is clearly regulated by AoXlnR, whereas this enzyme is known to be under control of another regulator, ArAr, in A. niger and not affected by XlnR. In contrast, M. oryzae Xlr1 does not significantly affect enzyme activities in this study. Based on extracellular protein profiles, disruption of Xyr1 results in the disappearance of only some bands in F. graminearum, while nearly all bands disappear in T. reesei Dxyr1. This comparison emphasizes the functional diversity of a fine-tuned (hemi-)cellulolytic regulatory system in filamentous fungi, which might be related to the adaptation of fungi to their specific biotopes.
Regulating the Aspergillus nidulans global nitrogen transcription factor AreA. Richard B. Todd. Department of Plant Pathology, Kansas State University, Manhattan, KS.

Nitrogen nutrient utilization genes are regulated in Aspergillus nidulans by the GATA DNA-binding transcription activator AreA. The transcriptional activity of AreA is regulated by multiple mechanisms including autogenous transcriptional regulation, differential areA transcript stability, interaction of AreA with the corepressor NmrA, and repression by the negative-acting GATA factor AreB. In addition, AreA shows regulated nuclear accumulation. AreA accumulates in the nucleus specifically during nitrogen starvation, and is rapidly exported to the cytoplasm upon addition of nitrogen nutrients to nitrogen-starved cells. I will focus on recent developments in our understanding of AreA nuclear import and nuclear export, the key control points of regulated AreA nuclear accumulation. We have shown that the six conserved nuclear localization signals (NLSs) in AreA show redundancy and collaborate to mediate nuclear import. In contrast, a single CrmA exportin-dependent nuclear export signal (NES) in AreA is required for nuclear export.

We have shown that fusion of the AreA NES to a constitutively nuclear protein confers nucleocytoplasmatic localization and a loss of function phenotype. We have exploited this phenotype to select mutants defective in the AreA-CrmA interaction.

Transcriptional analysis of oxalate degradation in the white rot basidiomycete Dichomitus squalens. Miia R. Mäkelä, Johanna Rytioja, Outi-Maaria Sieto, Sari Timonen, Annele Hatakka, Kristiina Hilden. Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland.

Basidiomycetous white rot fungi are the most efficient degraders of lignocellulose with a unique ability to mineralize the recalcitrant lignin polymer. Lignocellulose decay involves a complex enzymatic system, but is also suggested to be promoted by the fungal secretion of oxalic acid. White rot fungi synthesize oxalate as a metabolic waste compound and typically secrete it to their environment in millimolar quantities. As oxalate is a toxic compound, regulation of its intra- and extracellular concentration is extremely important for fungi and also for lignocellulose degradation since high oxalate levels are shown to inhibit the decomposition reactions. Therefore, specific oxalate-converting enzymes, namely oxalate decarboxylases (ODCs) that work in conjunction with formate-degrading formate dehydrogenases (FDHs), are recognized as key fungal enzymes in lignocellulose decay. Dichomitus squalens is a white rot fungus that degrades effectively all the wood polymers, i.e. cellulose, hemicelluloses and lignin, and secretes oxalic acid during its growth on wood. The genome of D. squalens harbours 5 putative ODC and 3 putative FDH encoding genes, while these numbers differ in other fungi based on comparative genomics. In order to enlighten the roles of the multiple oxalic-acid catabolising enzymes of D. squalens, the expression of the odc and fdh genes was followed with quantitative real-time RT-PCR when the fungus was grown on its natural substrate, i.e. Norway spruce (Picea abies) wood. In addition, the effect of organic acid (oxalic acid) and inorganic acid (HCl) supplementation on the relative transcript levels of the oxalate-catabolizing genes was examined in the submerged liquid cultures of D. squalens. The results show for the first time the sequential action of ODC and FDH at the transcript level in a white rot fungal species. The constitutive expression of odc1 suggests the pivotal role of the corresponding enzyme during the growth of D. squalens on wood. In addition, the strong upregulation of the transcription of odc2 in oxalic-acid amended cultures indicates the distinct roles of individual ODC isoenzymes.


Infectious growth of fungal pathogens is controlled by environmental cues, including nutrient status. The soilborne fungus Fusarium oxysporum produces vascular wilt disease in more than a hundred different crop species and can cause lethal systemic infections in immunodepressed humans. Previous work showed that the preferred nitrogen source ammonium causes repression of infection-related processes in F. oxysporum that could be reversed by rapamycin, a specific inhibitor of the conserved protein kinase TOR. Here we generated mutations in upstream components that should result in constitutive activation of TOR, including null mutants in tuberous sclerosis complex 2 (TSC2), a small GTPase that represses TOR activity, as well as strains expressing a dominant activating allele of the small GTPase Rag (ragAQ86L), an activator of TOR. The Dts2 mutants and, to a minor extent, the ragAQ86L strains showed defects in hyphal growth and colony morphology on several amino acids, as well as decreased efficiency in cellophane penetration and vegetative hyphal fusion. These phenotypes were exacerbated in Dts2ragAQ86L double mutants and could be reversed by rapamycin, suggesting that they are caused by hyperactivation of TOR. The mutants caused significantly lower mortality on tomato plants and on larvae of the animal model host Galleria mellonella. These results suggest that TOR functions as a negative regulator of fungal virulence on plant and animal hosts.

Transcriptional regulation of peptidases and nitrogen transporters during the assimilation of organic nitrogen by the ectomycorrhizal fungi Paxillus involutus. Firoz Shah, Francois Rineau, Tomas Johannson, Anders Tunlid. 1) Microbial Ecology Group, Department of Biology, Lund University, SE-22362, Lund, Sweden; 2) Centre for Environmental Sciences, Hasselt University, Building D, Agoralaan, 3590 Diepenbeek, Limburg, Belgium.

Proteins and amino acids form a major part of the organic nitrogen (N) sources in soils. Though a poorly characterized process, this N is mobilized and becomes available to plants due to the activity of ectomycorrhizal (ECM) fungi. We have examined the role of ectomycorrhizal extracellular peptidases and amino acid transporters in the degradation, uptake and transfer of various protein sources (BSA, Gliadin and pollen) as well as of plant litter material using the ECM model fungus Paxillus involutus. During N-deprived conditions, all substrates induced secretion of peptidase activities. The activity had acidic pH optimum (2.3-3.0), and it was mainly due to aspartic peptidases and with minor contribution of metallo and serine peptidases. The activity was partly and temporarily repressed by low concentrations of ammonium (1mg/L). Transcriptional analysis showed that P. involutus expressed a large array of proteins and enzymes involved in the assimilation of organic N including peptidases, N-transporters and enzymes of the N-metabolism. Extensive in-silico analysis revealed the presence of genes encoding 312 peptidases, 129 N transporters and 284 enzymes involved in amino acid metabolism. Out of these, 89 peptidases and 37 N-transporters and 109 amino acid metabolism enzymes encoding genes were significantly upregulated during organic N assimilation. The genes were encoding a variety of secreted (23) and non-secreted (20) peptidases which were differentially expressed depending on the medium with the highest expression of the aspartic and metallo peptidases. Apart from the YAAH/ATO family, upregulated genes were found in all the other families of transporters for amino acids, oligopeptides, ammonium, urea and allantoate/allantoin. The results shows that the expression levels of peptidases and transporters in P. involutus are coordinately regulated during the assimilation of organic N sources.
Regulation of glycolysis and gluconeogenesis by antisense transcription in Aspergillus nidulans? Michael Hynes1, Koon Ho Wong2, Sandra Murray1. 1) Dept Gen, Univ Melbourne, Parkville, Victoria, Australia; 2) Dept. of Biological Chemistry & Molecular Pharmacology, Harvard Medical School, Boston, MA.

The last step in glycolysis is carried out by pyruvate kinase, encoded by pkiA, converting phospho-enol-pyruvate to pyruvate for metabolism to oxaloacetate and acetyl-CoA. The key step in gluconeogenesis is conversion of oxaloacetate to phospho-enol-pyruvate by PEP carboxykinase, encoded by acuF. Simultaneous activity of these enzymes would generate a nasty futile cycle. A number of observations suggests that control of the expression of these two genes involves activation of sense transcription and negative control by activation of antisense transcription. For pkiA, ChIP studies have found binding of the gluconeogenic activators AcuK and AcuM and of the acetate dependent FacB activator in the downstream region. Cognate binding sites are conserved in filamentous ascomycetes. RNA Seq, polII ChIP and RT-PCR analysis indicates antisense transcript ion during growth on acetate or proline as carbon sources. Old data (de Graaf, van den Broek, Visser; Cur. Genetics 13: 315, 1988) showed that transformation of a construct lacking these 3' sites resulted in inappropriate pkiA expression on acetate. In response to growth on gluconeogenic carbon sources, the acuF gene is activated by AcuK and AcuM binding to sites in the 5' upstream region. Studies with an acuF-lacZ gene fusion indicate positive control by AcuK and AcuM but a loss of the glucose repression observed in Northernms suggesting negative regulation acting via 3' sequences in response to growth on glycolytic carbon sources. Support for this is provided by transcription studies. Modulation of the balance between the opposing activities of these two gene products is proposed to result from transcriptional interference involving collision of RNA polymerase molecules.
**Centrosome-Nuclear Disconnect Creates Mitotic Chaos in a Closed Mitosis System.** Michael Koonce, Irina Tikhonenko. Translational Medicine, Wadsworth Center, NYS Department of Health, Albany, NY.

In many fungi, protists, and unicellular algae, cells divide via a mitotic mechanism that functions within a closed nuclear compartment. Closed mitosis requires tight coordination between the centrosome and nucleus to ensure a smooth transition from cytoplasmic to nuclear activities. In many cases, the centrosome/spindle pole body is directly embedded into the nuclear envelope, an arrangement that facilitates this transition. However, in other organisms (e.g. Cryptococcus, S. Pombe, Dictyostelium), the centrosome is simply attached to the cytoplasmic side of the nucleus during interphase, gaining access to the intranuclear volume only during mitosis when this organelle builds the spindle. We have identified a kinesin motor protein (Kif9) that is responsible for maintaining the centrosome-nucleus interaction in Dictyostelium. In the absence of Kif9, centrosomes separate from nuclei and the two organelles move independently. We used this null mutant to examine what happens if centrosomes fail to engage nuclei when cells enter mitosis and to examine interactions in multi-nucleated arrangements. We find that centrosomes replicate and separate in the cytosol, but do not form a visible spindle apparatus. Nuclei that lack centrosomes import tubulin, but also fail to develop a spindle apparatus. Replicated daughter centrosomes are able to integrate into the nuclear envelope and can go on to form monopolar spindles. All centrosomes (nuclear or cytoplasmic) trigger cytokinesis activity. Moreover, the nuclear envelope is promiscuous and can dock multiple centrosomes if they are available. In these cases, multipolar or multiple independent spindles can arise which lead to aneuploid nuclear products. Our work illustrates the significance of maintaining a one centrosome-one nucleus relationship to ensure proper chromosome segregation. This is particularly important in multicellular syncitia where unpaired activities would result in multiple combinations of centrosome-nuclear engagements. In addition, a firm coupling between these two organelles links nuclei to microtubule force generating machinery that is crucial for nuclear transport and positioning. We will further present a model for how the Kif9 kinesin functions to maintain centrosome-nuclear paring. For our work, we gratefully acknowledge support from the NSF (MCB-1051612).


Non-scientists, even those with an interest in biology, tend to have a poor understanding of fungi; one example is the perception that mushrooms are “some kind of weird plant”. This lack of awareness regarding fungi and their importance allows non-majors students to enter the classroom without preconceptions or expectations. Fungi provide an excellent starting point to introduce students to broader biological themes such as cell biology, diversity of life, evolution, conservation, and molecular biology. In addition, the relative paucity of species classification for fungi when compared with other kingdoms provides an opportunity for non-majors students to engage in primary research projects. In a liberal arts college class entitled “Shrooms”, students collect mushrooms in the field, cultivate the fungi, extract DNA, and identify the species using DNA barcoding. Fungal identifications and sequences can contribute to ongoing fungal barcoding efforts. In future classes, students will expand this research to include identification of soil-borne fungi at a field station located close to gas-fracking operations.

**ComGen Authentic Research Experiences (C-ARE): Fungal genetic analysis.** Gita Bangera1, Andrea Gargas2. 1) Bellevue College, Bellevue, WA, USA; 2) Symbiology LLC, Middleton, WI, USA.

ComGen (Community College Genomics Research Initiative) teaches students the skills of self-directed learning, critical thinking, and analysis. Community college students in this program receive a mini-graduate school experience, following a single requisite course in cell biology. Students work on original research projects, learn to troubleshoot their experiments, organize lab meetings and student journal clubs, and network within the scientific community. In one research track students work with DNA from described fungal collections, learning DNA-based techniques including PCR amplification, DNA sequencing and sequence analysis. Student-gathered sequence information is used to advance identification and phylogenetic results for these collections. With NSF Award DUE #1125857 ComGen (C-ARE): Dissemination, Enrichment and Expansion Project the project will be expanding to community college partners throughout the Seattle/Tacoma region of Washington State.

**Wearing two hats: Tips for combining commitments to research and to university-wide initiatives in education.** Patricia J. Pukkila. Dept Biol, Univ North Carolina, Chapel Hill, NC.

In an editorial which appeared in the New York Times, Gary Gutting argued that the primary role of universities is to “nourish a world of intellectual culture; that is, a world of ideas, dedicated to what we can know scientifically, understand humanistically, or express artistically” (http://opinionator.blogs.nytimes.com/2011/12/14/what-is-college-for/). At research universities, faculty are expected to make substantial contributions to their disciplines, to society, and to educating students. It is important for faculty to seek pan-university roles, and making acknowledged contributions to changing your campus culture can be deeply satisfying. This session will encourage you to consider how you might productively multitask in ways that can actually benefit your research productivity in addition to improving the intellectual climate on your campus. Supported in part by the HHMI through the Precollege and Undergraduate Science Education Program.
Facilitating an Interdisciplinary Learning Community Amongst Undergraduate Research Fellows By Emphasizing Scientific Inquiry as the Unifying Thread. Virginia K. Hench\textsuperscript{1,2}, Patricia J. Pukkila\textsuperscript{1,2}. 1) Department of Biology, University of North Carolina at Chapel Hill, NC 27599; 2) Office for Undergraduate Research, University of North Carolina at Chapel Hill, NC 27599.

The HHMI-Future Scientists and Clinicians (HHMI-FSC) fellowship is 1 of 3 components of the HHMI Science Learning Communities program at UNC Chapel Hill. The HHMI-FSC program was designed to foster an intellectual community that empowers high-ability students from low-income backgrounds to engage in biomedical research for 2 summers. Each year, 12 new fellows are matched with mentors in labs spanning a range of biomedical areas. They work fulltime in labs on their own research project and meet weekly as a group to engage in interactive programming that targets skills critical for success in science beyond the bench. One area of emphasis has been the process of inquiry itself. The goal is for students to transition from being a pair of hands executing protocols to active learners invested in their own projects and able to speak with authority about why experiments are performed in particular ways and what conclusions can be drawn from data generated. This starts with coaching students to state the questions that they are trying to answer and think through whether an experimental setup is consistent with what they say they are trying to find out. Assignments and feedback are designed to reinforce this principle. One of the most satisfying aspects of doing science is getting to follow one’s own instinctive curiosities and develop the methodologies needed to navigate new terrains. Undergraduates are usually still trying to define their own specific curiosities. Pushing students to describe what they are curious and passionate about is one feasible strategy that can help students identify pursuits that fit their interests and talents. Another successful strategy has been to require returning second year fellows to share science learning experiences via 15-30 minute long talks for their peers. Some took the opportunity to become more immersed in their lab’s focus, while others branched into questions like what motivates scientists to work in foreign countries and what has genomic anthropology told us about human evolution. Project aims were developed through conversations between the fellow and instructor. The one constraint was for fellows to organize their presentations around questions. Feedback indicated that presenters benefited from having to give presentations and others enjoyed learning about a broader array of topics.

The Environmental Molecular Sciences Laboratory molecular analysis capabilities for fungal biology. S. E. Baker, Environmental Molecular Sciences Laboratory, Pacific Northwest Natl Lab, Richland, WA.

Tools for analysis of classical and reverse genetic mutants play an important role in fungal biology research. The Environmental Molecular Sciences Laboratory (EMSL) at the Pacific Northwest National Laboratory is a US Department of Energy national user facility. EMSL develops and utilizes cutting edge mass spectrometry, NMR, imaging and computational capabilities to accelerate research in a number of areas. We have used EMSL’s mass spectrometry capabilities to characterize glycosylation of secreted proteins of Aspergillus niger. In addition, we have explored the use of laser ablation and nano-DESI mass spectrometry for spatial localization of molecules associated with Trichoderma reesei mycelium. Finally, spores from wildtype and albino strains of Aspergillus carbonarius were characterized using helium ion microscopy. As a national user facility, the EMSL is open to the fungal biology community through a competitive, peer-reviewed proposal process.

Development and utilization of arrayed mutant sets for yeasts and filamentous fungi. Aric E Wiest, Kevin McCluskey. Fungal Genetics Stock Center, Kansas City, MO.

Advancements in high throughput functional genomics has allowed the generation of vastly increasing numbers of strains carrying single gene deletions. For some organisms these include mutations distributed across the genome. The FGSC has generated or acquired sets of arrayed mutants for several different yeast or filamentous fungal species including Neurospora crassa, Magnaporthe grisea, Cryptococcus neoformans, Candida albicans, Aspergillus nidulans, and Pichia pastoris. These arrayed sets allow rapid screening for desired traits across a broad number of gene deletions. Details of construction, replication and manipulation of these arrayed sets will be presented. Custom arrays, construction of functional sets, and cryopreservation will also be discussed.

Sequencing-based solutions to identify and characterize fungal developmental genes. Minou Nowrousian, Ines Teichert, Gabriele Wolff, Ulrich Kück. Dept. of General & Molecular Botany, Ruhr University Bochum, Bochum, Germany.

During sexual development, filamentous ascomycetes form complex, three-dimensional fruiting bodies for the protection and dispersal of spores. We are using a combination of classical genetics, next generation sequencing, molecular and microscopic methods to learn more about this differentiation process in the model organism Sordaria macrospora, and here we present data on the identification/characterization of transcription factors and signaling molecules that are involved in development. Whole genome sequencing of mutant pro44 was used to identify the mutation that causes sterility in the mutant strain. For Illumina/Solexa sequencing, pooled DNA from progeny of crosses of the mutant with the wild type was used, and we were able to pinpoint the causative mutation in the mutant strain through bioinformatics analysis. pro44 carries a mutation in a GATA-type transcription factor, and fertility can be restored by transformation with the wild-type allele. In a second approach, we used laser microdissection to isolate young fruiting bodies (protoperithecia) of the wild type and mutant pro1 that carries a deletion of another transcription factor gene essential for sexual development. Linear amplification of RNA from microdissected protoperithecia yielded enough material for RNA-seq analysis. A comparison with total mycelium revealed significant differences in in gene expression between protoperithecia and non-reproductive mycelia. Among the genes strongly up-regulated in protoperithecia were the pheromone precursor genes ppg1 and ppg2. This was confirmed by fluorescence microscopy of egfp expression under control of ppg1 regulatory sequences. In protoperithecia, many genes are under control of the transcription factor PRO1; thus, by combining laser microdissection and RNA-seq, we can now perform genome-wide analyses of genes that are dependent on a development-specific transcription factor for correct expression in a defined developmental structure in fungi. Among the genes that are dependent on PRO1 for correct expression in protoperithecia is pro44, which is among the 500 most strongly expressed genes in wild-type, but not pro1 protoperithecia. In summary, our data indicate that PRO1 and PRO44 are members of a transcription factor network that regulates gene expression and cell differentiation in developing fruiting bodies.

Aspergillus nidulans as an experimental system to identify novel cell wall growth and maintenance genes through identification of anti-fungal drug resistance mutations. Xiaoxiao Sean He, Shengnan Jill Li, Susan Kaminsky. Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Systemic fungal infections are estimated to contribute to ~10% of hospital deaths. Systemic fungal infections are most dangerous for the young, the old, and the already sick, since their immune systems are less vigorous. Most antifungal drugs in current clinical use target ergosterol (polyenes) or the ergosterol biosynthetic pathway (azoles and allylamines). Drugs against beta-glucan synthesis (echinocandins) are effective against aspergillosis and candidiasis. The use of compounds that target fungal enzymes inevitably leads to the development and natural selection of drug resistant fungal strains. Not only are the anti-fungal drugs in current clinical use losing efficacy in some situations, but in addition the high level of conservation between animal and fungal physiology leaves relatively few relevant targets to explore. However, it is likely that for any drug-enzyme combination there will be relatively few mutations that could increase drug resistance while still maintaining enzyme function. We are using Aspergillus nidulans as an experimental model system to assess the number and identity of mutations that lead to drug resistance. As proof of concept, we grew wild type A. nidulans on replicate plates containing a sub-lethal concentration of Calcifluor. These developed fast-growing sectors beginning at ~5 d (70 rounds of mitosis). Preliminary results show that many of these sectors harboured heritable, single-gene mutations. To date, mutated genes that confer robust, heritable resistance to Calcifluor that were identified by next generation sequencing have roles in cell wall synthesis, cell wall integrity regulation, or drug detoxification. We suggest this strategy will be useful for predicting genetically-mediated anti fungal resistance adaptation and help us to be ahead in the drug-resistance arms race.
CONCURRENT SESSION ABSTRACTS

Illumina-based genetic linkage map for wheat leaf rust. David L. Joly\(^1,2\), Barbara Mulock\(^3\), Christina A. Cuomo\(^4\), Barry J. Saville\(^2\), Brent D. McCallum\(^5\), Guus Bakkeren\(^6\). 1) Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada; 2) Forensic Science Program and Environmental & Life Sciences Graduate Centre, Trent University, Peterborough, ON, Canada; 3) Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, MB, Canada; 4) Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA 02142.

Few genetic maps have been made for rust fungi; yet they are useful in identifying candidate loci for phenotypic traits or in unravelling chromosomal arrangements. This lack of maps is, in part, due to the obligate biotrophic nature of rusts and the difficulties in manipulating their life cycle in a way that enables controlled crosses. Recently, the genome sequence of a wheat leaf rust (Puccinia triticina) isolate was determined and this prompted the sequencing of additional isolates using next-generation sequencing technologies. This has dramatically increased the amount of sequence information available at a substantially decreased per base cost. Fifty-seven F2 progeny of a wheat leaf rust sexual cross between race 9 (SBDG) and race 161 (FBDJ) were sequenced using Illumina. In order to generate a high-resolution genetic linkage map, genome-wide single-nucleotide polymorphisms (SNPs) were identified. Employing the genome sequence information from the two parents and the F1 isolate, more than 25,000 SNPs were selected and used to generate a genetic linkage map. Although they were obtained from different isolates, the genetic map and the reference genome were integrated, allowing the creation of pseudomolecules. Those represent a strong improvement over the currently fragmented status of the reference genome. Moreover, at least 9 seedling and 2 adult-plant avirulence genes were shown to segregate in this F2 population and candidate genes identified using the genetic map are currently being investigated.

Peering into the secretory life of Aspergillus nidulans with a little help from classical genetics. Miguel Penalva\(^1\), Areti Pantazopoulou\(^1\), Mario Pinar\(^2\), Herbert N. Arst, Jr.\(^3\). 1) Cellular and Molecular Biology, Centro de Investigaciones Biológicas CSIC, Madrid, Spain; 2) Department of Microbiology, Imperial College, London, UK.

Model fungi have survived the revolution of modern biology partly through their amenability to classical genetic analysis. Unquestionably, classical genetics lay at the root of the unmatched success of the yeast Saccharomyces cerevisiae, that exotic fungal visitor so pleasantly accepted into the parlour of true eukaryotic cells and in the conservatory of gene regulation that dominated the fungal community at the end of last millennium. Formerly fashionable, classical genetics became nearly extinct with the advent of the ‘omics era’, their demise confirmed with each of the uncountable developments of low-cost technology. However, we shall illustrate how extraordinarily powerful classical genetics can be, used in combination with sequencing techniques, to address general questions on the organization of the Golgi in eukaryotic cells. The Golgi is essential for the sorting and packaging of transport vesicles. Dysfunctional Golgi is associated with a variety of human diseases.

We have shown previously that rates of meiotic recombination are highly non-uniform along the assembled chromosomes of C. cinerea (Stajich et al. PNAS 107: 11889-11894, 2010). That study revealed an over-representation of paralogous multicycopy genes in regions with elevated levels of meiotic exchange. In addition, retrotransposon-related sequences were not found in large segments of the genome with low levels of meiotic exchange. However, the study was limited by the available markers, and only 31 Mb of the 36 Mb genome could be mapped. More recently, we have resequenced 45 meiotic segregants and 4 complete tetrads. We have developed a simple script to detect crossover and gene conversion events involving over 75,000 SNPs across the genome. In the parental strains, we detected a high level of meiotic recombination in the centromere region and a low level of recombination in the telomeres.

The secretory system in higher fungi. The Golgi complex plays a central role in the sorting and packaging of vesicles for transport. The Golgi is essential for the sorting and packaging of transport vesicles. Dysfunctional Golgi is associated with a variety of human diseases. A Hook protein is critical for dynein-mediated early endosome movement in Aspergillus nidulans. Jun Zhang\(^1\), Rongde Qiu\(^2\), Herbert Arst, Jr.\(^3\), Miguel Peña\(^1\), Xin Xiang\(^1\). 1) Department of Biochemistry and Molecular Biology, Uniformed Services University, Bethesda, Maryland, USA; 2) Department of Medicine, Imperial College London, London, UK; 3) Department of Molecular and Cellular Medicine, Centro de Investigaciones Biológicas CSIC, Ramiro de Maestu 9, Madrid, Spain.

It has been hypothesized that cytoplasmic linker proteins such as CLIP-170 facilitate motor-driven organelle transport by serving as an additional linker between the organelle and the microtubule track. However, mammalian and fungal cells lacking CLIP-170 do not exhibit any apparent defects in vesicle transport. We recently found that in the filamentous fungus Aspergillus nidulans, the Hook protein ortholog, HookA, is critical for dynein-mediated transport of early endosomes. HookA mutants were obtained from a genetic screen for mutants defective in dynein-mediated early endosome movement, and the HookA gene was identified by a combination of classical genetic and whole-genome-sequencing approaches. The HookA protein is homologous to human Hook proteins containing a N-terminal microtubule-binding domain, a coiled-coil domain and a C-terminal cargo-binding domain, an organization similar to that of CLIP-170. Both the N- and C-terminal domains of HookA are required for dynein-mediated early endosome transport, and HookA associates with early endosomes via its C-terminal domain in a dynein-independent manner. Importantly, HookA physically interacts with dynein/dynactin, and this interaction is independent of the C-terminal early-endosome-binding domain but dependent upon the N-terminal microtubule-binding domain. Together, our results suggest that HookA may facilitate cargo-motor-track interactions during dynein-mediated transport of early endosomes.
Fungi and Evolutionary Theory
Co-chairs: Hanna Johannesson and Duur Aanen

Reaching the wind: the fluid mechanics of spore discharge, and potential for dispersal mechanisms to shape the evolution of sporocarp and spore morphologies. Anne Pringle1, Michael Brenner2, Joerg Fritz2, Marcus Roper1, Agnese Seminara2. 1) Organismic & Evolutionary Biology, Harvard University, Cambridge, MA; 2) School of Engineering and Applied Sciences, Harvard University, Cambridge, MA; 3) Department of Mathematics, University of California, Los Angeles, CA.

Fungi play critical roles in human agriculture and Earth’s biogeochemistry, but mechanisms of fungal dispersal are poorly understood. Thinking has focused on the passive spread of spores by air and water, and neglected the biomechanics used by fungi to actively move spores to new habitats. In this talk we focus on terrestrial ascomycetes, a group including plant and animal pathogens, mycorrhizal fungi, and lichens. We build theory to catalog and explain the morphological features used by ascomycetes to shoot spores and facilitate the crossing of the boundary layer, a sheath of nearly still air surrounding sporocarps. Crossing the boundary layer is critically important to the fitness of a spore: a spore that cannot escape will fall back on the parent fungus, where probabilities of germination and survival are low. But after crossing the boundary layer, a spore must also travel in wind, and by explicitly modeling discharge and dispersal we identify a previously unsuspected trade-off constraining the sizes of spores. Large spores cross boundary layers more effectively, while small spores are more easily carried by wind. Spore dispersal shapes the epidemiology of disease, and will mediate range shifts in response to global change; understanding how and how quickly fungi move across landscapes will enable both management and conservation.


Following hybridization between species, parts of one species’ genome can be incorporated into the genome of the other. This transfer of genetic material, introgressive hybridization, is a well-known driver of speciation, diversification, and adaptive evolution. Introgression has occurred repeatedly in the fungus Neurospora tetrasperma and has resulted in the presence of large regions of DNA (< 4 Mbp tracts) from other species of Neurospora on the mating-type (mat) chromosomes of N. tetrasperma. The mat chromosomes of N. tetrasperma also contain large regions of suppressed recombination that are associated with the accumulation of mutations and possibly a reduction in biological fitness. It has been proposed that introgressions of DNA from other taxa, with freely recombining mat chromosomes, onto the mat chromosomes of N. tetrasperma could counteract the deleterious effects of mutation accumulation and “reinvigorate” fitness. Alternatively, interspecific introgression into N. tetrasperma mat chromosomes could be either neutral or deleterious to fitness, but are maintained due to lack of recombination between mat chromosomes. To test these hypotheses we have quantified physiological performance (linear growth rate, LGR) in homokaryons from eight strains of N. tetrasperma with mat chromosomes that differ in introgression history (e.g. introgressions from different species) and degree of degeneration. Differences in LGR between mating types and chromosome types (introgressed or degenerate) will inform our understanding how hybridization and chromosomal structure and content effect physiological performance and possibly fitness.

Nuclear arms races: sexual selection for masculine mushrooms. Bart Nieuwenhuis, Duur Aanen. Laboratory of Genetics, Wageningen University, Wageningen, Netherlands.

When many gametes compete to fertilize a limited number of compatible gametes, sexual selection will favor those traits that increase competitive advantage during mating. In animals and plants, sperm and pollen competition have yielded many interesting adaptations for increased mating success. In fungi, similar processes have not been directly shown yet. We test the hypothesis that sexual selection can increase competitive fitness during mating, using experimental evolution in the mushroom fungus Schizophyllum commune. Mating in S. commune occurs by donation of nuclei to a mycelium. These fertilizing ‘male’ nuclei migrate through the receiving ‘female’ mycelium. In our setup, an evolving population of nuclei was serially mated with a non-evolving female mycelium for 20 sexual generations. Four of the 12 tested strains had significantly increased competitive fitness and one had decreased fitness. The main characteristic that explained fitness change was the relative success in colonization of the female mycelium. In most cases, no trade-offs were found with other fitness components. Our results show that sexual selection can act in mushroom fungi and that sexual selection can lead to increased competitive ability during mating.

Genome-wide mutation dynamic within a long-lived individual of Armillaria. James B. Anderson. Department of Biology, Univ Toronto, Mississauga, Ontario, Canada.

Mutation is the ultimate source of all genetic variation in populations and yet the events themselves remain unobservable and buried in the past. Long-lived individuals of Armillaria gallica, a common opportunistic fungal pathogen of tree roots in temperate forests of the northern hemisphere, provide a spatial context for the mutational dynamic. Each individual of A. gallica arises in a single mating event between two haploid gametes and the resulting diploid genotype then grows vegetatively to occupy a discrete spatial territory including many adjacent tree root systems. In effect, this leaves a spatial record of growth over time within which mutations can be pinpointed. To identify mutations, the entire genomes of three spatially separated samples of one individual of A. gallica, approximately 200 by 60 m in size were sequenced and compared. In this comparison, mutations and chromosomal regions of loss of heterozygosity (LOH) were identified and then assayed in another 22 isolates from the same individual by conventional PCR and Sanger sequencing. The genotype network of all 10 mutations and two LOH events in the 90 Mb genome assembly was without internal conflict. Further, the spatial distribution of genotypes was non-random and appeared to reflect the vegetative expansion leading to the present-day individual. I will discuss the implications of the whole-genome data in estimating mutation rates and cellular generation times.
CONCURRENT SESSION ABSTRACTS

Rapid genetic change and plasticity in arbuscular mycorrhizal fungi is caused by a host shift and enhanced by segregation. C. Angelard, I. Sanders. University of Lausanne, Biophore, 1015 Lausanne, Switzerland.

Arbuscular mycorrhizal fungi (AMF) are among the most abundant symbionts of plants, improving plant productivity and diversity. They are clonal; a trait assumed to limit adaptability. However, AMF harbour genetically different nuclei. We hypothesized that AMF can respond rapidly to a change of environment through changes in the frequency of nuclei and by making genetically novel offspring. We subjected AMF parents and offspring to a host shift. We observed genetic changes in all AMF lines. Genetic and phenotypic responses were different among offspring and some displayed higher fitness than their parents. Our results demonstrate that AMF rapidly undergo genetic change in response to the environment and that nucleotype frequency plays a role in how they perform in the new environment. Even though clonal, AMF offspring display greater genetic change and plasticity in response to host shift. Such genetic and phenotypic flexibility is likely to be key to their ecological success.

Meiotic Drive: A Single Gene Conferring Killing and Resistance in Fungal Spore Killer. Pierre Grognet1,2*, Fabienne Malagnac1,2, Hervé Lalucque1,2, Philippe Silar1,2. 1) Univ Paris Diderot, Sorbonne Paris Cité, Laboratoire Interdisciplinaire des Energies de Demain, 75205 Paris CEDEX 13 France; 2) Univ Paris Sud, Institut de Génétique et Microbiologie, Bât. 400, 91405 Orsay cedex, France.

Meiotic drives (MD) are nuclear genetic loci ubiquitous in eukaryotic genomes that cheat the Mendel laws by distorting segregation in their favor. All known MD are composed of at least two linked genes, the disturter that acts as a toxin by disrupting the formation of gametes, and the responder that acts as an antitoxin and protects from the deleterious disturter effects. In fungi, MDs are known as Spore Killers (SK). In the model ascomycete Podospora anserina, MD has been associated with deleterious effect during ascospore formation of the Het-s prion and in Neurospora crassa a resistance gene (responder) to the Sk-2 and Sk-3 distorters has been identified. MDs are easily studied in P. anserina thanks to the ascus structure as SKs are identified by the presence of 2-spored asc in crosses between strains. Here, we identify and characterize by targeted deletion in P. anserina Spok1 and Spok2, two MD elements. We show that they are related genes with both spore-killing disturter and spore-protecting responder activities carried out by the same allele, unlike other known MD. These alleles act as autonomous elements and exert their effects in any region of the genome. Moreover, Spok1 acts as a resistance factor to Spok2 killing. As Spok1 and Spok2 belong to a multigene family, these Spore Killer genes represent a novel kind of selfish genes that proliferate in population through meiotic distortion.

Cryptic population subdivision, sympatric coexistence and the genetic basis of local adaptation in Neurospora discreta. Pierre Gladiieux, David Kowbel, Christopher Hann-Soden, John Taylor. Department of Plant and Microbial Biology, University of California, Berkeley, CA.

Identifying the genes for ecologically relevant traits is a central challenge in empirical population genetics. Species distributed across strong environmental gradients are excellent models to discover and identify the genetic targets of local selection as they are more likely to experience spatially heterogeneous selection pressures leading to local adaptation of ecologically important traits. We studied the origin of ecological differentiation in N. discreta phylogenetic species 4 (PS4), a species with a broad latitudinal distribution. We illumina-sequenced the complete genomes of 52 individuals representing 8 collections sites in Alaska, New Mexico, Washington, California, and Western Europe (average sequencing depth: 52X). Reads were mapped to the N. discreta PS4 reference genomes, and analyses were based on a final set of ca. 1.2 million high-quality SNPs. Phylogenetic analyses identified four well-supported clades. Papua New-Guinea individuals formed the most basal clade. Individuals from Alaska and Europe on the one hand, and from New Mexico on the other hand grouped into sister clades, and individuals from California were basal to these two clades. Individuals from Washington, sampled within the same site, grouped with either the New Mexico individuals, or the California individuals, indicating the coexistence in sympatry of two divergent populations. The observed pattern of population subdivision is being used as a reference to identify genes departing from the genome-wide background, and showing increased divergence consistent with divergent selective pressures, or decreased divergence consistent with gene-flow. Our findings emphasize the need to continue exploration to uncover divergent populations of Neurospora, and place N. discreta, along with N. crassa, among the handful of species that have the attributes to serve as outstanding evolutionary and ecological model organisms.

Ecological context in symbioses: when is your enemy also your friend? Georgiana May1,2, Paul Nelson3. 1) Dept Ecol, Evol, Behavior,#100, Univ Minnesota, St Paul, MN; 2) EEB graduate program University of Minnesota St. Paul MN.

Most plants are rife with fungal symbiotic partners with many of these having little apparent effect on the host’s health and fitness. In this work, we explore the degree to which the outcome of interactions between an endophytic fungus, pathogen and plant host depend on ecological context. In particular, we ask whether interactions between the endophyte of maize, Fusarium verticilliodes, with the pathogen Ustilago maydis, depend on host resistance to the pathogen. In the case of a host susceptible to the pathogen, the two fungal species should meet frequently, and compete over host resources, potentially driving greater virulence to the host in one or the other fungal species. In the case of a host resistant to the pathogen, the endophyte might be a “bystander” to the pathogen, because the two meet too infrequently to drive their co-evolutionary interaction. We show evidence that the two fungal species have evolved stronger antagonistic interactions in maize susceptible to the pathogen, and further, that this might be associated with greater virulence by the pathogen. Results of modeling will also be presented from which we predict longer term evolutionary trajectories for this 3-way interaction.
The molecular basis of extended dynein run-length. Sreedhar Kilaru, Martin Schuster, Gero Steinberg. School of Biosciences, Univ Exeter, EX4 4QD Exeter, UK.

Dynein is a minus-end directed motor that utilises ATP to transport organelles along microtubules. In fungi, a major "cargo" of dynein are early endosomes that are taken over long distance from the plus-ends near the growing apex to the central part of the hyphal cell. In cell-free assays it was shown that single dynein motors can only overcome 1 micrometer, and long-distance motility of organelles requires binding of several dynein motors that cooperate to extend the transport distance. We recently showed that this does not apply to the fungus Ustilago maydis. Here, single dynein motors move over 30 micrometers, raising the question of the underlying molecular mechanism for this extraordinary motor performance. This talk will provide a comprehensive explanation for this phenomenon.

The role of microtubule-based motors in the spatiotemporal control of autophagy. Martin Egan, Mark McClintock, Samara Reck-Peterson. Cell Biology, Harvard Medical School, Boston, MA.

Autophagy is a highly conserved eukaryotic process in which components of the cytoplasm, including damaged organelles and misfolded proteins, are sequestered into double membrane-bound vesicles called autophagosomes that are subsequently delivered to the vacuole for recycling. In fungi, autophagy is linked to cellular remodeling and differentiation, while in mammals dysfunction in the autophagy pathway has been implicated in cancer and neurodegenerative diseases. Here, we explore the role of microtubule-based motors in the spatiotemporal control of autophagy in the model filamentous fungus Aspergillus nidulans. Using a molecular genetic and live-cell imaging approach, we identify the motors responsible for autophagosome motility, and dissect their role in the delivery and fusion of autophagic vesicles with the vacuolar system. Furthermore, we examine the role of microtubule-based motors in the clearance of aggregation-prone proteins associated with motor neuron disease, and determine the effect of these aggregates on normal microtubule-based transport processes.

Microtubule-dependent co-transport of mRNPs and endosomes. Sebastian Baumann1,2, Thomas Pohlmann1,2, Andreas Brachmann1,2, Michael Feldbrügge1,2, 1) Heinrich-Heine University Düsseldorf, Institute for Microbiology, 40204 Düsseldorf, Germany; 2) Max Planck Institute for Terrestrial Microbiology, Department of Organismic Interactions, Karl-von-Frisch-Str. 10, 45043 Marburg, Germany; 3) Biocenter of the Ludwigs Maximilians University Munich, Genetics Section, Grosshaderner Str. 2-4, 82152 Planegg-Marienfeld, Germany.

Long-distance transport of mRNAs is determinant for polarization in eukaryotes. Molecular motors shuttle large messenger ribonucleoprotein complexes (mRNPs) containing mRNAs, RNA-binding proteins and associated factors along microtubules. However, precise mechanisms including the interplay of molecular motors and a potential connection to membrane trafficking remain elusive. In recent studies we identified the RNA-binding protein Rrm4 as the key player in microtubule-dependent mRNA transport in Ustilago maydis. Combining in vivo CLIP and RNA-live imaging revealed a subset of mRNAs that are bound by Rrm4 and transported processively throughout the hyphae. Studying the molecular motors revealed that shuttling is mediated by Kin3 and Dyn1/2. The same set of motors acts in endosome trafficking and indeed, studying the SNARE Yup1 and the small GTPase Rab5 we found co-transport with endosomes as a novel mechanism for mRNP transport. Currently, we address the link between mRNAs and endosomes.

Role of tea1 and tea4 homologs in cell morphogenesis in Ustilago maydis. Flora Banuett, Woraratandadharm Tad, Lu Ching-yu, Valinluck Michael. Biological Sciences, California State University, Long Beach, CA.

We are interested in understanding the molecular mechanisms that govern cell morphogenesis in Ustilago maydis. This fungus is a member of the Basidiomycota and exhibits a yeast-like and a filamentous form. The latter induces tumor formation in maize (Zea mays) and teosinte (Zea mays subsp. parviglumis and subsp. mexicana). We used a genetic screen to isolate mutants with altered cell morphology and defects in nuclear position. One of the mutants led to identification of tea4. Tea4 was first identified in Schizosaccharomyces pombe, where it interacts with Tea1 and other proteins that determine the axis of polarized growth. Tea4 recruits a formin (For3), which nucleates actin cables towards the site of growth, and thus, polarizes secretion (Martin et al., 2005). Tea1 and Tea4 have been characterized in Aspergillus nidulans and Magnaporthe oryzae (Higashitsuiji et al., 2009; Patkar et al., 2010; Takeshita et al., 2008; Yasin et al., 2012). Here we report the characterization for the first time of the Tea4 and Tea1 homologs in the Basidiomycota. The U. maydis tea4 ORF has coding information for a protein of 1684 amino acid residues that contains a Src homology (SH3) domain, a RAS-associating domain, a phosphatase binding domain, a putative NLS, and a conserved domain of unknown function. All Tea4 homologs in the Basidiomycota contain a RA domain. This domain is absent in Tea4 homologs in the Ascomycota, suggesting that Tea4 performs additional functions in the Basidiomycota. We also identified the Umtex1 homolog, which codes for a putative protein of 1698 amino acid residues. It contains three Kelch repeats. The Tea1 homologs in the Ascomycota and Basidiomycota contain variable numbers of Kelch repeats. The Kelch repeat is a protein domain involved in protein-protein interactions. The tea1 gene was first identified in S. pombe and is a key determinant of directionality of polarized growth (Mata and Nurse, 1997). To understand the function of tea1 and tea4 in several cellular processes in U. maydis, we generated null mutations. We demonstrate that tea4 and tea1 are necessary for the axis of polarized growth, cell polarity, normal septum positioning, and organization of the microtubule cytoskeleton. We also determined the subcellular localization of Tea1::GFP and Tea4::GFP in the yeast-like and filamentous forms.
Aspergillus nidulans septin interactions and post-translational modifications. Yainitz Hernandez-Rodriguez, Shunsuke Masuo, Darryl Johnson, Ron Orlando, Michelle Momany. 1) Plant Biology, University of Georgia, Athens, GA, US; 2) Laboratory of Advanced Research AS15, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai, Tsukuba, Ibaraki, JP; 3) Department of Chemistry, University of Georgia, Athens, GA, US; 4) Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, US.

Septins are cytoskeletal elements found in fungi, animals, and some algae, but absent in higher plants. These evolutionarily conserved GTP binding proteins form heterooligomeric complexes that seem to be key for the diverse cellular functions and processes that septins carry out. Here we used Aspergillus nidulans, a model filamentous fungus with well defined vegetative growth stages to investigate septin-septin interactions. A. nidulans has five septins: AspA/Cdc11, AspB/Cdc3, AspC/Cdc12 and AspD/Cdc10 are orthologs of the “core-filament forming-septins” in S. cerevisiae; while AspE is only found in filamentous fungi. Using S-tag affinity purification assays and mass spectrometry we found that AspA, AspB, AspC and AspD strongly interact in early unicellular and multicellular vegetative growth. In contrast, AspE appeared to have little or no interactions with core septins in unicellular stages before septation. However, after septation AspE interacted with other septins, for which we postulate an accessory role. AspE localized to the cortex of actively growing areas and to septa, and localizations are dependent on other septin partners. Interestingly, core septin localizations can also depend on accessory septin AspE, particularly post-septation. In addition, LC-MS/MS showed acetylation of lysine residues in AspA before septation and AspC after septation. Western blot analysis using an anti-acetylated lysine antibody showed that AspC is highly acetylated in all stages examined, while other septins showed acetylation post-septation. Though LC-MS analysis failed to detect phosphorylation of septins, this modification has been widely reported in fungal septins. Using phosphatase treatments and Western Bloting, we found phosphorylation of AspD, but no other septins. This is interesting because AspD belongs to a special group of septins that lack a C-terminal coiled-coil found in other septins. However, septin localization is not affected by the absence of AspD/Cdc10, but by the absence of filamentous fungi specific septin AspE. Our data suggests that septin interactions and modifications change during development and growth in A. nidulans, and that some modifications are septin specific.

Altered Ras1 trafficking impairs the pathogenicity of Cryptococcus neoformans. Connie B. Nichols, Teresa O’Meara, Kyla Selvig, Sandra Breeding, J. Andrew Alspaugh. Dept. of Medicine, Duke University Medical Center, Durham, NC, USA.

Cryptococcus neoformans is an opportunistic human fungal pathogen. The ability to cause disease is linked to several different determinants, one of which is the ability to grow at high temperature. Previously we found that one branch of the Ras1 signaling cascade mediates cell morphology and cytokinesis in response to mild stress, such as growth at high temperature. Inactivation of Ras1 and other components of this signaling branch negatively impacts C. neoformans pathogenicity. Additionally, this branch of the Ras1 signaling cascade is dependent on the trafficking of Ras1 from the endomembranes to the plasma membrane and is mediated by palmitoylation of the Ras1 protein. We have identified and characterized several C. neoformans protein acyltransferases (PAs), the enzymes responsible for palmitoylation, to further understand the role of palmitoylation and trafficking on Ras1 function and activity during high temperature growth and pathogenesis. Although there is some degree of functional redundancy in this protein family, we identified individual PATs that are required for stress response and virulence in models of cryptococcal disease.

Quantification of the thigmotropic response of Neurospora crassa to microfabricated slides with ridges of defined height and topography. Karen Stephenson, Fordyce Davidson, Neil Gow, Geoffrey Gadd. 1) Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Dundee, United Kingdom; 2) Division of Mathematics, University of Dundee, Dundee, United Kingdom; 3) Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom.

Thigmotropism is the ability of an organism to exhibit an orientation response to a mechanical stimulus. We have quantified the thigmotropic response of Neurospora crassa to microfabricated slides with ridges of defined height and topography. We show that mutants that lack the formin BNI-1 and the Rho-GTPase CDC-42, an activator of BNI-1, had an attenuated thigmotropic response. In contrast, null mutants that lack cell end-marker protein TEA-1 and KIP-A, the kinesin responsible for its localisation, exhibited significantly increased thigmotropism. These results indicate that vesicle delivery to the hyphal tip via the actin cytoskeleton is critical for thigmotropism. Disruption of actin in the region of the hyphal tip which contacts obstacles such as ridges on microfabricated slides may lead to a bias in vesicle delivery to one area of the tip and therefore a change in hyphal growth orientation. This mechanism may differ to that reported in Candida albicans in so far as it does not seem to be dependent on the mechanosensitive calcium channel protein Mid1. The N. crassa Dmid-1 mutant was not affected in its thigmotropic response. Although it was found that depletion of exogenous calcium did not affect the thigmotropic response, deletion of the spray gene, which encodes an intracellular calcium channel with a role in maintenance of the tip-high calcium gradient, resulted in a decrease in the thigmotropic response of N. crassa. This predicts a role for calcium in the thigmotropic response. Our findings suggest that thigmotropism in C. albicans and N. crassa are similar in being dependent on the regulation of the vectorial supply of secretory vesicles, but different in the extent to which this process is dependent on local calcium-ion gradients.


In the yeast Saccharomyces cerevisiae the dynein pathway has a specific cellular function. It acts together with the Kar9 pathway to position the nucleus at the bud neck and to direct the pulling of one daughter nucleus into the bud. Nuclei in the closely related multinucleated filamentous fungus Ashbya gossypii are in continuous motion and nuclear positioning or spindle orientation is not an issue. A. gossypii expresses homologues of all components of the Kar9/Dyn1 pathway, which apparently have adapted novel functions. Previous studies with A. gossypii revealed autonomous nuclear divisions and, emanating from each MTOC, an autonomous cytoplasmic microtubule (cMT) cytoskeleton responsible for pulling of nuclei in both directions of the hyphal growth axis. We now show that dynein is the sole motor for bidirectional movements. Surprisingly, deletion of Kar9 shows no phenotype. Dyn1, the dynactin component Jnm1, the accessory proteins Dyn2 and Ndl1, and the potential dynein cortical anchor Num1 are involved in the dynamic distribution of nuclei. In their absence, nuclei aggregate to different degrees, whereby the mutants with dense nuclear clusters grow extremely long cMTs. Like in budding yeast, we found that dynein is delivered to cMT +ends, and its activity or processivity is probably controlled by dynactin and Num1. Together with its role in powering nuclear movements, we propose that dynein also plays (directly or indirectly) a role in the control of cMT length. Those combined dynein actions prevent nuclear clustering in A. gossypii and thus reveal a novel cellular role for dynein.
ChIP-seq: an inexpensive and powerful method for studying genome-wide chromatin remodeling and transcription regulation in fungi. **Koon Ho Wong**, Kevin Struhl, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA.

Chromatin immuno-precipitation (ChIP) is a commonly used technique for studying protein-DNA interactions. When coupled with the Next Generation Sequencing (NGS) technology, ChIP-seq can map and measure genome-wide locations and occupancies of any protein-of-interest at very high resolution, and is an invaluable technique for studying chromatin-associated processes including transcription regulation. However, owing to the fact that NGS experiments are expensive, this powerful technique has yet been widely applied to fungal studies. The output of current sequencing technologies vastly exceeds the sequencing depth requirement of ChIP-seq experiments as well as many NGS applications in fungi. We have developed a multiplex sequencing method that allows up to 96 different samples to be included in a single sequencing reaction, providing a means to obtain whole-genome data at a highly affordable cost. Using multiplex sequencing, ChIP-seq and a technique called Anchor-Away for conditional depletion of proteins from the nucleus, we have gained important insights into different aspects of transcription regulation including the repression mechanism of the Cy8-Tup1 co-repressor complex in *Saccharomyces cerevisiae*. Examples on how ChIP-seq applications may be broadly addressed to apply common questions regarding transcription regulation will also be presented.

Protein Binding Microarrays and high-throughput real-time reporters studies: Building a four-dimensional understanding of transcriptional networks in *Neurospora crassa*. **A. Montenegro-Montero**, A. Goity*, C. Olivares-Yahez*, A. Stevens-Lagos*, M. Weirauch*, A. Yang*, T. Hughes*, L. F. Larrondo*. 1) Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Santiago, Chile; 2) CAGE, Cincinnati Children’s Hospital Medical Center, University of Cincinnati, U.S.A; 3) Banting and Best Department of Medical Research, University of Toronto, Canada.

It has been suggested that ~20% of the Neurospora transcriptome may be under circadian control. Nevertheless, there is scarce information regarding the regulators that are involved in the rhythmic expression of clock-controlled genes (ccgs). We are using a high-throughput platform, based on various codon-optimized luciferase transcriptional- and translational-reporters, to monitor time-of-day-specific gene expression and to identify key elements mediating circadian transcriptional control. Thus, we have identified transcription factors -such as SUB-1- that affect the expression of known and novel ccgs, among which there are transcriptional regulators that give access to a group of third-tier ccgs. In addition, we are characterizing several rhythmic bZIP-coding genes as potential nodes of circadian regulation. In order to characterize regulatory networks in which these and all Neurospora transcription factors participate, we are using double-stranded DNA microarrays containing all possible 10-base pair sequences to examine their binding specificities and in that way, predict possible targets on a genome-wide manner. Currently, these Protein Binding Microarray studies have provided DNA-binding specificities for over 120 Neurospora transcription factors granting an unprecedented and powerful tool for transcriptional network studies. Finally, we have generated graphic tools to explore the different similarities observed in the temporal control of gene expression. Funding: Conicyt/Fondcyt/regual 1090513.


The 3’ end polyadenylation of pre-mRNAs is a two-step process. First, pre-mRNAs are cleaved at their 3’ end. The second step involves the addition of the poly(A) tail by RNA polymerases. Presence of multiple potential 3’ end cleavage sites is common in eukaryotic genes, and the selection of the right site is regulated during development and in response to cellular cues. This mechanism of alternative (or non-canonical) polyadenylation generates mRNA isoforms with different exon content or 3’ UTR lengths and regulates the presence of cis elements in the mRNA. Proteins involved in alternative polyadenylation (APA) include Cleavage Factor I in metazoans (CFIm), Hrp1 in yeast and Rbp3S in filamentous fungi. The cis elements present in the 3’ UTRs such as miRNA target sites modulate gene expression by affecting cytoplasmic polyadenylation, subcellular localization, stability, translation and/or decay of the mRNA. Therefore, the selection of a proper 3’ end cleavage site represents an important step of regulation of gene expression. Using Direct RNA Sequencing (DRS), we are carrying out in the rice blast fungus *Magnaporthe oryzae* a comprehensive map of genome wide polyadenylation sites and

**CONCURRENT SESSION ABSTRACTS**

**Thursday, March 14 3:00 PM–6:00 PM**

**Fred Farr Forum**

Nucleic Acid-Protein Interactions that Impact Transcript and Translation

Co-chairs: Michael Freitag and Mark Caddick

**ChIP-seq: an inexpensive and powerful method for studying genome-wide chromatin remodeling and transcription regulation in fungi.** **Koon Ho Wong**, Kevin Struhl, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA.

Chromatin immuno-precipitation (ChIP) is a commonly used technique for studying protein-DNA interactions. When coupled with the Next Generation Sequencing (NGS) technology, ChIP-seq can map and measure genome-wide locations and occupancies of any protein-of-interest at very high resolution, and is an invaluable technique for studying chromatin-associated processes including transcription regulation. However, owing to the fact that NGS experiments are expensive, this powerful technique has yet been widely applied to fungal studies. The output of current sequencing technologies vastly exceeds the sequencing depth requirement of ChIP-seq experiments as well as many NGS applications in fungi. We have developed a multiplex sequencing method that allows up to 96 different samples to be included in a single sequencing reaction, providing a means to obtain whole-genome data at a highly affordable cost. Using multiplex sequencing, ChIP-seq and a technique called Anchor-Away for conditional depletion of proteins from the nucleus, we have gained important insights into different aspects of transcription regulation including the repression mechanism of the Cy8-Tup1 co-repressor complex in *Saccharomyces cerevisiae*. Examples on how ChIP-seq applications may be broadly addressed to apply common questions regarding transcription regulation will also be presented.

**Protein Binding Microarrays and high-throughput real-time reporters studies: Building a four-dimensional understanding of transcriptional networks in *Neurospora crassa*.** **A. Montenegro-Montero**, A. Goity*, C. Olivares-Yahez*, A. Stevens-Lagos*, M. Weirauch*, A. Yang*, T. Hughes*, L. F. Larrondo*. 1) Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Santiago, Chile; 2) CAGE, Cincinnati Children’s Hospital Medical Center, University of Cincinnati, U.S.A; 3) Banting and Best Department of Medical Research, University of Toronto, Canada.

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quantifying their usage under different nutritional conditions (rich and minimal media, carbon and nitrogen starvation) in the wild type and the Drp3S mutant. Results of these polyadenylation maps will be presented, including candidate APA targets, sequence motifs present in long 3' UTRs, Rbp3S-dependent mRNA isoforms, and conservation of significant mRNA isoforms in other filamentous fungi.

Post-transcriptional gene regulation contributes to host temperature adaptation and virulence in Cryptococcus neoformans. Amanda L. Misener Bloom1,2, Kurtis Downey1, Nathan K. Wool1, John C. Panepinto1,2. 1) Microbiology/Immunology, SUNY University at Buffalo, Buffalo, NY; 2) Witebsky Center for Microbial Pathogenesis and Immunology, SUNY University at Buffalo, Buffalo, NY.

In response to the hostile host environment, pathogens must undergo rapid reprogramming of gene expression to adapt to the stresses they encounter. Upon exposure to host temperature, Ribosomal protein (RP) transcripts are rapidly repressed in C. neoformans. We are interested in investigating specific mechanisms involved in this response, as this repression may be a critical process in host temperature adaptation. Using a mutant null of the major deadenylase, Ccr4, we have discovered that this repression is in part due to enhanced degradation of RP-transcripts. Ccr4 lacks a nucleic acid binding domain and therefore must be recruited to mRNA targets via RNA binding proteins. Using MEME analysis and chromatographic techniques, we have identified a shared cis element in the 3'UTR of RP transcripts that is recognized by the zinc knuckle protein, Gis2. We are currently investigating the importance of this protein-RNA interaction in the expression of RP genes.

Host temperature-induced enhanced degradation of RP transcripts is also dependent on the dissociable RNA polymerase II subunit, Rpb4. Specifically, we demonstrated that in an rpb4Δ mutant, RP-transcript deadenylation is impaired, suggesting that Rpb4 may be required for Ccr4-targeted degradation. In addition, we observed that upon a shift to 37°C, Rpb4 travels from the nucleus to the cytoplasm, supporting a role for Rpb4 in coupling transcription and degradation. Interestingly, this coupling is not restricted to the RP transcripts, as Rpb4 is also involved in enhanced decay of ER stress transcripts following their peak induction, one hour after a shift to host temperature. We have demonstrated that signaling through PKH enhances the degradation of the RP-transcripts in response to host temperature, but not the ER stress transcripts, highlighting the complexity of this system. We report that when transcription and degradation are uncoupled by the loss of Rpb4, growth at host temperature is impaired and virulence in a mouse model of disseminated cryptococcosis is attenuated. Our data suggests that coupling of transcription and degradation via Rpb4 allows the cell to control the intensity and duration of different responses at specific times following exposure to host temperature, contributing to the ability of C. neoformans to adapt to this stress.

Dual targeting of glycolytic enzymes by alternative splicing and translational read-through. Johannes Freitag, Julia Ast, Alina Stiebler, Michael Bölker. Department of Biology, Philipps-Universität Marburg, Marburg, Germany.

Processing of mRNA is a highly conserved process in eukaryotes involving three major steps. Nascent transcripts are capped at their 5'end, introns are removed by splicing and the 3'end is cleaved and polyadenylated. In the plant pathogenic fungus Ustilago maydis, several genes show hallmarks of differential splicing and alternative polyadenylation resulting in the production of C-terminally extended proteins. We detected that this process leads to generation of an extended glyceraldehyde-3-phosphate dehydrogenase (GAPDH) isomor harboring a C-terminal peroxisomal targeting sequence (PTS1). We could also detect peroxisomal isoforms of two further glycolytic enzymes, phosphoglycerate kinase (PGK) and triosephosphate isomerase (TPI). Remarkably, peroxisomal isoforms of PGK and TPI are generated by translational read-through in U. maydis. Further analysis revealed that dual targeting of glycolytic enzymes to peroxisomes and the cytoplasm is not restricted to U. maydis but occurs in a variety of fungal species. Interestingly, in different species variable mechanisms to generate extended peroxisomal isoforms of glycolytic enzymes are operating. In the ascomycete Aspergillus nidulans the PTS1-motif of PGK is derived from alternative splicing and polyadenylation, while translational read-through is used to generate a peroxisomal isoform of GAPDH. We could also show that some enzymes are partially targeted to peroxisomes by means of weak peroxisomal targeting signals. Dual localization of glycolytic enzymes to peroxisomes and the cytoplasm appears to be widespread in fungi. This indicates that fungal peroxisomes are endowed with a more complex metabolism than previously assumed. Thus, the consideration of alternative splicing and translational read-through will be of importance in future proteomic and metabolomic studies of organelles.

Non-optimal codon usage determines the expression level, structure and function of the circadian clock protein FREQUENCY. Mian Zhou1, Jinhu Guo3, Joonseok Cha1, Michael Chae2, She Chen2, Jose Barral1, Matthew Sachs2, Yi Liu1. 1) Department of Physiology, UT Southwestern Medical Center, Dallas, TX; 2) National Institute of Biological Sciences, Beijing, China; 3) Departments of Neuroscience and Cell Biology and Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, TX; 4) Departments of Biology, Texas A&M University, College Station, TX; 5) School of Life Sciences, Sun Yat-sen University, Guangzhou, China.

Codon usage bias has been observed in the genomes of almost all organisms and is thought to result from selection for efficient and accurate translation of highly expressed genes 1-3. In addition, codon usage is also implicated in the control of transcription, splicing and RNA structure 4-6. Many genes, however, exhibit little codon usage bias. The lack of codon bias for a gene is thought to be due to lack of selection for mRNA translation. Alternatively, however, non-optimal codon usage may also have biological significance. The rhythmic expression and the proper function of the Neurospora FREQUENCY (FRQ) protein are essential for circadian clock function. Here, we show that, unlike most genes in Neurospora, frq exhibits non-optimal codon usage across its entire open reading frame. Optimization of frq codon usage results in the abolition of both overt and molecular circadian rhythms. Codon optimization not only increases FRQ expression level but surprisingly, also results in conformational changes in FRQ protein, impaired FRQ phosphorylation, and impaired functions in the circadian feedback loops. These results indicate that non-optimal codon usage of frq is essential for its circadian clock function. Our study provides an example of how non-optimal codon usage is used to regulate protein expression levels and to achieve optimal protein structure and function.

A transcriptome-wide view on microtubule-dependent mRNA transport. Carl Haag1, Julian Konig2, Kathi Zarnack3, Michael Feldbrugge1. 1) Institut for Microbiology, Heinrich-Heine University, Düsseldorf, NRW, Germany; 2) MRC LMB Cambridge, UK; 3) EBI Hinxton, UK.

Long distance transport of mRNAs regulates spatio-temporal gene expression during polar growth. In filaments of U. maydis, for example, microtubule-dependent shuttling of mRNAs is crucial to determine the axis of polarity. The key component of this transport system is the RNA-binding protein Rrm4 that binds a distinct set of target mRNAs. Recently, we discovered a novel mechanism for mRNA transport, namely the co-transport of Rrm4 and associated mRNAs with endosomes. Here, new insights on mRNA transport will be presented using the improved in vivo UV-crosslinking technique: iCLIP. This technique allows identification of target mRNAs at the transcriptome-wide level with single nucleotide resolution.
INTERACTIONS BETWEEN FUNGI AND ANIMALS

Elicitation of host damage occurs in a temporally programmed manner during Aspergillus fumigatus infections. Elaine M. Bignell, Microbiology Section, Imperial College London, London, United Kingdom.

Background: In tissue-invasive lung infections caused by the mould Aspergillus fumigatus the molecular basis of host damage remains unclear. It has long been hypothesised that the secretion of proteolytic enzymes by invading A. fumigatus hyphae provides a mechanism by which epithelial damage is mediated. However, in whole animal studies of disease it has not been possible to substantiate an important role of fungal proteases since A. fumigatus mutants lacking individual or multiple enzyme functions retain the ability to cause fatal infections. One of the first cellular lines of defence against A. fumigatus infection is the monolayer of epithelial cells which line the mammalian airway. Epithelial cells provide a physical barrier against endothelial damage and initiate an inflammatory immune response upon contact with A. fumigatus spores. Here we show that the A. fumigatus pH-responsive transcription factor, PacC, which governs expression of secreted proteases and secondary metabolism genes, is required for invasion of the murine pulmonary epithelium, and pathogenicity. Results: We determined, via murine and epithelial infection assays, that DpacC mutants are defective in elicitation of early-phase host damage which occurs, in wild type isolates, via a novel contact-dependent mechanism. Transcriptomic analyses of murine aspergillosis revealed aberrant cell wall biosynthesis in infecting DpacC isolates, suggesting a novel role for the A. fumigatus cell wall in pathogen-mediated host damage. Concordant with these findings FucC null mutants were shown to have significantly heightened chitin content in the fungal cell wall and were hypersensitive to cell wall perturbing agents, including caspofungin. The mechanistic relevance of cell wall-mediated host damage was verified by comparative analysis of damage elicited by cell wall extracts and heat-killed hyphae from wild type and DpacC isolates. Conclusion: A. fumigatus elicits host damage in a biphasic manner, initially via a novel contact-dependent mechanism involving cell wall components, and later via soluble mediators. A. fumigatus mutants deficient in the pH-responsive transcription factor PacC suffer deficits in both mechanisms. On the basis of this functional transcriptomic analysis we propose a new model of biphasic host damage during A. fumigatus infections.

Exploiting innate recognition of fungi for vaccine development. Stuart Levitz, Medicine, University of Massachusetts, Worcester, MA.

Most licensed vaccines work by promoting protective antibody responses. However, some populations, such as the elderly and the immunocompromised, generally have poor antibody responses to conventional vaccines. Moreover, for many infectious and neoplastic diseases, vaccines that arm adaptive T cell responses appear necessary. Thus, a major challenge in vaccinology is the development of platforms and adjuvants that effectively promote protective T cell and antibody responses. The immune system has evolved to innately recognize components of the fungal cell wall, particularly b-glucans. Research in my laboratory, in collaboration with Gary Ostroff, has focused on how this innate recognition of the fungal cell wall can be exploited for vaccine development. To achieve this aim, we have used glucan particles (GPs) as a novel vaccine platform. GPs are hollow, highly purified microparticles prepared from Saccharomyces cerevisiae cell walls. GPs are composed predominantly of b-1,3-glucan and are recognized by b-glucan receptors (particularly Dectin-1) on dendritic cells and other phagocytes. GPs also potently activate complement, resulting in opsonization and recognition by complement receptors. GPs can be loaded with antigens and immunomodulators such that the “payload” is released following phagocytosis. We have demonstrated robust and long-lasting antigen-specific T cell (Th1- and Th17-biased) and antibody responses following immunization of mice with GPs “encapsulated” with antibody. Moreover, vaccination of mice with GPs loaded with fungal antigens can protect mice against lethal challenges with the pathogenic fungi Cryptococcus neoformans and Histoplasma capsulatum.

Regulatory circuits governing Candida albicans proliferation in a mammalian host. Jose C. Perez1, Carol A. Kumamoto2, Alexander D. Johnson1. 1) Microbiology and Immunology, UCSF, San Francisco, CA; 2) Molecular Biology and Microbiology, Tufts University, Boston, MA.

The fungus Candida albicans resides in the gastrointestinal tract of most, if not all, human adults and is also a leading cause of life-threatening fungal infections in immunocompromised individuals. C. albicans has no known environmental reservoir suggesting that it has extensively co-evolved to thrive in its host. To uncover the C. albicans gene circuits governing its proliferation in a host, we used mouse models of intestinal colonization and systemic infection to screen a set of ~75 transcription regulator deletion strains. These mutant strains were chosen because they showed no gross phenotypes when cultured under a variety of laboratory growth conditions. We identified eight transcription regulators that play roles in intestinal colonization, systemic infection or both. Through genome-wide chromatin immunoprecipitation and transcriptional profiling experiments, we determined the target genes and the general circuity controlled by these regulators. Our results reveal multiple biological functions necessary for C. albicans to inhabit a mammalian host, the acquisition of carbon and nitrogen sources being prominent among them. These findings highlight common challenges faced by bacterial and eukaryotic (fungal) species when colonizing the mammalian intestine and illustrate how evolution has tinkered with the C. albicans regulatory circuity to meet these demands.

Dramatic ploidy change as an adaptive strategy in Candida albicans... Meleah A. Hickman, Ben Harrison, Darren Abbey, Anja Forche, Carsten Paulson, Kathleen Matter, Judith Berman, Dept Gen, Cell Biol & Dev, Univ Minnesota, Minneapolis, MN.

For over 100 years, Candida albicans has been considered an obligate diploid, although it clearly tolerates single chromosome aneuploidy as well as long tracts of homozygosity. We recently identified tetraploid, triploid as well as intriguing reductions to below diploid C. albicans cells, some from the clinic, others from a mouse host and others following stress exposure in vitro. Tetraploidy arises either through parasex (mating between diploid cells) or defects in mitosis. Stress conditions, including exposure to the antifungal drug fluconazole, increase the frequency of tetraploid formation. The polyploid state is relatively unstable even under standard laboratory growth conditions and loss of a heterozygous marker increases by an order of magnitude as compared to diploid populations. A small subset of tetraploid cells return to a near diploid state very rapidly even without exposure to the stresses usually used to induce concerted chromosome loss. The diploid derivatives of polyploid cells exhibit a wide range of chromosome aneuploidies and homozygosities, thus generating a wide range of genetic diversity within a single population. Evolution experiments with fluconazole suggest that diploid cells undergo transient polyploidization in response to fluconazole and that polyploid cells adapt to stress conditions more rapidly.
Nematode-trapping fungi eavesdrop on nematode pheromones. Yen-Ping Hsueh1, Parag Mahanti1, Frank Schroeder2, Paul Sternberg2. 1) Howard Hughes Medical Institute and Division of Biology, California Inst of Technology, Pasadena, CA; 2) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY.

The recognition of molecular patterns associated with specific pathogens or food sources is fundamental to ecology and plays a major role in the evolution of predator-prey relationships. Recent studies showed that nematodes produce an evolutionarily highly conserved family of small molecules, the ascarosides, which serve essential functions in regulating nematode development and behavior. Here we show that nematophagous fungi, natural predators of soil-dwelling nematodes, can detect and respond to ascarosides. Nematophagous fungi use specialized trapping devices to catch and consume nematodes, and previous studies demonstrated that most fungal species do not produce traps constitutively but rather initiate trap-formation in response to their prey. We found that ascarosides, which are constitutively secreted by many species of soil-dwelling nematodes, represent a conserved molecular pattern used by nematophagous fungi to detect prey and trigger trap formation. Ascaroside-induced morphogenesis is conserved in several closely related species of nematophagous fungi and occurs only under nutrient-deprived condition. Our results demonstrate that microbial predators eavesdrop on chemical communication among their metazoan prey to regulate morphogenesis, providing a striking example of predator-prey co-evolution. We anticipate that these findings will have broader implications for understanding other inter-kingdom interactions involving nematodes, which are found in almost any ecological niche on Earth.

A morphogenesis regulator controls cryptococcal neurotropism. Xiaorong Lin1, Bing Zhai1, Karen Wozniak2, Srijana Upadhyay1, Linqui Wang3, Shuping Zhang4, Floyd Wormley2. 1) Biology, Texas A&M University, TAMU-3258, TX; 2) Biology, the University of Texas at San Antonio, San Antonio, Texas, USA; 3) Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, USA.

Cryptococcus neoformans is the major causative agent of cryptococcal meningitis, a disease that is responsible for more than 600,000 deaths each year. This ubiquitous environmental pathogen enters host lungs through inhalation and typically establishes asymptomatic latent infections. However, extrapulmonary dissemination often occurs in individuals with weakened immunity and Cryptococcus has a predilection to infect the brain. Brain infections are fatal and formidable to treat due to the poor penetration of most antifungals to the brain. Unfortunately, little is known about cryptococcal factors that control its neurotropism. Here we report that a morphogenesis regulator Znf2 controls the tissue tropism of cryptococcal infection. In particular, activation of Znf2 abolishes Cryptococcus extrapulmonary dissemination and consequently leads to the absence of fatal brain infections in the inhalation infection model. Although Znf2 overexpression strains are avirulent in this animal model, these strains are capable of proliferating in the animal lungs during the early stages of infections. Histological examinations and cytokine profiling revealed that the Znf2 overexpression strain causes enhanced monocyte infiltration in the animal lungs. Consistently, the Znf2 overexpression strain stimulates pro-inflammatory host responses while suppresses deleterious Th2 host responses during early stage of infection in the pulmonary infection model. Such protective host defense responses might have prevented the extrapulmonary dissemination of Cryptococcus. In the intravenous infection model where the lung infection was bypassed and there was uniform hematogenous dissemination, the Znf2 overexpression strain showed a specific defect in the brain infection. Taken together, our data indicate that Znf2 helps polarize the host immunity towards protection and that it mediates cryptococcal tissue tropism during infection.

Sit and wait: Special features of Aspergillus terreus in macrophage interactions and virulence. M. Brock1, I.D. Jacobsen2. 1) Microbial Biochemistry/Physiology, Friedrich Schiller University and Hans Knöll Institute, Jena, Germany; 2) Molecular Pathogenicity Mechanisms, Hans Knöll Institute Jena, Germany.

While Aspergillus fumigatus is known as the main cause of invasive pulmonary aspergillosis in immunocompromised patients, Aspergillus terreus is an emerging pathogen prevalent in some local hot spots. When tested in embryonated egg or murine infection models, A. terreus required substantially higher infectious doses compared to A. fumigatus to cause high mortality rates. Furthermore, when A. fumigatus and A. terreus infections were followed by in vivo imaging using bioluminescent reporter strains, germination and tissue invasion of A. terreus was significantly delayed. To elucidate differences in more detail, the interaction of A. terreus and A. fumigatus with macrophages was compared. A. terreus was phagocytosed significantly faster, which appears mainly due to higher exposure of galactomannan and glucans on the surface of conidia. Additionally, although phagocytosis of both species resulted in phagolysosome maturation, A. fumigatus efficiently inhibited acidification, which was not the case for A. terreus. However, within this acidic environment of phagolysosomes, A. terreus showed long-term persistence without significant inactivation of conidia. Further analyses revealed that inefficient blocking of acidification by A. terreus was due to differences in the spore colour pigment of both species. Recombinant production of a naphthopyrone synthase from Aspergillus nidulans enabled A. terreus to inhibit the acidification to a similar extent as observed for A. fumigatus. This alteration of the phagolysosomal environment resulted in an increased escape from macrophages and was accompanied by increased virulence in a murine infection model. We speculate that the long-term persistence of A. terreus wild-type strains in acidified phagolysosomes might be responsible for high dissemination rates observed in infected human patients, because A. terreus might hitchhike inside immune effector cells to reach secondary sites of infection.
The mutational landscape of gradual acquisition of drug resistance in clinical isolates of Candida albicans. Jason Funt1, Darren Abbey7, Luca Issi5, Brian Oliver3, Theodore White9, Reeta Rao5, Judith Berman6, Dawn Thompson1, Aviv Regev1,2. 1) Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02142; 2) Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Ave, Cambridge, MA 02140; 3) Seattle Biomedical Research Institute, Seattle, WA; 4) School of Biological Sciences, University of Missouri at Kansas City, MS; 5) Worcester Polytechnic Institute, Department of Biology and Biotechnology, 100 Institute Road, Worcester MA 01609; 6) Tel Aviv University, Ramat Aviv, 69978 Israel; 7) University of Minnesota, Minneapolis MN 55455 USA.

Candida albicans is both a member of the healthy human microbiome and a major pathogen in immunocompromised individuals1. Infections are most commonly treated with azole inhibitors of ergosterol biosynthesis. Prophylactic treatment in immunocompromised patients2,3 often leads to the development of drug resistance. Since C. albicans is diploid and lacks a complete sexual cycle, conventional genetic analysis is challenging. An alternative approach is to study the mutations that arise naturally during the evolution of drug resistance in vivo, using isolates sampled consecutively from the same patient. Studies in evolved isolates have implicated multiple mechanisms in drug resistance, but have focused on large-scale aberrations or candidate genes, and do not comprehensively chart the genetic basis of adaptation5. Here, we leveraged next-generation sequencing to systematically analyze 43 isolates from 11 oral candidiasis patients, collected sequentially at two to 16 time points per patient. Because most isolates from an individual patient were clonal, we could detect newly acquired mutations, including single-nucleotide polymorphisms (SNPs), copy-number variations and loss of heterozygosity (LOH) events. Focusing on new mutations that were both persistent within a patient and recurrent across patients, we found that LOH events were commonly associated with acquired resistance, and that persistent and recurrent point mutations in over 150 genes may be related to the complex process of adaptation to the host. Conversely, most aneuploidies were transient and did not directly correlate with changes in drug resistance. Our work sheds new light on the molecular mechanisms underlying the evolution of drug resistance and host adaptation.
Fungal Volatiles and Organic Compounds as Signaling Agents

Co-chairs: Joan Bennett and Richard Splivallo

Fungi reacting to rhizobacterial volatiles. Birgit Piechulla, Piyali Das, Uta Effmert. University of Rostock, Rostock, Germany.

Microorganisms, similar as other organisms are able to synthesize and release volatile organic compounds (VOCs), which are responsible for characteristic blends or aromas of for example foodstuff such as wine and cheese as well as spoiled meat. The capability of microorganisms to emit complex volatile mixtures is tremendous. More than 800 volatiles are presently known that are emitted by microorganisms (database of volatiles of microorganisms DOVE-MO). Beside the wealth of volatile emissions, to date not much is known about the biological functions of these compounds. To study volatile-mediated interactions of plant associated bacteria and fungi, various rhizobacteria and phytopathogenic fungi were co-cultivated in bipartite Petri dishes, which allow only volatiles to traverse from one to the other compartment. The volatiles of Serratia, Stenotrophomonas, Pseudomonas, Burkholderia and Staphylococcus inhibited the growth of Aspergillus, Fusarium, Microdochium, Neurospora, Rhizoctonia, Phaeolomyces, Penicillium, Phoma, Sclerotinia, Trichoderma and Verticillium in species specific manner. The reactions of Sclerotinia sclerotiorum to Serratia sp. 4Rx13 volatiles were studied in more detail, e.g. radial growth, biomass formation, catalase activity and lipid peroxidation. Furthermore, the volatile mixture of Serratia sp. 4Rx13 was studied using headspace collection systems and GCMS analysis. Ca. 100 volatiles were separated, some of them were identified, most of them remain unknowns or structures have to be elucidated. References: Kai et al. (2007) Arch. Microbiol. 187:351-360 Vespermann et al. (2007) Appl. Environ. Microbiol. 73:5639-5641 Kai et al. (2010) Appl. Microbiol. Biotechnol. 88:965-976 Effmert et al. (2012) Chem. Ecol. 38:665-703.

Enhancement of plant growth and stress resistance by Fusarium volatile organic compounds: A novel mechanism mediating plant-fungal interactions. Seogchan Kang1,2, Vasileios Bitas1,2, Nate McCartney1,2, Jim Tumlinson1,2. 1) Plant Pathology & Environmental Microbiology, Pennsylvania State Univ, University Park, PA; 2) Entomology, Pennsylvania State Univ, University Park, PA; 3) Center for Chemical Ecology, Pennsylvania State Univ, University Park, PA.

Every organism employs an elaborate network of signaling pathways for sensing stimuli from surrounding environments and neighboring organisms and translating them into specific molecular and cellular responses. Production and perception of a vast array of secreted proteins and metabolites plays key roles in this mechanism. A group of secreted molecules that are ubiquitous but often overlooked is volatile organic compounds (VOCs). VOCs can travel far from their point of production through the atmosphere as well as porous soils, making them ideal signaling molecules for mediating organismal interactions without physical contact. Roles of animal- and plant-derived VOCs in directing animal behaviors and roles of plant VOCs in chatters of “talking trees” are well known and serve critical roles in diverse ecological processes. In contrast, the available knowledge of microbial VOCs as semiochemicals is limited and mostly circumstantial. Multiple isolates of Fusarium oxysporum, a soil-borne, cosmopolitan fungus that often resides in the rhizosphere of many plants, produce unknown VOCs that drastically enhance the growth and stress resistance of Arabidopsis thaliana. Other Fusarium species also promoted Arabidopsis growth. Molecular and cellular changes underpinning the Fusarium VOC-mediated signaling will be discussed. Given the vast diversity of fungi in nature and the critical importance of fungal communities for the ecology and fitness of plants, VOC-mediated signaling is a mostly uncharted frontier, waiting for systematic exploration.

The Role of Quorum-sensing Molecules in Interactions between Candida albicans and its Host. Jessica C. Hargarten1,2, Thomas M. Petro1, Kenneth W. Nickerson1, Audrey L. Atkin1, 1) School of Biological Sciences, University of Nebraska, Lincoln, Lincoln, NE; 2) Department of Oral Biology, University of Nebraska Medical Center, Lincoln, NE.

Candida albicans is a polymorphic fungus that is capable of causing the life threatening disease Candidiasis once it reaches the bloodstream of a susceptible host. The capability to switch between morphologies, and its ability to synthesize and secrete the quorum sensing molecule (QSM) farnesol are known virulence factor. Previously, we showed that C. albicans mutants that produced less farnesol are less pathogenic to mice than their parental strain in a tail vein assay. Also, oral administration of farnesol to the mice prior to infection increased mortality. In contrast, farnesol blocks the yeast to mycelia transition in vitro, which should have a protective effect. These observations pose the dilemma of finding a mechanism whereby a molecule which blocks the yeast to mycelia transition can also act as a virulence factor. We hypothesize that farnesol functions as a virulence factor by modulating the host innate immune response. Distinct Candida morphologies elicit different host immune responses. Both white and opaque cells stimulate leukocyte movement, but only white cells secrete a small molecular weight chemoattractant that draws the leukocyte directly towards the white cell and stimulates engulfment by mouse macrophages. The white cells are also less susceptible to killing by human macrophages and neutrophils than opaque cells, possibly due to their increased capabilities of escape once phagocytosed. The chemical identity of this chemoattractant is currently unknown, but the reason behind its continued secretion by white cells is intriguing. One likely candidate is farnesol because opaque cells, unlike white cells, do not accumulate detectable levels of farnesol. Macrophages are capable of detecting and responding to exogenous farnesol. Earlier our group reported that farnesol stimulates the expression of both pro-inflammatory and regulatory cytokines by mouse macrophage. The production of these warning signals is an important indicator of how the body ultimately hopes to clear the infection. Others have shown that farnesol suppresses the anti-Candida activity of macrophages through its cytotoxic effects, thus making it all the more difficult to eliminate the fungus early in infection. Here we report the in vitro role of farnesol and other known QSM in macrophage chemotaxis and relative phagocytosis of C. albicans.
The Trichoderma reesei polyketide synthase gene pkts1 is necessary for yellow-green pigmentation of conidia and is involved in the establishment of environmental fitness. Lea Atanasova1, Benjamin P. Knox1, Christian P. Kubicek1, Scott E. Baker1, Irina S. Druzhinina1. 1) Microbiology Group, Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, 1060 Vienna, Austria; 2) Chemical and Biological Process Development Group, Pacific Northwest National Laboratory, Richland, WA, USA.

The economically important genus Trichoderma (Hypocreales, Ascomycota, Dikarya) is well known for its mycorrhizal lifestyle and for the broad range of biotrophic interactions with plants and animals. Moreover it contains several cosmopolitan species characterized by their outstanding environmental opportunism. These properties have given rise to the use of several species in agriculture as biopesticides and biofertilizers while T. reesei is applied for production of bioenergy-related enzymes. The molecular basis of the opportunistic success of Trichoderma is not yet well understood. While there is some evidence for a role of secreted enzymes and proteins, less is known about a possible role of secondary metabolites. Recently it was predicted that the PKS encoding gene pkts1 from T. reesei and its orthologues are most likely responsible for the characteristic yellow-green pigmentation of conidia. To reveal the full function of the gene we deleted it from the wild-type strain QM 6a what resulted in complete loss of the green coloration of conidia. The ecophysiological profiling of Dpks1 showed that the gene is also involved in multiple functions at different stages of the T. reesei life cycle. Testing the antagonistic antifungal potential of the T. reesei Dpks1 mutant against several host/prey fungi suggested that the loss of pkts1 reduced the ability to combat them by means of both mechanisms: the pre-contact inhibition and direct overgrowth. However the overall analysis of mycoparasitic interactions suggests that the gene is most likely involved in protection against other fungi rather than in attacking them. Interestingly, we noticed the increased production of volatile compounds by the Dpks1 strains. The phenotype microarrays showed that PKS1 encoding gene restricts T. reesei from conidiation on a number of the best utilized carbon sources but does not influence the sexual development except the alteration of stromata pigmentation. The data for transcriptional response of genes putatively involved in above mentioned processes will be presented.

Semiochemicals and signaling: plant responses to Trichoderma volatile organic compounds. Richard Hung, Plant Biology, Rutgers, The State University of New Jersey, New Brunswick, NJ.

Volatile organic compounds (VOCs) produced by Trichoderma viride have recently been shown to have plant growth promoting effects on Arabidopsis thaliana. This finding adds a new facet to the multiple methods which fungi in the genus Trichoderma promote plant health and are beneficial to humans. Both above and below ground growth was greater in A. thaliana exposed to naturally produced T. viride VOCs as compared to controls. The average root mass of control plants was 0.36g and the average mass of VOC exposed plants was 0.77g showing a 113% increase in plant mass. In addition there was a 60% increase in chlorophyll concentration (5.5mg/g control, 8.8mg/g test). GCMS analysis of the VOCs produced by T. viride has resulted in 51 identified compounds. Several compounds from the GCMS data were chosen to determine the effects of individual compounds on the health of A. thaliana. The compound trans-2-octenol at concentrations of 1ppm caused decreased dry weight (14% less than control) and extended root length (16% longer than control), indicative of stress. At 1 and 10ppm, the compound 2,5-dimethylfuran, which has been reported to be produced by Trichoderma but was not found in the aforementioned GCMS analysis, caused only visual differences. The exposed A. thaliana had extended stems as compared to controls but no other differences. In summary, the individual compounds of the T. viride volatile profile that were tested, did not promote plant growth.


Fusarium oxysporum is a soilborne pathogen that causes vascular wilt disease on a wide range of plant species, including tomato (Solanum lycopersicum). The host signals that trigger fungal infection are currently unknown. A chemotropic response of tomato root exudate towards chemoattractants. To purify the chemoattractant compound(s) from tomato root exudate, we applied a series of purification methods including extraction with organic and inorganic solvents, fractionation by size exclusion and ion exchange chromatography. The compound(s) showing chemoattractant activity were found in the hydrophilic fraction, had a molecular weight between 30 and 50 kDa and were sensitive to boiling and treatment with protease K, suggesting that they correspond to one or several secreted tomato proteins. Polycyclamide gel electrophoresis of the active fraction revealed multiple protein bands of the expected size, two of which displayed chemoattractant activity when eluted from the gel. Identification of the active protein(s) by LC-ESI-MS is currently ongoing. Identification of the secreted chemoattractant(s) from tomato roots will advance our understanding of the molecular events that trigger fungus-root interactions.
The mixed fungal and bacterial origin of truffle aroma. Richard Splivallo\textsuperscript{1}, Aurélie Deveau\textsuperscript{2}, Nayuf Valdez\textsuperscript{1}, Nina Kirchhoff\textsuperscript{1}, Pascale Frey-Klett\textsuperscript{2}, Petr Karlovsky\textsuperscript{1}. \textsuperscript{1}Molecular Phytopathology and Mycotoxin Research, Georg-August University of Goettingen, Grisebachstrasse 6, Germany; \textsuperscript{2}UMR1136 INRA Université de Lorraine "Interactions Arbres/Micro-organismes", Labex ARBRE, IFR110 EFABA, Centre INRA of Nancy, 54280 Champenoux, France.

Truffles are symbiotic ectomycorrhizal fungi which develop on plant roots. Their fruiting bodies are highly appreciated by humans for their aroma, which typically comprises twenty to fifty volatiles per truffle species. The biosynthetic routes leading to characteristic truffle volatiles have not yet been fully characterized. By similarity to yeasts, volatile synthesis in truffles most likely involves amino acid and fatty acid catabolism. Truffle fruiting bodies further contain a diverse microbial community which might be able to generate volatiles or biotransform volatile-precursors on its own. Our aim was to investigate the formation of sulphur containing volatiles in truffles, because sulphur volatiles play a major role in the truffle ecology and are determinant of their quality (for humans). We demonstrate that sulphur volatiles characteristic of the white truffle \textit{T. borchii} are actually produced by bacteria colonizing truffle fruiting bodies. Under laboratory bioassays, sulphur containing compounds (thiophenes volatiles) resulted from the transformation by bacteria of non-volatile precursor(s) into volatiles. Interestingly in our assays thiophene volatiles were detectable only from bacteria and not from truffle mycelium, while other compounds such as dimethyl disulfide were detectable from both organisms. This indicates that some volatiles might be produced by both truffle mycelium and bacteria, but thiophene volatiles most likely originate from bacteria only. Characterization of the bacterial population by Fluorescence In Situ Hybridization highlighted that the concentration of thiophene volatiles correlated with the bacterial density inside fruiting bodies. This gives further ground to the bacterial origin of thiophene volatiles. Additionally the production of thiophene volatiles was suppressed upon treating truffle fruiting bodies with antibacterial or antifungal agents, suggesting that the precursors of thiophene volatiles might be synthesized by both fungi and bacteria. These unexpected results disprove the earlier belief that truffles were able to synthesize their aroma on their own. They add a new dimension to plant-fungal interactions by highlighting the importance of the bacterial community associated to truffle fruiting bodies.
Genomics and Biochemistry of Degradation of Complex Molecules in the Environment
Co-chairs: Jonathan Walton and Dan Cullen

Fungal transcriptome as database for proteome and refinement tool of gene annotation. K. Igarashi1, C. Hori1, M. Ishiguro1, Y. Uemura2, A. K. Takeda2, S. Kaneko3, M. Samejima1. 1) University of Tokyo, Tokyo, Japan; 2) Genaris, Inc., Kanagawa, Japan; 3) National Food Research Institute, Ibaraki, Japan.

So far, wood-rotting basidiomycetes, such as white-rot and brown-rot fungi, are the organisms known to grown on wood. They produce various enzymes to outside of their cell, extracellular part of the mycelia, in order to degrade major components of plant cell wall such as cellulose, hemicellulose and lignin. There are many enzymes, which can be utilized for the biomass conversion, in those fungi, as well as the proteins helping and/or accelerating the degradation of the plant cell wall. Therefore, combination of correct annotation of these genes and the proteome analysis of the extracellular enzymes are quite important for biomass utilization. In the present study, we have cultivated the white-rot basidiomycetes Flammulina velutipes (Enoki-take, winter mushroom) and Phanerochaete chrysosporium in various biomass-degrading culture, and the transcriptome databases were constructed by sequencing of the cDNA library using 454 sequencer. In F. velutipes, we identified 19 novel biomass-degrading enzymes including 12 carbohydrate-active enzymes (CAZymes) by 2-dimensional gel electrophoresis of extracellular proteins from cellulose-grown culture, using the transcriptome data as a reference sequence. In the case of P. chrysosporium, the transcriptome sequence data was also used to improve the gene annotation, and more than 1,000 genes are newly annotated by the algorithms refined by cDNA sequences. The improvement of gene annotation caused accurate prediction of introns and showed unique monodispersed distribution of intron length in this fungus.

Developmental regulation and cellulase gene expression in Trichoderma reesei. Irina S. Druzhinina1,2, Razieh Karimi-Aghcheh1, Lea Atanasova1, Christian P. Kubicek1,2, 1) Microbiology Group, Institute of Chemical Engineering, Vienna, Austria; 2) Austrian Center of Industrial Biotechnology, c/o Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria.

We have recently shown that expression of cellulase and hemicellulase encoding genes in Trichoderma reesei (teleomorph Hypocreja jecorina) is obligatorily dependent on the function of the protein methyltransferase LAE1. Its orthologue in Aspergillus nidulans, LaeA, is a part of the VELVET protein complex consisting of LaeA, VeA and VelB that regulates secondary metabolism and sexual reproduction. Here we have investigated a possible role of VEL1, the T. reesei orthologue of A. nidulans VeA, in expression cellulase genes and the development of the fungus. The T. reesei vel1 gene is not expressed in the darkness and is expressed at a relatively low level under illumination. Deletion of the vel1 locus causes a complete loss of conidiation and essential alteration in sexual development such as loss of formation of perithecia. Overexpression of vel1 under the constitutive expression signals of tef1 did not enhance conidiation in light or darkness. However it led to irregular formation of fertile perithecia in the darkness. Deletion of vel2 did not affect cellulase gene expression, but vel2 overexpression strongly enhanced it. Consistent findings were also obtained for expression of xylanase and b-xylanolase genes. The stimulation of cellulase gene expression by overexpressing vel1 was dependent on a functional lae1 gene. Our data show that VEL1 controls photoinduced sexual development and light-independent conidiation. In addition, while vel1 overexpression stimulates cellulase gene expression, is dispensable for this process and its action is therefore different from that of LAE1.

Parallel losses of genes associated with saprotrophy in ectomycorrhizal Agaricomycotina lineages. D. Flandas1, L. Nagy1, A. Kohler1, A. Kuo1, I. Grigoriev1, F. Martin2, D. Hibbett1. 1) Biology, Clark Univ, Worcester, MA; 2) Lab of Excellence ARBBRE, Tree-Microbes Department, INRA-Nancy, 54280 Champenoux, France; 3) DOE Joint Genome Institute, Walnut Creek, CA.

Mushroom forming fungi (Agaricomycotina) play pivotal roles in the cycling of nutrients in terrestrial ecosystems. Agaricomycotina exhibit diverse lifestyles including saprotrophs and symbionts, such as mutualistic ectomycorrhizas. Previously, as part of the Saprotrophic Agaricomycotina Project (SAP), we performed analyses of fungal genomes focusing on wood decayers, which suggested that white rot is the pleiomorphic nutritional strategy of Agaricomycetes and emerged 300 million years ago at the end of Carboniferous era. Our analyses also suggested that the brown rot mechanism and the mycorrhizal lifestyle of Laccaria bicolor have emerged from white rot ancestors. The transitions from white rot to brown rot have taken place several times in Agaricomycotina and were accompanied by losses of genes encoding enzymes involved in lignin and crystalline cellulose degradation. A similar pattern was reconstructed for the transition from a saprotrophic towards a mycorrhizal lifestyle in L. bicolor, which was the first mycorrhizal species in the Agaricomycotina to have its genome sequenced. However, L. bicolor represents only one of many ectomycorrhizal lineages recognized across Agaricomycotina. Here, we present data from eleven newly sequenced mycorrhizal genomes of Agaricomycotina, generated under the auspices of the Mycorrhizal Genomes Initiative (MGI), in addition to 8 new genomes of decayers from the SAP. With these new genomes in hand, we are able to explore how the emergence of mycorrhizal lifestyles is associated with changes in numbers of genes encoding enzymes involved in degradation of plant biopolymers. The results suggest that ectomycorrhizal lifestyles have emerged multiple times from both white rot and brown rot ancestors in Agaricomycotina. The transitions to the ectomycorrhizal lifestyle show parallelism in gene losses between L. bicolor and other mycorrhizal Agaricomycotina lineages. However, patterns of retention of genes encoding lignocellulolytic enzymes vary across ectomycorrhizal lineages. For example, cellulohydrolases, which are involved in the degradation of crystalline cellulose, have been retained in several mycorrhizal lineages. The results suggest that the emergence of ectomycorrhizal lineages in Agaricomycotina has been associated with different degrees of reduction of their saprotrophic ability.
Co-expression analysis of _Phanerochaete carnosa_ during growth on hardwood and softwood species to predict proteins with unknown function relevant to biomass conversion. Hitoshi Suzuki\(^1\), Chi Yip Ho\(^2\), Kin Chan\(^2\), Philip Wong\(^1\), Yunchen Gong\(^2\), Elisabeth Tillier\(^2\), Emma Master\(^1\). 1) University of Toronto, Toronto, Ontario, Canada; 2) Mount Sinai Hospital, Toronto, Ontario, Canada.

Softwood is the predominant form of land plant biomass in the Northern hemisphere and is among the most recalcitrant biomass resource to bioprocess technologies. The white rot fungus _Phanerochaete carnosa_ has been isolated almost exclusively from softwoods, while most known white-rot species, including the model fungus _Phanerochaete chrysosporium_, were mainly isolated from hardwoods. Growth studies of _P. carnosa_ and _P. chrysosporium_ on sawpulp and hardwood from deciduous and coniferous species revealed comparable growth of _P. carnosa_ on all wood samples, while _P. chrysosporium_ grew poorly on hardwood from conifers. A contributing factor to growth on extractive-rich hardwood samples could be the comparatively high number of P450 monoxygenases encoded by _P. carnosa_. Notably, genome sequencing revealed that _P. carnosa_ possesses one of the largest P450 contingents (239 P450s) among the sequenced and annotated wood-rotting basidiomycetes. However, like most sequencing efforts, a significant fraction of the _P. carnosa_ genome comprises genes that encode proteins with unknown function. Moreover, transcripts from several of these genes were identified in mycelia collected at a single time point from _P. carnosa_ cultivations growing on woody biomass. Accordingly, the aim of the current study was to analyze co-expression patterns of known and unknown genes to identify those with unknown function that might be most relevant to biomass conversion. Our approach was to separately cultivate _P. carnosa_ on ball-milled trembling aspen (_Populus tremuloides_) and ball-milled white spruce (_Picea glauca_) and to collect mycelia at five time points over a one-month cultivation period. RNA collected from all cultures at each time point was sequenced separately using the Illumina HiSeq platform. Co-expression patterns will be described and used to predict new gene products that are particularly interesting to target for detailed biochemical characterization.

**Functional Analysis of the _Pleurotus ostreatus_ Manganese-Peroxidase Gene Family.** Tomer Salame, Doriv Knop, Dana Levinson, Oded Yarden, Yitzhak Hadar, Microbiology and Pathology, Hebrew University, Rehovot, Israel.

Mn amendment to _P. ostreatus_ cultures enhances degradation of recalcitrant aromatic compounds. Manganese peroxidase (MnP) isoenzymes are key players in these processes. The MnP gene family is comprised of five Mn-dependent peroxidases (mnp3, 6, 7, 8 and 9) and four versatile-peroxidases (mnp1, 2, 4 and 5; VPs). In liquid medium, Mn amendment resulted in a drastic up-regulation of the predominantly expressed mnp3 and mnp9, and down-regulation of mnp4. To obtain direct evidence for the role of these enzymes, we produced genetically-modified (knockout, knockdown and/or over-expression) strains in mmps and studied their degradation capacity. The compounds studied were: azo-dyes such as orange II and reactive black, recalcitrant pharmaceutical compounds found in treated waste water such as Carbamazepine and lignocellulosic agricultural waste. We engineered a transformant, constitutively expressing mnp4 a VP naturally repressed by Mn (designated OEmnp4) under the control of the b-tubulin promoter. Now, despite the presence of Mn in the medium, OEmnp4 produced mnp4 transcript as well as VP activity as soon as four days after inoculation. OEmnp4 decolorized the azo-dyes two days earlier relative to the wild type in Mn amended medium. RNAi silencing targeting mnp3 resulted in a delay in the decolorization capacity which occurred concomitantly along with a marked reduction of the expression level of all mmps, particularly mnp3 and mnp9. This observation supported the conclusion that MnPs are involved in the process but could not determine the specific contribution of the different genes to the outcome. Therefore we produced a Dku80 strain, exhibiting a 100% homologous DNA recombination rate, to enable specific gene replacement. Subsequently, homokaryon mnp2, 3, 4 and 9 knockout strains were produced. In Mn amended GP, orange II decolorization was not significantly inhibited by any of these strains, indicating on functional redundancy. In Mn deficient GP, inactivation of mnp4 proved that it encodes the key VP responsible for Mn dependent and Mn independent peroxidase activity, as well as resulted in reduction of the azo dye reactive black 5 decolorization capacity. The tools and protocols developed increase the amenability of _P. ostreatus_ to genetic manipulations and expand options for gene function analyses.

**Carbon source and light dependent regulation of gene clusters in _Trichoderma reesei_ (Hypocrea jecorina).** Doris Tisch\(^3\), Monika Schmoll\(^4\). 1) Health and Environment, Bioresources, Austrian Institute of Technology AIT, Tulln, Austria; 2) Vienna University of Technology, Institute of Chemical Engineering, Vienna, Austria.

_Trichoderma reesei_ (anamorph of _Hypocrea jecorina_) is one of the most prolific producers of plant cell wall degrading enzymes. Regulation of the genes encoding these enzymes occurs in response to the nutrient sources available in the environment and many of them are responsive to light as well. Cellulose as the natural substrate induces the most complete enzyme set, while induction of cellulosases also occurs on sophorose and lactose. In contrast, no cellulosases are induced on glycerol and the respective genes are repressed on glucose. We therefore investigated the transcriptome on these five carbon sources in light and darkness and aimed to identify genes specifically expressed under cellulase inducing conditions. These c genes are repressed on glucose. We therefore investigated the transcriptome on these five carbon source conditions are characterized by a significant enrichment of genes involved in C-compound and carbohydrate degradation and transport among the upregulated gene set. Genes down-regulated under inducing conditions show a significant enrichment in amino acid metabolism and energy metabolism. We were further interested whether light dependent regulation is clustered in the genome and if the carbon source is relevant for activation of light dependent clusters. We found that light dependent regulation predominantly occurs upon growth on cellulose, with the most significant regulation in a gene cluster comprising env1. This cluster appears on glucose as well, but is not down regulated in mutants of blr1 or blr2. Also cbl2, the arabinofuranosidase gene abf2 and the histone acetyltransferase gene gcn5 are part of light dependent clusters. Hierarchical clustering of gene expression patterns was performed to reveal functional divergence of gene regulation with respect to light response or carbon specific regulation. Glycoside hydrolase genes follow the whole transcriptome pattern with carbon source being superior to light in terms of regulation. ENV1 in in part the G-protein beta subunit GB1 were found to be crucial for carbon source specific regulation of G-protein coupled receptors, genes involved in secretion, sulphur metabolism and oxidative processes as well as transporters. We conclude that clustered regulation of light responsive gene preferentially occurs upon growth cellulose and that ENV1 and to a lesser extent GN1 play a role in carbon source dependent regulation of specific gene groups in light.
Genome-wide analysis of eleven white- and brown-rot Polyporales provides insight into mechanisms of wood decay. Chiaki Hori, Kiyohiko Igarashi, David Hibbett, Bernard Henriussat, Masahiro Samejima, Dan Cullen. 1) Graduate School of Agricultural and Life sciences, University of Tokyo, Tokyo, Japan; 2) Forest Products Laboratory, USDA, Madison, WI; 3) Biology Department, Clark University, Worcester, MA; 4) CNRS, Marseille, France.

Many efficient wood decay fungi belong to the Polyporales, and these can be categorized as white-rot fungi or brown-rot fungi, based on decay patterns. White-rot fungi degrade cell wall polysaccharides such as cellulose and hemicellulose as well as the more recalcitrant phenylpropanoid polymer, lignin. In contrast, brown-rot fungi depolymerize the polysaccharides but the modified lignin remains in the wood. Comparative analysis of white- and brown-rot gene repertoires and expression profiles have revealed substantial variation but considerable uncertainty persists with respect to precise mechanisms. Addressing this issue, we performed genome-wide analysis of carbohydrate-active enzymes (CAZy) and some oxidative enzymes related to polysaccharides degradation in eleven white- and brown-rot fungi. This analysis included classifying and enumerating genes from three recently sequenced polyporales Bjerkandera adusta, Ganoderma sp. and Phlebia brevisspora. Furthermore, comparative secretomic analysis of seven Polyporales grown on wood culture were conducted. Summarizing, the average number of genes coding CAZy in the genomes of white-rot fungi was 373, significantly more than the 283 observed in brown-rot fungi. Notably, white-rot fungi have genes encoding cellulase and hemicellulase such as those belonging to glycoside hydrolase (GH) families 6, 7, 9 and 74, whereas these are lacking in genomes of brown-rot polyporales. White-rot genes encoding oxidative enzymes potentially related to cellulose degradation such as cellobiose dehydrogenase (CDH), polysaccharides monooxygenase (PMO, formerly GH61), cytochrome b562 with cellulose-binding module, are also increased relative to brown-rot fungi. Indeed, secretomic analysis identified GH6, GH7, CDH and PMO peptides only in white-rot fungi. Overall, these results show that, relative to brown rot fungi, white rot polyporales maintain greater enzymatic diversity supporting lignocellulose attack.

Transcription factor shuttling during cellulase induction in Trichoderma reesei. Alex Lichius, Christian P. Kubicek, Verena Seidl-Seiboth. Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria.

For economically feasible production of liquid fuels and other value-added compounds from lignocellulosic plant material, strategies are required to boost cellulolytic and hemicellulolytic enzyme production by industrially relevant fungi. One promising approach is to modulate the transcriptional control mediating release from carbon catabolite repression (CCR) and induction of cellulase, hemicellulase and xylanase gene expression. To better understand the underlying molecular dynamics during induction, we characterized nucleo-cytoplasmic shuttling of the two transcription factors carbon catabolite repressor 1 (CRE1) and xylanase regulator 1 (XYR1) of Trichoderma reesei by means of live-cell imaging. In submerged cultures, nuclear import and export of CRE1 upon repression and induction, respectively, occurred within minutes and therefore was generally faster than shuttling of XYR1. Under CCR conditions XYR1 expression levels were very low, and its nuclear signal required up to one hour to significantly increase upon replacement into an inducing carbon source. Cultured directly under inducing conditions, nuclear accumulation of XYR1 was detectable after about 20h post inoculation, and strongly increased within the following 24 hours. CRE1 under the same conditions was localized exclusively to the cytoplasm. In plate cultures, nuclear recruitment of CRE1 and XYR1 differed within the central area, the subperiphery and the periphery of the colony depending on the provided carbon source. Most interestingly, under inducing conditions we found evidence for increased nuclear recruitment of CRE1 in the central area, correlating with strong nuclear import of XYR1 in the same region. Notably, the cytoplasmic signal of CRE1 was usually elevated in leading hyphae, whereas XYR1 was never significantly recruited to the colony periphery. Taken together our data provide the first temporal resolution of transcription factor shuttling during the induction of cellulase gene expression in Trichoderma reesei, and reveal some interesting differences between the subcellular localization of CRE1 and XYR1 in submerged and plate cultures, respectively. These differences indicate that the mycelial organization during fungal growth might be another important regulatory element to consider for the industrial scale production of cellulolytic enzymes.
Pathogenic Signaling via Effector Proteins

Co-chairs: Brett Tyler and Sebastien Duplessis

Dissecting nuclear immunity using Arabidopsis downy mildew effector as probes. Marie-Cecile Caillaud1, Lennart Wirthmüller1,2, Shuta Asai3, Sophie Piquerez2, Georgina Fabro1,3, Jonathan Jones1. 1) The Sainsbury Laboratory, Norwich, United Kingdom; 2) John Innes Centre, Norwich, United Kingdom; 3) Present address: CIQUIBIC-CONICET, Universidad Nacional de Cordoba, Argentina.

An important role in plant defence has been attributed to nuclear dynamics, since a growing number of reports reveal that the nuclear localization of key components of plant immunity is essential for disease resistance. Recent studies suggest that effectors may manipulate host transcription or other nuclear process for the benefit of the pathogen. However, the specific mechanisms by which these effectors promote susceptibility remain unclear. The interaction between Arabidopsis and Hyaloperonospora arabidopsidis (Hpa) has been studied intensively during the past twenty years, and it has become one of the most well-understood model systems to help us understand pathogen effector biology and the plant immune system. The recent identification of 15 nuclear-localized Hpa effectors (HaRxLs) provides a powerful tool to dissect plant nuclear immunity. When stably expressed in planta, nuclear-HaRxLs cause diverse developmental phenotypes which highlight their interferences with fundamental plant regulatory mechanisms. Remarkably, nuclear HaRxLs-plant targets are often transcriptional regulators, which may act in complex with immunity co-factors. Here, we report recent insights into our understanding of the arms race between obligate pathogen and its host.

The mutualistic fungus Laccaria bicolor uses the effector protein MiSSP7 to alter host jasmonate signaling and establish symbiosis. Claire Venneault-Fourrey1, Jonathan Plett1,2, Yohann Daguerre3, Aurélie Deveau4, Annegret Kohler5, Jennifer Morrell-Falvey1, Annick Brun1, Francis Martin1. 1) UMR1136 LaM_INRA/UHP, Lorraine Univ / INRA, Lab of Excellence ARBBRE, Nancy, France; 2) Oak Ridge National Laboratory, Oak Ridge, TN 37831-6422, USA; 3) Hawkesbury Institute for the Environment, University of Western Sydney, Australia.

Roots of most trees form a nutrient-acquiring symbiosis with mutualistic fungi. Mycorrhiza-induced Smal Secreted protein MiSSP7, a fungal effector protein necessary for the mutualistic interaction between the ectomycorrhizal fungus Laccaria bicolor and Populus spp. host trees, is secreted by the fungus in contact with plant tissues and is taken up via endocytosis into plant cells where it localizes to the nucleus and targets plant transcription through an unknown mechanism. Here we demonstrate that MiSSP7 interacts with the jasmonic acid receptor tIAZ6 of Populus trichocarpa and that PtIAZ6 interacts with a number of other nuclear localized proteins that likely form a DNA binding complex. MiSSP7 is able to block jasmonic acid signaling in both L. bicolor host and non-host plants, likely through its interaction with a jasmonate receptor. Loss of MiSSP7 expression in L. bicolor can be complemented by transiently expressing the transcription of PtIAZ6 or through inhibiting jasmonic acid biosynthesis in poplar roots. We conclude that MiSSP7, in contrast to arbuscular mycorrhizal fungi and pathogenic bacteria that promote jasmonate signaling to colonize host tissues, is a novel effector used to promote mutualism by blocking jasmonic acid signaling. In addition to MiSSP7, L. bicolor expresses other MiSSPs to communicate with its host-plant. In particular, we demonstrate that MiSSP8, an apoplastic effector is required for symbiosis.


Identification and characterization of an RXLR-like effector family from medically relevant fungi. Shiv D. Kale1*, Kelly C. Drews1,2, Helen R. Clark1,2, Hua Wise3,4, Vincenzo Antignani2, Tristan A. Hayes1,3, Christopher B. Lawrence1,2, Brett M. Tyler1,5. 1) Virginia Bioinformatics Institute, Virginia Tech., Blacksburg, VA; 2) Department of Biological Sciences, Virginia Tech., Blacksburg, VA; 3) Department of Biochemistry, Virginia Tech., Blacksburg, VA; 4) Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR; 5) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR.

Fungal infections have become an increasingly significant problem for immunocompromised individuals, transplant recipients, the elderly, several cases involving healthy individuals. There is a significant growth in incidences of morbidity and mortality associated with medically important fungi, specifically Aspergillus species. Aspergillus fumigatus virulence has been attributed to production of pigments, adhesins on the surface of the cell wall, secreted proteases, and mycotoxins. Current treatments consist of oral corticosteroids, antifungal medications, and/or surgery to remove aspergillomas. Many of these treatments have substantial shortcomings. Detection and diagnosis is also weighty problem as most clinical tests take weeks for results allowing the infection to proceed. Appropriately, the paradigm for human fungal interactions has been focused on the host deficiencies mediating virulence of opportunistic fungal pathogens. There has been substantial progress in identifying and characterizing secreted proteins (effectors) from bacterial, oomycete, and fungal plant pathogens. A subset of these effector proteins are able to enter host cells and modulate host intracellular functions. Using our bioinformatics pipeline we have been able to identify a family of secreted proteins from A. fumigatus sharing a conserved N-terminal RXLR-like motif. We found this family is expanded amongst primary fungal pathogens. The RXLR and RXLR-like motifs from known intracellular effectors of plant pathogenic and mutualistic oomycetes and fungi have been shown to facilitate effector entry into plant cells via binding external phosphatidylinositol-3-phosphate (PI3P). Here we describe AF2, a candidate effector from A. fumigatus that contains a N-terminal RXLR-like motif. Through the use of confocal microscopy and flow cytometry we show AF2 is rapidly able to enter several primary and immortalized mammalian cell lines. Through the use of isothermal titration calorimetry and liposome binding assays we show AF2 has nanomolar binding affinity for PI3P, and does not bind other mon or poly-PIPs that we have tested thus far. Based on our bioinformatics and biochemical analysis we postulate AF2 is a secreted effector protein capable of rapidly translocating into mammalian cells. We will present our latest findings on the physiological relevance of AF2.

Phytophthora sojae is a notorious oomycete pathogen producing a great loss on global soybean production annually. The disease outcome between soybean and P. sojae depends on whether hosts could recognize pathogen avirulence effectors. Recently identified oomycete avirulence effectors are characterized by N-terminal host entry motif (RxLR motif), sequence and transcripational polymorphisms between virulent and avirulent strains. Benefit from 454 genome sequencing and solexa transcriptome sequencing of P. sojae strains, eight RxLR effectors are bioinformatically identified, genetic mapping suggested that two of them perfectly matched Avr3b and Avr1d phenotype respectively. Transient expression of the ORF from avirulence strain on soybean specifically triggered Rps3b and Rps1d mediated program cell death, respectively. confirming that it encodes avirulence effector Avr3b and Avr1d. Transient expression of Avr3b and Avr1d on Nicotiana benthamiana could promote the infection of Phytophthora capsaci, suggesting both avirulence effectors could suppress plant immunity and contribute to pathogen infection. Silencing of Avr3b impaired the virulence of Phytophthora sojae. Our progress in elucidating the mechanism under the inhibiting plant immunity by these effectors will be presented.

Fungal lipoxigenases: a novel instigator of asthma? Gregory J. Fischer1,2, Katharyn Affeldt3, Erwin Berthier4, Nancy P. Keller1,2,3,1) Department of Genetics, University of Wisconsin-Madison, Madison, WI; 2) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI; 3) Department of Bacteriology, University of Wisconsin-Madison, Madison, WI.

Statement of Purpose: Fungi have long been associated with asthmatic diseases, yet the exact mechanism(s) by which fungi induce asthma is unknown. We propose that fungal lipoxigenase enzymes and their eicosanoid products are involved in asthmatic diseases. Human 5-lipoxygenase derived leukotrienes induce inflammation, mucus secretion, vasodilation, and bronchial constriction. We hypothesize that the fungal pathogen Aspergillus fumigatus is capable of secreting a 5-lipoxygenase homolog, LoxB, that participates in eicosanoid production, including leukotrienes. This secreted homolog is translocated into plant epithelial cells, participates in the production of leukotriene and other eicosanoids, and exacerbates asthmatic responses, such as bronchoconstriction. Together, this work will help delineate the role fungal products play in asthmatic diseases. Methods: We are assessing fungal interactions with lung epithelial cells using a microfluidic in-vitro platform followed by murine asthma model research. To assess the effects of LoxB overexpression, mass spectrometry was used to identify eicosanoid oxylipins within culture supernatants. Results: We have identified an Aspergillus fumigatus lipoxigenase, LoxB, with high identity to human 5-lipoxygenase. Moreover, we have identified a motif in LoxB that may mediate entry into lung epithelial cells. To fully understand the impact of LoxB in asthma, we have developed an Aspergillus fumigatus strain that overexpresses LoxB. Overexpression of LoxB results in increased levels of various eicosanoids that are known to cause airway hyperresponsiveness and increased mucus production. Future work will focus on characterizing the effect these eicosanoid products have on the airway and whether fungal effector translocation result in increased leukotriene levels.

Magnaporthe oryzae has evolved two distinct mechanisms of effector secretion for biotrophic invasion of rice. Martha C. Giraldo1, Yasin F. Dadgas2, Yogesh K. Gupta3, Thomas A. Mentlik4, Mihwa Y1, Hiromasa Saitoh5, Ryohel Terauchi5, Nicholas J. Tabot5, Barbara Valent1. 1) Plant Pathology, Kansas State University, Manhattan, KS, USA; 2) School of Biosciences, University of Exeter, Exeter, EX4 4QD, UK; 3) Iwate Biotechnology Research Center, Kitakami, Iwate, 024-0003 Japan; 4) Cambridge Consultants Ltd, Cambridge, CB4 0DW, U.K.

Pathogens secrete effector proteins into the host tissue to suppress immunity and cause disease. Pathogenic bacteria have evolved different secretion systems to target specific effector proteins during pathogenesis, but it was not previously known if fungal pathogens require different secretory mechanisms. We present evidence that the blast fungus Magnaporthe oryzae possesses different secretion systems for delivering effector proteins during biotrophic invasion of rice cells. M. oryzae secretes cytoplasmic effectors targeted for delivery inside rice cells and apoplastic effectors targeted to the extracellular space. Cytoplasmic effectors preferentially accumulate in the biotrophic intercellular complex (BIC), a novel in planta structure located beside the tip of the initially filamentous invasive hypha and then remaining next to the first differentiated bulbous invasive hypha cell. In contrast, apoplastic effectors remain in the extracellular compartment uniformly surrounding the invasive hypha inside the invaded cell. Disruption of the conventional ER-Golgi secretion pathway by Brefeldin A (BFA) treatment blocked secretion of apoplastic effectors, which were retained in the ER, but not secretion of cytoplasmic effectors. Fluorescence Recovery After Photobleaching experiments confirmed that cytoplasmic effectors continued to accumulate in BICs in the presence of BFA. Analysis of mutants showed that the BIC is associated with a novel form of secretion involving exocyst components, Exo70 and Sec5, and the t-SNARE Sso1, which are required for efficient delivery of effectors into plant cells and are critical for pathogenicity. By contrast, effectors which function between the fungal cell wall and plant plasma membrane are secreted from invasive hyphae to the apoplast by the ER-Golgi secretory pathway conserved in eukaryotes. We propose a model for the distinct secretion systems that the rice blast fungus has evolved to achieve tissue invasion.


The genome of the corn smut fungus Ustilago maydis codes for a large repertoire of secreted effectors. Some of them play crucial roles for virulence and establishment of the biotrophic phase. The chorismate mutase Cmu1 is one such secreted translocated effector of U. maydis. cmu1 deletion strains are attenuated in virulence that is attributed to higher salicylate levels in plants infected with the mutant strain, most likely through alterations in the channeling of chorismate from the plastids to the cytosol. Here we identify the motif in Cmu1 that is necessary for the translocation of the protein across the plant plasma membrane and present a mutational analysis of this region. To test for uptake we assayed the ability of mutant proteins to complement a cmu1 mutant strain as well as the retained ability to complement the growth defect of a Daro7 strain of S. cerevisiae in minimal medium. By deletion analysis a region of 20 amino acids adjacent to the signal peptide was shown to be essential for the translocation. Microscopic analysis of maize tissue infected with U. maydis strains expressing Cmu1-mcherry fusion proteins with or without the probable uptake motif revealed that the 20 amino acid motif allows binding of the protein to an as yet unknown plant plasma membrane component. We hypothesize that the translocation of Cmu1 across the plant plasma membrane is a two step process; initiated by binding followed by translocation across the membrane. In addition, we present results where the 20 amino acid motif is substituted by motifs from other effectors.
Penetration-specific effectors from Phytophthora parasitica favour plant infection. Edouard Evangelisti1*, Benjamin Govetto2, Naima Minet-Kebdani1, Marie-Line Kuhn1, Agnes Attard1, Franck Panabieres1, Mathieu Gourgues1. 1) UMR Institut Sophia Agrobiotech, INRA/CNRS/Université de Nice, Sophia Antipolis, France; 2) Institut Méditerranéen de Biodiversité et d’Écologie marine et continentale (IMBE), CNRS-INEE - IRD - Aix Marseille Université - Université d’Avignon - Institut Pytheas.

Oomycetes are major crop pests which cause million dollars loss every year. To date only a few efficient chemicals are available against these filamentous microorganisms. A better understanding of the molecular events occurring during plant-oomycete interactions will help to propose new strategies for crop protection. We performed a transcriptional analysis in order to identify oomycete penetration-specific genes and identified a set of penetration-specific effectors (PSE) bearing a RXLR motif. This motif was previously shown to promote effector import into plant cells during the biotrophic stage in feeding structures called haustoria. Here we report the functional analysis of three candidate genes, referred to as PSE1, PSE2 and PSE3. The three effectors were able to abolish plant defense responses when transiently expressed in Nicotiana plants. Moreover, constitutive expression of PSE1 and PSE3 in A. thaliana led to an enhanced susceptibility to P. parasitica infection suggesting a role for these proteins in P. parasitica pathogenicity. Transgenic Arabidopsis lines accumulating PSE1 protein showed several developmental perturbations that were associated with altered auxin physiology. Root growth inhibition assays showed that auxin signaling pathway is not altered by PSE1 accumulation. Nevertheless, the coiled-root phenotype and the enhanced susceptibility of PSE1-expressing lines to P. parasitica were reverted by synthetic auxin 2,4-D supply, or treatment with the auxin efflux inhibitor TIBA suggesting that a reduced auxin accumulation is responsible for these phenotypes. This hypothesis was confirmed by a reduced activity of the pDR5 auxin sensitive promoter at the root apex. The alteration of the expression pattern observed for two auxin efflux carriers, PIN4 and PIN7 suggests that a perturbation of auxin efflux could be responsible for the PSE1 associated defects. We proposed that PSE1 could favour P. parasitica virulence by interfering with auxin content. Our results show that penetration specific effectors can modulate general plant functions to facilitate plant infection. Perturbation of hormone physiology was previously reported for other plant pathogens, including nematodes and bacteria, supporting the hypothesis that infection strategies from distant pathogens species could converge onto a limited set of plant targets.
The function of Rho type small GTPases for cell polarity in *Ustilago maydis*. Britta Tillmann1, Michaela Wehr2, Sonja Frieser1, Kay Oliver Schink2, Johannes Freitag3, Michael Böker1. 1) Dept Biol, Univ Marburg, Marburg, Germany; 2) Institute for Cancer Research, the Norwegian Radium Hospital, Oslo University Hospital, Montebello, 0310 Oslo, Norway.

Establishment of cell polarization requires the coordinated transport and localized fusion of secretory vesicles. This process is controlled by Rho-type GTPases that act as molecular switches. Temporal and spatial activation of Rho-GTPases depends on specific guanine nucleotide exchange factors (GEFs). Inactivation of Rho proteins is achieved via interaction with GTPase activating proteins (GAPs) that stimulate the low intrinsic GTPase activity of Rho proteins. During its life cycle, *U. maydis* switches between budding and filamentous growth. The Rho type GTPase Rac1 is the main regulator of this morphogenic transition. The highly related Cdc42 is required for cell separation after mitosis and for formation of retraction septa during filamentous growth. We could show that the activator of Rac1, the Rho-GEF Cdc24 is subject to a negative autoregulatory feedback loop. Active Rac1 triggers Cla4 dependent multivalent phosphorylation of a C-terminal destruction box. This results in rapid degradation of Cdc24 and release of a ternary complex containing active Rac1, the scaffold protein Bem1 and the Rac1 effector kinase Cla4. The active Rac1 is subsequently inactivated by GAPs that localize in a ring-like fashion underneath of the tip. Both destruction of Cdc24 and inactivation of Rac1 serve to delimit Rac1 activity to the very tip of the fungal hypha. Sustained polarized growth is further supported by recycling of inactive Rac1 to the hyphal tip. This is achieved either by interaction with the Rho protein GDP dissociation inhibitor Gdi1 or via endocytosis. Active Rho-GTPases recruit specific effectors that trigger the localized fusion of secretory vesicles at the hyphal tip. We found that both Cdc42 and Rac1 interact with Sec3, a subunit of the multiprotein exocyst complex. We have identified several homologs of exocyst subunits in *U. maydis* and tested them for functions during polar growth. We could demonstrate that Rac1 is critical for proper localization of the exocyst landmark protein Sec3. We have identified the *U. maydis* homolog of Smg-GDS, an unconventional activator of Rho GTPases in mammals. Smg-GDS contains a number of armadillo repeats and interacts with both Cdc42 and Rac1. Deletion of the Smg-GDS gene reduces significantly mating and filament formation, indicating that it contributes to regulation of cell polarity.


We present a quantitative three dimensional treatment of fungal hyphal growth which adapts previous theoretical treatments in the light of advances in our knowledge of the components of polarised growth and their location as revealed by GFP fusions. The model is based on the proposition that vesicle fusion with the hyphal tip at a rate determined by the experimentally observable local density of exocyst components. Enzymes such as β,1,3 glucan synthase are embedded in the plasma membrane by this process and continue to synthesize cell wall until they are removed from the membrane by endocytosis. The time development of the spatial distribution of the syntheses molecules arises from the model. We test the model in the hyphae of the human fungal pathogen *Candida albicans* by quantitative measurements of the distribution of exocyst components and membrane components such as GFP-Rho1, the regulatory subunit of β,1,3 glucan synthase, Rom2-GFP, the GEF for Rho1, and the location of actin cortical patches. We show that the predicted shape and width of the hyphae are in good agreement with that predicted by the model, provided that endocytosis acts to remove cell wall synthesizing enzymes at the subapical band of cortical actin patches. Thus the pattern of tip growth of fungal hyphae can be satisfactorily explained by a simple but quantitative model rooted within the known molecular processes of polarized growth. At the same time the model exposes the areas of uncertainty which need to be addressed by future experimentation.

Cell wall integrity signaling in *Aspergillus fumigatus*. Johannes Wagener, Karl Dichtl, Christoph Helmschrott, Sweta Samantaray, Franziska Dirr, Michael Neubauer. Max von Pettenkofer-Institut, University of Munich, Munich, Germany.

*Aspergillus fumigatus* is an opportunistic pathogen and the most frequent cause of a severe invasive infection termed invasive aspergillosis. Similar to other fungi, this mold is surrounded by a robust cell wall that defines its shape and protects it from physical stress. We have characterized the cell wall integrity (CWI) pathway of *A. fumigatus*. It comprises at least three major membrane anchored cell wall stress sensors with partially overlapping functions (Wsc1, Wsc3 and Midd), the guanine nucleotide exchange factor Rom2, a Rho GTPase, protein kinase C and a MAP kinase signaling module. We have shown that the principal CWI components are well conserved from yeasts to filamentous fungi. Though, the importance of the individual components for the fungal physiology, e.g., cell polarity and conidiation, may significantly differ. Our data stress the importance of the CWI pathway for the antifungal drug susceptibility and virulence of this pathogen.

Optimization of polarity establishment through coupling of multiple feedback loops. Roland Wedlich-Soldner1, Tina Freisinger1, Ben Klunder2, Nikola Mueller2, Gisela Beck2, Garwin Pichler1, Jared Johnson1, Richard Gerion1, Erwin Frey2. 1) Cellular Dynamics adn Cell Patterning, Max Planck Institute of Biochemistry, Martinsried, Germany; 2) Arnold Sommerfeld Center for Theoretical Physics, Ludwig Maximilians University Munich, Munich, Germany; 3) Department of Chemistry and Chemical Biology, Baker Laboratory, Cornell University, Ithaca, NY, USA; 4) Department of Biology II, Ludwig Maximilians University Munich, Martinsried, Germany.

Establishment of cell polarity - or symmetry breaking - relies on local accumulation of polarity regulators. While simple positive feedback is sufficient to drive symmetry breaking, it is highly sensitive to stochastic fluctuations typical for living cells. By integrating mathematical modeling with quantitative experimental validations we now show that in the yeast Saccharomyces cerevisiae only a combination of actin- and Guanine nucleotide Dissociation Inhibitor (GDI)-dependent recycling of the central polarity regulator Cdc42 is capable of establishing robust cell polarity at a single site during yeast budding. The GDI pathway consistently generates a single polarization site, but requires Cdc42 to cycle rapidly between its active and inactive form, and is therefore highly sensitive to perturbations of the GTPase cycle. Conversely, actin-mediated recycling of Cdc42 induces robust symmetry breaking but...
**CONCURRENT SESSION ABSTRACTS**

**Cell wall structure and biosynthesis in oomycetes and true fungi: a comparative analysis.** Vincent Bulone. Sch Biotech, Royal Inst Biotech (KTH), Stockholm, Sweden.

Cell wall polysaccharides play a central role in vital processes like the morphogenesis and growth of eukaryotic micro-organisms. Thus, the enzymes responsible for their biosynthesis represent potential targets of drugs that can be used to control diseases provoked by pathogenic species. One of the most important features that distinguish oomycetes from true fungi is their specific cell wall composition. The cell wall of oomycetes essentially consists of (1®3)-b-glucans, (1®6)-b-glucans and cellulose whereas chitin, a key cell wall component of fungi, occurs in minute amounts in the walls of some oomycete species only. Thus, the cell walls of oomycetes share structural features with both plants [cellulose; (1®3)-b-glucans] and true fungi [(1®3)-b-glucans, (1®6)-b-glucans and chitin in some cases]. However, as opposed to the fungal and plant carbohydrate synthases, the oomycete enzymes exhibit specific domain compositions that may reflect polyfunctionality. In addition to summarizing the major structural differences between oomycete and fungal cell walls, this presentation will compare the specific properties of the oomycete carbohydrate synthases with the properties of their fungal and plant counterparts, with particular emphasis on chitin, cellulose and (1®3)-b-glucan synthases. The significance of the association of these carbohydrate synthases with membrane microdomains similar to lipid rafts in animal cells will be discussed. In addition, distinguishing structural features within the oomycete class will be highlighted with the description of our recent classification of oomycete cell walls in three different major types. Genomic and proteomic analyses of selected oomycete and fungal species will be correlated with their cell wall structural features and the corresponding biosynthetic pathways.

**Cellular morphogenesis of Aspergillus nidulans conidiophores: a systematic survey of protein kinase and phosphatase function.** Lakshmi Preethi Yerra, Steven Harris. University of Nebraska-Lincoln, Lincoln, NE.

In the filamentous fungus Aspergillus nidulans, the transition from hyphal growth to asexual development is associated with dramatic changes in patterns of cellular morphogenesis and division. These changes enable the formation of airborne conidiophores that culminate in chains of spores generated by repeated budding of phialides. Our objective is to characterize the regulatory modules that mediate these changes and to determine how they are integrated with the well-characterized network of transcription factors that regulate conidiation in A. nidulans. Because protein phosphorylation is likely to be a key component of these regulatory modules, we have exploited the availability of A. nidulans post-genomic resources to investigate the roles of protein kinases and phosphatases in developmental morphogenesis. We have used the protein kinase and phosphatase deletion mutant libraries made available by the Fungal Genetics Stock Center to systematically screen for defects in conidiophore morphology and division patterns. Our initial results implicate ANID_11101.1 (=yeast Hsl1/Gin4) in phialide morphogenesis, and also reveal the importance of ANID_07104.1 (=yeast Yak1) in the maintenance of cell integrity during asexual development. Additional deletion mutants with reproducible defects have been identified and will be described in detail. We will also summarize initial results from double mutant analyses that attempt to place specific protein kinase deletions within the regulatory network that controls conidiation.

**Septum formation starts with the establishment of a septal actin tangle (SAT) at future septation sites.** Diego Delgado-Álvarez¹, S. Seiler², S. Bartnicki-García³, R. Mourriño-Pérez⁴. 1) CICESE, Ensenada, Mexico; 2) Georg August University, Göttingen, Germany.

The machinery responsible for cytokinesis and septum formation is well conserved among eukaryotes. Its main components are actin and myosins, which form a contractile actomyosin ring (CAR). The constriction of the CAR is coupled to the centripetal growth of plasma membrane and deposition of cell wall. In filamentous fungi, such as Neurospora crassa, cytokinesis in vegetative hyphae is incomplete and results in the formation of a centrally perforated septum. We have followed the molecular events that precede formation of septa and constructed a timeline that shows that a tangle of actin filaments is the first element to conspicuously localize at future septation sites. We named this structure the SAT for septal actin tangle. SAT formation seems to be the first event in CAR formation and precedes the recruitment of the anillin Bud-4, and the formin Bni-1, known to be essential for septum formation. During the transition from SAT to CAR, tropomyosin is recruited to the actin cables. Constriction of the CAR occurs simultaneously with membrane internalization and synthesis of the septal cell wall.

**Visualization of apical membrane domains in Aspergillus nidulans by Photoactivated Localization Microscopy (PALM).** Norio Takeshita¹, Yuji Ishitsuka², Yiming Li³, Ulrich Nienhaus⁴, Reinhard Fischer⁵. 1) Dept. of Microbiology, Karlsruhe Institute of Technology, Karlsruhe, Germany; 2) Institute for Applied Physics, Karlsruhe Institute of Technology.

Apical sterol-rich plasma membrane domains (SRDs), which can be viewed using the sterol-binding fluorescent dye filipin, are gaining attention for their important roles in polarized growth of filamentous fungi. The size of SRDs is around a few mm, whereas the size of lipid rafts ranges in general between 10-200 nm. In recent years, super-resolution microscope techniques have been improving and breaking the diffraction limit of conventional light microscopy whose resolution limit is 250 nm. In this method, a lateral image resolution as high as 20 nm will be a powerful tool to investigate membrane microdomains. To investigate deeply the relation of lipid membrane domains and protein localization, the distribution of microdomains in SRDs were analyzed by super-resolution microscope technique, Photoactivated Localization Microscopy (PALM). Membrane domains were visualized by each marker protein tagged with photoconvertible fluorescent protein mEosFP for PALM. Size, number, distribution and dynamics of membrane domains, and dynamics of single molecules were investigated. Time-laps analysis revealed the dynamic behavior of exocytosis.
**CONCURRENT SESSION ABSTRACTS**

**Friday, March 15  3:00 PM–6:00 PM**

**Heather**

**Sexual Regulation and Evolution in the Fungi**

Co-chairs: Frances Trail and Nicolas Corradi

**Clonality and sex impact aflatoxigenicity in Aspergillus populations.** Ignacio Carbone\(^1\), Bruce W. Horn\(^2\), Rodrigo A. Olarte\(^3\), Geromy G. Moore\(^3\), Carolyn J. Worthington\(^4\), James T. Monace\(^5\), Rakhi Singh\(^4\), Eric A. Stone\(^6\,\(^7\), Kerstin Heil\(^8\), Sofia N. Chulze\(^9\), German Barros\(^4\), Graeme Wright\(^4\), Manjunath K. Naik\(^5\).

\(^1\) Department of Plant Pathology, NC State University, Raleigh, NC, USA; \(^2\) National Peanut Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Dawson, GA, USA; \(^3\) Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, LA, USA; \(^4\) Bioinformatics Research Center, NC State University, Raleigh, NC, USA; \(^5\) Department of Genetics, NC State University, Raleigh, NC, USA; \(^6\) International Institute of Tropical Agriculture, Cotonou, Republic of Benin; \(^7\) Departamento de Microbiología e Immunología, Universidad Nacional de Rio Cuarto, Cordoba, Argentina; \(^8\) Department of Primary Industries, Queensland, Kingaroy, Australia; \(^9\) Department of Plant Pathology, College of Agriculture, Karnataka, India.

Species in Aspergillus section *Flavi* commonly infect agricultural staples such as corn, peanuts, cottonseed, and tree nuts and produce an array of mycotoxins, the most potent of which are aflatoxins. *Aspergillus flavus* is the dominant aflatoxin-producing species in the majority of crops. Populations of aflatoxin-producing fungi may shift in response to: (1) clonal amplification that results from strong directional selection acting on a nontoxin- or toxin-producing trait; (2) disruptive selection that maintains a balance of extreme toxigenicities and diverse mycotoxin profiles; (3) sexual reproduction that results in continuous distributions of toxigenicity; and (4) female fertility/sterility that impacts the frequency of sexual reproduction. Population shifts that result in changes in ploidy or nuclear DNA composition (homokaryotic versus heterokaryotic) may have immediate effects on fitness and the rate of adaptation in subsequent fungal generations. We found that *A. flavus* populations with regular rounds of sexual reproduction maintain higher aflatoxin concentrations than predominantly clonal populations and that the frequency of mating-type genes is directly correlated with the magnitude of recombination in the aflatoxin gene cluster. Genetic exchange within the aflatoxin gene cluster occurs via crossing over between divergent lineages in populations and between closely related species. During adaptation, specific toxin genotypes may be favored and swept to fixation or be subjected to drift and frequency-dependent selection in nature. Results from mating experiments in the laboratory indicate that fertility differences among lineages may be driving genetic and functional diversity. Differences in fertility may be the result of female sterility, changes in heterokaryotic state, DNA methylation, or other epigenetic modifications. The extent to which these processes influence aflatoxinanlash: is largely unknown, but is critical to understand for both fundamental and practical applications, such as biological control. Our work shows that a combination of population genetic processes, especially asexual/sexual reproduction and fertility differences coupled with ecological factors, may influence aflatoxin anlash: in these agriculturally important fungi.

**Toolkit for sexual reproduction in the genome of Glomus spp; a supposedly ancient asexual lineage.** Nicolas Corradi. Department of Biology, University of Ottawa, Ottawa, Ontario, Canada.

Arbuscular mycorrhizal fungi (AMF) are involved in a critical symbiosis with the roots of most land plants; the mycorrhizal symbiosis. Despite their importance for terrestrial ecosystems worldwide, many aspects of AMF evolution and genetics are still poorly understood, resulting in notorious scientific frustrations and intense debates; especially regarding the genetic structure of their nuclei (heterokaryosis vs homokaryosis) and their mode of propagation (long-term clonality vs cryptic sexuality). This will aim address the latter aspect of their biology - i.e. their mode of reproduction - by cataloguing and highlighting emerging evidence, based on available genome sequence data, for the presence of a cryptic sexual cycle in the AMF. In particular, investigations along available genome and transcriptome data from several AMF species have unravelled the presence of a battery of genes that are commonly linked with sexually-related processes in other fungal phyla. These include a gene-set required for the initiation and completion of a conventional meiosis, as well as many other genomic regions that are otherwise found to play a pivotal role in fungal partner recognition. The origin, diversity and functional analysis of some of these sexually-related genes in AMF will be discussed.

**Comparative transcriptomics identifies new genes for perithecium development.** Frances Trail\(^1\), Usha Sikhakolli\(^1\), Kayla Fellows\(^1\), Nina Lehr\(^2\), Jeffrey Townsend\(^3\).

In recent years, a plethora of genomic sequences have been released for fungal species, accompanied by functional predictions for genes based on protein sequence comparisons. However, identification of genes involved in particular processes has been extremely slow, and new methodologies for identifying genes involved in a particular process have not kept pace with the exponential increase in genome sequence availability. We have performed transcriptional profiling of five species of Neurospora and Fusarium during six stages of perithecium development. We estimated the ancestral transcriptional shifts during the developmental process among the species and identified genes whose transcription had substantially and significantly shifted during the evolutionary process. We then examined phenotypes of knockouts of genes whose expression greatly increased in *Fusarium graminearum* perithecium development. In numerous cases, gene disruption resulted in substantial changes in perithecium. These genes were not previously identified as candidates for function in perithecium development, illustrating the utility of this method for identification of genes associated with specific functional processes.

In animals and plants, sex-biased gene expression plays a major role in gene evolution. In particular, reproductive genes with male-biased expression tend to exhibit rapid protein evolution and reduced codon bias as compared to female-biased or unbiased genes. Minimal data are available for fungi. Here, we demonstrate that sex-biased expression is associated with gene evolution in the filamentous fungus *Neurospora crassa*, but in contrast to animals and plants, the rapid evolution occurs for female-biased genes. Based on analyses of >25,000 expressed sequence tags (ESTs) from male (conidial), female (protophthallic) and vegetative (mycelial) tissues, we show that reproductive genes with female-biased expression exhibit faster protein evolution and reduced optimal codon usage than male-biased genes and vegetative genes. Furthermore, our data suggest that female-biased genes are also more apt to experience selective sweeps. The sex-biased expression effects are observable at the species and population level. We argue that the rapid molecular evolution of female-biased genes is best explained by sexual selection via female-female competition, but could also result from mate-choice and/or directional natural selection.

**Self-attraction can not bypass the requirement for two mating type genes during sexual reproduction in *Neurospora crassa*.** Katherine A. Borkovich, Hyojeong Kim, Sara Wright, Gyungsook Park, Shouqiang Ouyang, Svetlana Krystolfova, Plant Pathology and Microbiology, University of California, Riverside, Riverside, CA.

The pheromone receptor PRE-2 is highly expressed in male and female reproductive structures of mat a strains in *Neurospora crassa*. Trichogynes from Dpre-2 mat a protoperithecia do not respond chemotropically to mat A conidia or form mature fruiting bodies or meiotic progeny. Strains with swapped identity due to heterologous expression of pre-2 or its cognate pheromone ccc-4 behave normally in crosses with opposite mating-type. Coexpression of pre-2 and ccc-4 in the mat A background leads to self-attraction and development of barren perithecia that lack ascospores. Further perithecial development is achieved by inactivation of Sad-1, a gene required for meiotic gene silencing in *N. crassa*. Results from studies using forced heterokaryons of opposite mating-type strains show that the presence of one receptor and its compatible pheromone is necessary and sufficient for perithecial development and ascospore production. Taken together, the results demonstrate that although receptors and pheromones control sexual identity, the mating-type genes (mat A and mat a) must be in two different nuclei to allow meiosis and sexual sporulation to occur in *N. crassa*.

**Fertility in *Aspergillus fumigatus* and the identification of an additional ‘supermater’ pair.** Céline M. O’Gorman1, Sameea S. Swilaiman1, Janyce A. Sugui2, Kyung J. Kwon-Chung3, Paul S. Dyer1. 1) School of Biology, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom; 2) Molecular Microbiology Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA.

*Aspergillus fumigatus* is an opportunistic human pathogen that causes a range of allergic and invasive diseases in severely immunocompromised individuals, with a very high mortality rate typically in excess of 50%. A functional sexual cycle was discovered in 2009 and a highly fertile ‘supermater’ pair, AFB62 and AFR928, was later identified from a collection of 50 isolates. Here we describe the results of a larger, worldwide fertility screen and present an additional ‘supermater’ pair. A set of 126 clinical and environmental *A. fumigatus* isolates were crossed against two Irish reference strains of each mating type. A subset of the eight most-fertile strains was then tested in all pairwise combinations. The pairing of isolates 47-169 x 47-154 had consistently high mating efficiency and outcrossing ability after four weeks, therefore it was chosen as an additional ‘supermater’ pair for community use in mating projects. It is important to have alternative tester strains to allow for unexpected mating differences when crossing isolates of diverse genetic origins. This is because factors such as heterokaryon incompatibility (het) loci and single nucleotide polymorphisms, can considerably influence sexual compatibility. The worldwide fertility screen found that approximately 85% of isolates are sexually fertile, indicating that sexual reproduction should be possible in nature when suitable environments are present. Next, the plasticity of sexual crossing conditions was tested, to determine whether they could be manipulated to increase fertility in crosses involving low-fertility strains of interest. A range of environmental and growth conditions were examined, including incubation temperature, CO2 level, and oatmeal agar type. Fertility levels were significantly affected by certain parameters. Work is ongoing to integrate these factors to further optimize fertility in the ‘supermater’ pairs.

**Sexual reproduction and mating type function in the penicillin producing fungus *Penicillium chrysogenum*.** Julia Böhme1, Birgit Hoff2, Simon Wolfers1, Céline O’Gorman2, Paul Dyer1, Stefanie Pöggeler1, Ulrich Kück1. 1) Christian Doppler Laboratory for Fungal Biotechnology, Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum, Deutschland; 2) School of Biology, University of Nottingham, University Park, Nottingham, NG7 2RD, U.K; 3) Abteilung Genetik eukaryotischer Mikroorganismen, Institut für Mikrobiologie und Genetik, Georg-August Universität Göttingen, 37077 Göttingen, Deutschland.

*Penicillium chrysogenum* is a filamentous fungus of major medical and historical importance, being the original and present day industrial source of the antibiotic penicillin with a world market value of about 600 million € per year. The species has been considered asexual for over 100 years and despite concerted efforts it has not been possible to induce sexual reproduction. However, we recently were able to detect mating type loci in different strains, indicating a sexual lifecycle. Isolates, carrying opposite mating types, were found in near-equal proportion in nature and we observed transcriptional expression of mating type loci as well as pheromone and pheromone receptor genes [1]. Utilising knowledge of mating-type (MAT) gene organization we now describe conditions under which a sexual cycle can be induced leading to the production of cleistothecia and meiotic ascospores, which were similar to those described recently for *Eupenicillium crustaceum* [2]. Evidence of recombination was obtained using both molecular and phenotypic markers. The newly identified heterothallic sexual cycle was used for strain development purposes, generating offspring with novel combinations of traits relevant to penicillin production.

Furthermore, the MAT1-1-1 mating-type gene, known primarily for a role in governing sexual identity, was also found to control transcription of a wide range of genes including those regulating penicillin production, hyphal morphology and conidial formation, all traits of biotechnological relevance. For functional characterization MAT1-1-1 knockout and overexpression strains were generated and analyzed. These discoveries of a sexual cycle and MAT gene function are likely to be of broad relevance for manipulation of other asexual fungi of economic importance.

The \textit{Sclerotinia sclerotiorum} mating type locus (MAT) contains a 3.6-kb region that is inverted in every generation. Patrik Inderbitzin\textsuperscript{1}, Periasamy Chitrampalam\textsuperscript{2}, Karunakaran Maruthachalam\textsuperscript{2}, Bo-Ming Wu\textsuperscript{3}, Krishna Subbarao\textsuperscript{1}. \textsuperscript{1) Department of Plant Pathology, University of California-Davis, Davis, CA, USA; \textsuperscript{2) Department of Plant Sciences, University of Arizona, Tucson, AZ, USA; \textsuperscript{3) Department of Plant Pathology, China Agricultural University, 2 West Yuanmingyuan Rd., Haidian District, Beijing, China.}

\textit{Sclerotinia sclerotiorum} is a filamentous ascomycete in the \textit{Sclerotiniaceae} (Pezizomycotina) and a necrotrophic pathogen of more than 400 hosts worldwide, including many important agricultural crops. In California, the biggest lettuce producer in the United States, \textit{S. sclerotiorum} is a causal agent of lettuce drop that reduces overall annual lettuce yield by 15%. Little is known about the details of sexual reproduction in \textit{S. sclerotiorum}, but the structure of the \textit{S. sclerotiorum} mating type locus \textit{MAT}, the master regulator of sexual reproduction in ascomycetes, has previously been reported. As in other homothallic (self-fertile) ascomycetes, \textit{S. sclerotiorum} \textit{MAT} contains both idiomorphs (divergent alleles) fused end-to-end at a single locus. Using 283 isolates from lettuce in California and from other states and hosts, we investigated the diversity of \textit{S. sclerotiorum} \textit{MAT}, and identified a novel version of \textit{MAT} that differed by a 3.6-kb inversion and was designated Inv+, as opposed to the previously known \textit{S. sclerotiorum} \textit{MAT} that lacked the inversion and was Inv-. The inversion affected three of the four \textit{MAT} genes: \textit{MAT1-2-1} and \textit{MAT1-2-4} were inverted and \textit{MAT1-1-1} was truncated at the 3\textsuperscript{\textprime} end.

Expression of \textit{MAT} genes differed between Inv+ and Inv- isolates. In Inv+ isolates, only one of the three \textit{MAT1-2-1} transcript variants of Inv- isolates was detected, and the alpha1 domain of Inv+ \textit{MAT1-1-1} transcripts was truncated. Both Inv- and Inv+ isolates were self-fertile, and the inversion segregated in a 1:1 ratio regardless of whether the parent was Inv- or Inv+. This suggested the involvement of a highly regulated process in maintaining equal proportions of Inv- and Inv+, likely associated with the sexual state. The MAT inversion region, defined as the 3.6-kb \textit{MAT} inversion in Inv+ isolates and the homologous region of Inv- isolates, was flanked by a 250-bp inverted repeat on either side. The 250-bp inverted repeat was a partial \textit{MAT1-1-1} that through mediation of loop formation and crossing over, may be involved in the inversion process. Inv+ isolates were widespread, and in California and Nebraska constituted half of the isolates examined. We speculate that a similar inversion region may be involved in mating type switching in the filamentous ascomycetes \textit{Chromocrea spinulosa}, \textit{Sclerotinia trifoliorum} and in certain \textit{Ceratocystis} species.

The ability of Candida albicans to colonize or invade multiple host environments requires that it rapidly adapts to different conditions. Our group has been exploiting ChIP-chip and transcription profiling technologies, together with computer modeling, to provide a better understanding of select transcription factor (TF) networks. We used DNA microarrays to measure the changes in transcriptional profiles that occur immediately following the transfer of C. albicans to hypoxic growth conditions. The impressive speed of this response is not compatible with current models of fungal adaptation to hypoxia that depend on the inhibition of sterol and heme biosynthesis. Functional interpretation of these profiles was achieved using Gene Set Enrichment Analysis, a method that determines whether defined groups of genes exhibit a statistically significant bias in their distribution within a ranked gene list. The Sit4p phosphatase, Ccr4p mRNA decaylase and Sko1p TF were identified as novel regulators of the early hypoxic response. While cells mutated in these regulators exhibit a delay in their transcriptional responses to hypoxia their ability to grow in the absence of oxygen is not impeded. Promoter occupancy data on 26 TFs was combined with the profiles of 375 significantly-modulated target genes in a Network Component Analysis (NCA) to produce a model of the dynamic and highly interconnected TF network that controls this process. The NCA also allowed us to observe correlations between temporal changes in TF activities and the expression of their respective genes, thus allowing us to identify which TFs are potentially subjected to post-transcriptional modifications. The TF network is centered on Tye7p and Upc2p which are associated with many of the genes that exhibit the fastest and strongest up-regulations. While Upc2p only associates with downstream promoters, Tye7p is acting as a hub, its own promoter being bound by itself and 7 additional TFs. Rap1 and Ahr1 appear to function as master regulators since they bind to a greater proportion of TF gene promoters. However, the abundance of enzymes of the pentose-phosphate shunt pathway decreased, whereas enzymes involved in ethanol fermentation significantly increased. On the other hand, thioredoxin and other redox proteins involved in the regulation of the transcription factor Yap1p mediates response to oxidative stress via nuclear re-localization and activation of genes coding for detoxification enzymes. In this study, we investigate the role of Yap1p homolog in F. graminearum, Fgap1, in response to oxidative stress and its eventual role in the regulation of trichothecene production. A deleted mutant and a strain expressing a constitutively activated form of the Fgap1 factor in F. graminearum were constructed. We cultured these mutants in GYEP liquid medium supplemented with H2O2 to evaluate their sensitivity to oxidative stress and analyse their toxin production. The nuclear localization of constitutively activated Fgap1p under oxidative stress by H2O2 was analyzed. Expression profiles of genes encoding oxidative stress response enzymes potentially controlled by Fgap1p and of genes involved in the biosynthesis of type B trichothecenes were analyzed by Q-RT-PCR. Trichothecene accumulation is strongly enhanced in the deleted strain, with an increase in Tri genes expression. On the other hand, Tri genes expression and toxin accumulation are drastically repressed in the mutant in which Fgap1p is constitutively activated. Moreover, the level of expression of two genes encoding catalases is modulated in both mutants. The involvement of Fgap1p in other types of stress has also been investigated. In particular, cadmium and osmotic stress affect growth in the deleted strain.
The role of NADPH oxidases in *Neurospora crassa* cell fusion. Naielly Cano-Dominguez1, Ernestina Casto-Longoria1, Jesus Aguirre2. 1) Departamento de Microbiologia, CICESE, Ensenada, Baja California, Mexico; 2) Departamento de Biologia Celular y Desarrollo. Instituto de Fisiologia Celular UNAM, Mexico City, D.F. Mexico.

Hansberg and Aguirre proposed that reactive oxygen species (ROS) play essential roles in cell differentiation in microorganisms. ROS are generated mainly during mitochondrial electron transport and by the action of certain enzymes. The NADPH oxidases (NOX) are enzymes that catalyze the production of superoxide by transferring electrons from NADPH to oxygen. *Neurospora crassa* contains the NADPH oxidases NOX-1 and NOX-2 and a common regulatory subunit NOR-1. NOX-1 is essential for ascospore germination, while NOX-1 is required for sexual and asexual development, polar growth and cell fusion. NOR-1 is essential for all these NOX functions. We have found that a functional NOR-1::GFP fusion is localized throughout the cytoplasm, enriched at the hyphal tip and sometimes in aggregates. This suggests that the functional NOX complexes are probably not localized at the plasma membrane. Up to now NOX function in fungi has been evaluated in mutants that completely lack NOX proteins. We generated nox-1 alleles that result in NOX-1 proteins carrying substitutions of proline 362 by histidine or cysteine 542 by arginine, which affect NADPH-binding. Equivalent mutations in phagocytic Nox2/gp91phox do not affect protein stability but completely lack oxidase activity. P382H and C542R mutants did not produce sexual fruiting bodies and showed a decreased growth and differentiation of aerial mycelia, without affecting production of conidia. These results indicate that sexual development depends on ROS production by NOX-1, whereas during asexual differentiation NOX-1 plays an important role independently of its catalytic activity. Dnox-1, Dnor-1, P382H NOX-1 and CS42R NOX-1 mutants were all able to produce some conidial anastomosis tubes (CATs) but they were unable to complete cell-cell fusion. All these mutants are also impaired in vegetative hyphae-hyphae fusion, which might explain the growth defects in Dnox-1 and Dnor-1 strains. CATs production is delayed in the presence of antioxidant N-acetyl cystein (NAC) and Dsod-1 strains show an increase in CATs fusions. The results suggest that some ROS may be implicated in signaling CATs homing and vegetative fusion.


Peroxidoxins (Ppx) are extremely abundant antioxidant enzymes with important roles in protecting against oxidative stress, ageing and cancer. The thioredoxin peroxidase activity of eukaryotic typical 2-Cys Prx detoxifies hydrogen peroxide but, enigmatically, is highly sensitive to inactivation by peroxide-induced hyperoxidation of a catalytic cysteine residue. It has been proposed that hyperoxidation might allow hydrogen peroxide to act as a signal and/or promote an alternative activity of Prx as a chaperone [1, 2]. However, any advantage to be gained by inhibiting the thioredoxin peroxidase activity and preventing Prx from removing peroxides under oxidative stress conditions has remained obscure. The fission yeast Schizosaccharomyces pombe contains a single 2-Cys Prx, Tpx1. Our previous work has established that, counterintuitively, Tpx1 is vital for adaptive transcriptional responses to hydrogen peroxide due to essential roles in the hydrogen peroxide-induced activation of the p38/INK/Hog1-related MAPK Styl and AP-1-like transcription factor Pap1 [3, 4]. In seeking to understand why the thioredoxin peroxidase activity of Tpx1 should be important for Pap1 activation, we have identified that Tpx1 is the major cellular substrate for thioredoxin. Accordingly, in hydrogen peroxide-treated cells, Tpx1 competitively inhibits the activity of thioredoxin towards other substrates, including Pap1, and the methionine sulphoxide reductase A, Mxr1. Consequently, we show that the oxidative inactivation of the thioredoxin peroxidase activity of Tpx1 is important to maintain active Mxr1, repair oxidative protein damage and maintain cell viability following exposure to toxic levels of hydrogen peroxide [5]. Based on these discoveries in yeast, we propose that an important function for the reversible hyperoxidation of eukaryotic 2-Cys Prx is to regulate thioredoxin and thus thioredoxin-mediated signalling and repair processes. I will present further data supporting this conclusion and discuss its implications for hydrogen peroxide signal transduction.

NADPH oxidases regulate septin-mediated cytoskeletal re-modeling during plant infection by the rice blast fungus Magnaporthe oryzae. Lauren S. Ryder1, Yasin F. Dagdas1, Thomas A. Mentlak1, Michael J Kershaw1, Martin Schuster1, Christopher R Thornton1, Jisheng Chen2, Zonghua Wang2, Nicholas J Talbot1. 1) Dept Biosciences, Univ Exeter, Exeter, United Kingdom; 2) Fujian agricultural university.

NADPH oxidases (Nox) are flavoenzymes that function by transferring electrons across biological membranes to catalyze reduction of molecular oxygen to superoxide. In animal cells, Nox enzymes are implicated in cell proliferation, cell signalling and apoptosis, while in plants Nox are necessary for programmed cell death, the response to environmental stresses, pathogen infection, and polarized growth of root hairs. In filamentous fungi, Nox are necessary for cell differentiation during sexual reproduction and for developmental processes that involve transitions from non-polarized to polarized cell growth, such as tissue invasion by mutualistic and pathogenic fungi, and fungal virulence. The underlying function of Nox enzymes in these diverse developmental processes remains unclear. The rice blast fungus *Magnaporthe oryzae* infects plants with a specialized cell called an appressorium, which uses turgor to drive a rigid penetration peg through the rice leaf cuticle. Here, we show that NADPH oxidases (Nox) are necessary for septin-mediated re-organization of the dynamic F-actin cytoskeleton to facilitate cuticle rupture and plant cell invasion. We report that the Nox2-NoxR complex spatially organises a heterologomeric septin ring at the appressorium pole, required for assembly of a toroidal F-actin network at the point of penetration peg emergence. Maintenance of the cortical F-actin network during plant infection independently requires Nox1, a second NADPH oxidase, which is necessary for penetration hypha elongation. Organisation of F-actin and septins in appressoria are disrupted by application of anti-oxidants, while latrunculin-mediated depolimerisation of appressorial F-actin is competitively inhibited by reactive oxygen species (ROS), providing evidence that regulated synthesis of ROS by fungal NADPH oxidases directly controls septin and F-actin dynamics.
CONCURRENT SESSION ABSTRACTS

Redox regulation of an AP-1-like transcription factor, YapA, in the fungal symbiont Epichloë festucae. Gemma M. Cartwright, Barry Scott, Yvonne Becker. Molec Biosci, Massey Univ, Palmerston Nth, New Zealand. Reactive oxygen species (ROS) are emerging as important regulators required for the successful establishment and maintenance of the mutualistic association between the fungal endophyte Epichloë festucae and its grass host Lolium perenne. The generation of reactive oxygen species (ROS) by the fungal NADPH oxidase, NoxA has previously been shown to regulate hyphal growth of E. festucae in planta; a result that has led to the hypothesis that fungal-produced ROS are key second messengers in the symbiosis. However, the highly reactive nature of these molecules dictates that cells possess efficient sensing mechanisms to maintain ROS homeostasis and prevent oxidative damage to cellular components. The Saccharomyces cerevisiae Gpx3-Yap1 and Schizosaccharomyces pombe Tpx1-2, two-component H2O2 sensors, serve as model redox relays for coordinating the cellular response to ROS. While proteins related to the Yap1 and Pap1 basic-leucine zipper (bZIP) transcription factors have been identified in a number of filamentous fungi, the components involved in the upstream regulation remain unclear. This study investigated the role of the E. festucae Yap1 homologue, YapA, and putative upstream activators GpxC and TpxA, homologues of Gpx3 and Tpx1, respectively, in responding to ROS. YapA is involved in responding to ROS generated at the wound site following inoculation into ryegrass seedlings. However, deletion of yapA did not impair host colonization indicating redundancy in systems used by E. festucae to sense and respond to plant-produced ROS. In culture, deletion of E. festucae yapA, renders the mutants sensitive to only a subset of ROS and this sensitivity is influenced by the stage of fungal development. In contrast to the H2O2-sensitive phenotype widely reported for fungi lacking the Yap1-like protein, the E. festucae yapA mutant maintains wild-type mycelial resistance to H2O2, but conidia of the yapA mutant are very sensitive to H2O2. Using a degron-tagged GFP-CL1 as a reporter, we found YapA is required for the expression of the spore specific catalase, cata. Moreover, YapA is activated by H2O2 independently of both GpxC and TpxA, suggesting a novel mechanism of regulation exists in E. festucae. This work provides a comprehensive analysis of the role and regulation of the AP-1 transcription factor pathway in a filamentous fungal species.

Interaction between phenolic and oxidant signaling in Cochliobolus heterostrophus. Benjamin A Horwitz1, Samer Shalaby1, Olga Larkov1, Mordechai Ronen2, Sophie Lev2. 1) Department of Biology, Technion - IIT, Haifa, Israel; 2) Department of Plant Science, Tel Aviv University, Ramat Aviv, Israel; 3) Centre for Infectious Diseases and Microbiology, University of Sydney at Westmead Hospital, Westmead, NSW 2145, Australia. The transcription factor ChAP1 is an ortholog of yeast YAP1 in the maize pathogen Cochliobolus heterostrophus. ChAP1 migrates to the nucleus upon exposure to oxidative stress, inducing antioxidant genes such as thioredoxin and glutathione reductase [1]. ChAP1 also localizes to nuclei on contact with the leaf and during invasive growth. Though reactive oxygen species are encountered on the host, ChAP1 nuclear retention can occur without oxidative stress. One of the signals responsible is provided by phenolic compounds [1-3]. Using a genetically-encoded ratiometric reporter of the redox state, we showed that leaf extract and phenolics, despite their antioxidant properties, promote nuclear accumulation of ChAP1. To study this dual role of ChAP1 we identified genes expressed in response to phenolics. Intradiol dioxygenase CCHD1 is rapidly upregulated, independent of ChAP1 [2]. Coumaric acid caused rapid and simultaneous upregulation of most of the b-ketoadipate pathway genes. Deletion of CCHD1 provided genetic evidence that protocatechuate acid is an intermediate in catabolism of many aromatics [3]. The activity of a structure series showed complementary requirements for upregulation of CCHD1 and ChAP1 nuclear retention. The ability to metabolize a compound and ChAP1 nuclear retention are inversely correlated. To find additional genes induced by phenolics, microarrays designed from the predicted coding sequences of the C. heterostrophus genome [4] were hybridized to probes made from RNA of cultures exposed to coumaric acid, or controls. Expression of about 90 genes from different pathways primarily for metabolism, for example, the b-ketoadipate, quinic acid and shikimic acid pathways, as well as transporters from different families was altered in response to coumaric acid. The ability to respond to phenolics and detoxify or metabolize them via the b-ketoadipate pathway confers an advantage to plant pathogens, and explains the presence of at least two response pathways detecting these compounds. [1] Lev et al. (2005) Eukaryot. Cell 4:443-454; [2] Shanmugam et al. (2010) Cell. Microbiol. 12:1421-1434; [3] Shalaby et al. (2012) MPMI 25: 931-940; [4] Ohm et al. (2012) PLoS Pathog 8: e1003037. Supported in part by the Israel Science Foundation. We thank Michal Levin and Itai Yanai for help with microarray hybridization.
Phylogenomics
Co-chairs: Jason Stajich and Joey Spatafora

Characterizing Gene Tree Incongruence on a Genome Scale. Dannie Durand, Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

Gene families evolve through gene duplication and loss, and lateral gene transfer. Reconstructing these events is a powerful approach to understanding the co-evolution of genes and species and the emergence of novel protein function. Gene duplication, loss, and transfer can all result in a gene tree that disagrees with the species tree. This incongruence can be exploited to infer the history of these events, as well as the ancestral lineage in which each event took place. This is achieved by fitting the gene family tree to the associated species tree, a process called reconciliation. I will discuss the benefits and challenges of gene tree reconciliation, with special attention to genome scale analyses. The use of gene tree reconciliation will be compared with non-phylogenetic analyses of gene family expansion and contraction. The problem of determining whether the observed incongruence is due to gene duplication, lateral transfer, or incomplete lineage sorting will also be discussed. I will present analyses of several large gene tree data sets from well-studied species lineages, as a practical demonstration of this approach. Our algorithms have been implemented in [http://www.cs.cmu.edu/~durand/Notung], a freely available software tool.

Early fungi and their carbohydrate active enzymes. Mary L. Berbee1, Satoshi Sekimoto2, Joseph Spatafora3, Timothy M. Porter2, Rytao Vilgalys1. 1) Dept Botany, Univ British Columbia, Vancouver, B.C., Canada; 2) Department Of Biological Sciences, The University Of Alabama, Tuscaloosa, AL; 3) Oregon State University, Dept of Botany & Plant Pathology, 2082 Cordley Hall, Corvallis, OR.

Early fungi are intermingled with some of the oldest fossils from vascular plants, dated at 400 Ma. However, what the fungi were doing for their nutrition besides land plants were available has been difficult to reconstruct because in phylogenies of the earliest diverging fungal lineages, saprotrophs and parasites of plants as well as animals are intermingled, and which fungal life style came first is ambiguous. We are using phylogenetic analysis of enzymes involved in carbohydrate metabolism to reconstruct the enzymatic capabilities of some of the early terrestrial fungi. Our community sequencing proposal to the US Joint Genome Institute resulted in four new genome sequences for evolutionarily divergent lineages including aquatic fungi, the chytrids and Blastocladiomycota, and zygomycetes. Analysis of the genomes suggests that cellulosases and pectinases to degrade plant wall carbohydrates were already present in the earliest fungal lineages but largely lost from the zygomycetes. This implies that fungi evolved in association with the green algal/green plant lineage. Even with complete genome sequences, the branching order among the aquatic fungi and zygomycetes remains problematical, and branching order conflicts from one analysis to another. The conflicts may reflect difficulties involved in modeling evolutionary processes across lineages. Alternatively, the conflicts may indicate that fungi, like animals, underwent a 'Cambrian Explosion' perhaps facilitated by rapid expansion of nutritional resources offered by radiation of multicellular plants and animals.

Better evolution through gene clustering. Jason Slot2, Matthew Campbell1, Han Zhang1, Martijn Staats2, Jan van Kan4, Antonis Rokas1. 1) Biological Sciences, Vanderbilt University, Nashville, TN; 2) Botany, University of Hawai`i, Manoa, HI; 3) Biosystematics group, Wageningen University, Wageningen, The Netherlands; 4) Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands.

The recent availability of a large number of fungal genomes has facilitated systematic investigations of metabolic pathway evolution across the kingdom. Through combining phylogenetic and genomic techniques, we have recently examined the evolution of metabolic pathways across a well-sampled fungal phylogeny, and gained new insight into the role of metabolic gene clusters in fungal evolution. The occasional occurrence of horizontal gene transfer of entire pathways between distantly related fungi via gene clusters suggests that fungal species have access to larger pan-genomes than previously thought. Furthermore, analysis of gene cluster decay suggests these transfers are underestimated by analyses of single strains, and that evolution within clustered pathways is constrained by natural selection. Increased evolvability in fungi is also implied by the discovery of chromosomal loci that maintain large alternative secondary metabolite gene clusters within recombinating lineages. Together, these phylogenomic analyses in fungi illustrate a multi-faceted role of gene clustering in fungal evolution.

Phylogenomics unveils secondary metabolites specific to mycoparasitic lineages in Hypocreales. C. Alisha Owensby, Kathryn E. Bushley, Joseph W. Spatafora. Botany & Plant Pathology, Oregon State University, Corvallis, OR.

Hypocreales is an order characterized by a dynamic evolutionary history of interkingdom host jumping, with members that parasitize animals, plants, and other fungi. The monophyly of taxa attacking members of the same kingdom is not supported by molecular phylogenetics, however. For example, Trichoderma spp. and Elaphocordycipps spp. are both mycoparasitic, but are members of different families within Hypocreales, Hypocreaceae and Ophiocordycipitaceae, respectively. In fact, both genera are more closely related to insect pathogens, than they are to each other. Multiple species of Trichoderma have sequenced genomes, and recently genomes of several insect pathogens in Hypocreales have been completed (e.g. Metarhizium spp. and Tolyposciadium inflatum). The genus Elaphocordycipps represents a unique clade within Hypocreales, because whereas most species in the family Ophiocordycipitaceae are insect pathogens, most Elaphocordycipps parasite truffles of the ectomycorrhizal genus Elaphomyces [Eurotiales, Ascomycota]. To compare genes of a truffle pathogen with hypocrealean insect pathogens and mycoparasites, we sequenced the genome of Elaphocordycipps ophioglossoides. Our draft assembly of the E. ophioglossoides genome is ~32 MB and has 10,779 gene models, 36 of which are predicted to produce secondary metabolites. We have identified three very large genes in E. ophioglossoides related to peptaibol producing nonribosomal peptide synthetase (NRPS) genes. Peptaibols, which disrupt osmoregulation by forming ion channels through lipid bilayers, have antibiotic and antifungal activity and are best described in Trichoderma spp. E. ophioglossoides and its beetle-pathogenic congener, T. inflatum, both possess three putative peptaibol synthetases which we identified through analysis of NRPS adenylation domains. Of the three peptaibol-specific domain clades, one is predicted to encode for the nonproteinogenic a-aminoisobutyric acid residues. We also show that, despite being very closely related, E. ophioglossoides and T. inflatum each possess
three different peptoid-like genes, only two of which appear to be located in syntenic regions. The current distribution of fungi possessing peptoid-like genes is restricted to mycoparasitic lineages of Hypocreales and is generating hypotheses about the role of secondary metabolites in mycoparasitism.

**Comparative analysis of 35 basidiomycete genomes reveals diversity and uniqueness of the phylum.** Robert Riley1, Asaf Salamov2, Robert Ottillar1, Kirsten Fagnan1, Bastien Boussau1, Daren Brown1, Bernard Henriuss2, Anthony Levesque1, Benjamin Held1, Laszlo Nagy1, Dimitris Floudas1, Emmanuelle Morin1, Gerard Manning1, Scott Baker2, Robert Blanchette2, Francis Martin1, David Hibbert1, Igor Grigoriev1. 1) Joint Genome Institute, Lawrence Berkeley National Lab, Walnut Creek, CA; 2) Clark University, Worcester, MA; 3) UC Berkeley, Berkeley, CA; 4) USDA, Peoria, IL; 5) AFMB, Marseille, France; 6) UMN, St. Paul, MN; 7) INRA, France; 8) Salk Institute, La Jolla, CA; 9) Pacific Northwest National Lab, Richland, WA.

Fungi of the phylum Basidiomycota (basidiomycetes), make up some 37% of the described fungi, and are important in forestry, agriculture, medicine, and bioenergy. This diverse phylum includes symbionts, pathogens, and saprobes including wood decaying fungi. To better understand the diversity of this phylum we compared the genomes of 35 basidiomycete fungi including 6 newly sequenced genomes. The genomes of basidiomycetes span extremes of genome size, gene number, and repeat content. A phylogenetic tree of Basidiomycota was generated using the Phylodog software, which uses all available protein sequence data simultaneously infer gene and species trees. Analysis of core genes reveals that some 48% of basidiomycete proteins are unique to the phylum with nearly half of those (22%) comprising proteins found in only one organism. Phylogenetic patterns of plant biomass-degrading genes suggest a continuum rather than a sharp dichotomy between the white rot and brown rot modes of wood decay among the members of Agaricomycotina. Based on phylogenetically-informed PCA analysis of such profiles, we predict that that Botryobasidium botryosum and Jaapia argillacea have properties similar to white rot species, although neither has lignolytic class II fungal peroxidases. Furthermore, we find that both fungi exhibit wood decay with white rot-like characteristics in growth assays. Analysis of the rate of discovery of proteins with no or few homologs suggests the high value of continued sequencing of basidiomycete fungi.

**Genome evolution of fungal pathogens from the Magnaporthe oryzae/grisea clade.** Helene Chiapello1,2, Ludovic Mallet1,2, Cyprien Guérin1, Gabriela Aguileta3, François Rodolphe4, Annie Gendrault1, Jonathan Kreplak3, Joelle Amsele5, Enrique Ortega-Abboud6, Marc-Henri Lebrun7, Didier Tharreau1, Elisabeth Fournier1. 1) INRA, UR MIG, 78352 Jouy-en-Josas, France; 2) INRA, UR BIA, 31326 Castanet-Tolosan, France; 3) INRA, URGI, 78026 Versailles, France; 4) CRG, Barcelona, Spain; 5) CIRAD, UMR BGPI, TA 54K, 34398 Montpellier, France; 6) INRA, UMR BIOGER, 78850 Thiverval-Grignon, France; 7) INRA, UMR BGPI, TA 54K, 34398 Montpellier, France.

The GEMO project aims at characterizing genomic determinants of pathogenicity and evolutionary events involved in adaptation of 9 isolates from the M. oryzae/grisea clade differing in their host specificity. Eight strains from M. oryzae species complex and one of the related species M. grisea have been sequenced and assembled. De novo structural gene annotation was carried out using Eugene (Schick, 2001) to predict genes and REPET (Flutre, 2011) to annotate Transposable Elements (TES) in these 9 genomes. Four of them exhibited large supplementary genomic regions potentially acquired from an unknown bacterial strain of the Burkholderia genus. An original strategy based on Gotham software (Ménigaud, 2012) was used to accurately quantify these regions in all the affected genome scaffolds. Functional gene annotations were performed using InterProScan. Databases and interfaces relying on the GMD2 tools (gmd2.org) were set up to browse annotations and facilitate further evolutionary analyses. In order to identify gene families, the entire set of the predicted and known proteins of the M. oryzae/grisea genomes were clustered using OrthoMCL (Li, 2003). A total of 20443 clusters (15326 assigned to M. oryzae/grisea and 5117 to Burkholderia) were obtained, including 8154 clusters comprising single copy genes shared by all genomes (core genome) and variable number of genome specific gene families (305-1550). Genes encoding putative Secreted Proteins (SPs) were identified in 2522 genes and interfaces relying on the phylogenetic reference genealogy of the M. oryzae/grisea complex. We also evaluated the ability of individual genes to recover the same topology as that supported by most of the genes by using a recent method based on multiple co-inertia analysis (de Vienne, 2012). Most of the genes exhibit a concordant topology with the reference tree except a small set of ‘outliers’. Further investigations are currently being performed to determine possible causes for incongruities. Finally, we present preliminary results regarding the comparison of TE distribution in M. oryzae/grisea species taking into account the reference genealogy of the strains.

**Leptosphaeria maculans 'brassicae': 'Transposable Elements changed my life, I feel different now'.** Jonathan Grandaubert1, Conrad Schoch2, Hossein Borhan3, Barbara Howlett4, Thierry Rouxel1. 1) INRA-BIOGER, Thiverval-Grignon, France; 2) NCBI, National Institutes of Health, Bethesda, MD, USA; 3) AAF Saskatchewan, Canada; 4) School of Botany, University of Melbourne, Australia.

The Dothideomycetes phytopathogen Leptosphaeria maculans and Leptosphaeria biglobosa form a complex of 4 species and putative subspecies suggested to have diverged “recently”. In 2007, the sequencing of an isolate of *Leptosphaeria maculans* ‘brassicae’ (Lmb) provided the first reference genome for this fungus. The 45-Mb genome has an unusual bipartite structure, alternating large GC-equilibrated and AT-rich regions. These AT-rich regions comprise one third of the genome and are mainly composed of mosaics of truncated Transposable Elements (TEs) postulated to have “invaded” the genome 5-10 MYA; they also comprise 5% of the predicted genes of which 20% encode putative effectors. In these regions, both genes and TEs are affected by Repeat Induced Point mutation (RIP). To investigate when and how genome expansion took place in the evolutionary series, and the consequences it had on fungal adaptability and pathogenicity, the genomes of five members of the species complex showing contrasted host range and infection abilities were sequenced. In silico comparison of the reference genome with that of 30-32-Mb genome of *L. maculans* ‘lepidii’ (Lml), *L. biglobosa* ‘brassicace’, *L. biglobosa* ‘thlaspiii’ and *L. biglobosa* ‘canadensis’, showed these species have a much more compact genome with a very low amount of TEs (<4%). The TE annotation allowed us to identify 121 TE families, all RIP-affected including the expected presence of lineage-specific TEs. Unexpectedly, two of the most expanded TE families in Lmb have been present in the Dothideomycete lineage for 100 million years. This questions the possible causes for incongruities. In these families, while they have been actually RIPped, managed to expand recently in Lmb. Interestingly, the comparison between the TE-rich genome of Lmb and the TE-poor genome of Lml, estimated to have diverged 5.5 MYA, indicated a nearly perfect synteny at the chromosomal level, suggesting low incidence of TE expansion on genome reorganisation. The gene annotation produced a similar gene number in each genome (~11000), but compared to the reference genome, less than 20% of the effector genes and 50% of other genes in AT-rich regions are present in the other genomes, suggesting that TEs were key players in gene innovation and that the genome environment promoted rapid sequence diversification and selection of genes involved in pathogenicity.

The mammalian pathogens *Coccidioides immitis* and *C. posadasii* are the only dimorphic fungal pathogens that form spherules in the host. Furthermore, all of *Coccidioides*’ closest known relatives are non-pathogenic. In this project, we are interested in genome changes between the *Coccidioides* lineage and its relatives, and how these changes compare to recently published comparative and population genomics, and transcriptomics studies in *Coccidioides*. *Coccidioides* and its closest sequenced relative, *Uncinocarpus reesii*, are estimated to have diverged 75-80 million years ago. Here, we have sequenced the genomes of four species more closely related to *Coccidioides* than *U. reesii*: *Bysssoonygena ceratinophila*, *Chrysosporium queenslandicum*, *Amauroascus niger* and *A. mutatus*. For each of these four species, we prepared genomic DNA Illumina sequencing libraries; the resulting genome assemblies ranged from 23-34Mb, with N50 of 90kb-205kb. Predicted genes were confirmed by RNAseq; the total number of genes ranged from 8,179-9,184. We assessed individual gene gain/loss, and gene family expansion/contraction in *Coccidioides* using these new genomes and other recently published genomes from the Onygenales order, including the yeast-forming dimorphic pathogens *Histoplasma* and *Paracoccidioides*, and the dermatophytes *Microsporum* and *Trichopyton*. We have compared these results to genes identified in recently published *Coccidioides* “omics” studies that show evidence of positive selection, introgression and/or differential expression.
Engineering Aspergillus oryzae for high level production of L-malic acid. 

Debbie S Yaver, S. Brown, A. Berry

1) Expression Technology, Novozymes, Inc., Davis, CA; 2) Microbial Physiology, Novozymes, Inc., Davis, CA. In the last decade, there has been widespread interest and investment in developing processes for the production of bulk and specialty chemicals from renewable feedstocks by fermentation. During this period, Novozymes has successfully developed technology for production of a specialty molecule (hyaluronic acid) by Bacillus fermentation and has been very active in developing technologies for the production of bulk chemicals by metabolic engineering and fermentation using several different microorganisms. An example of the latter is L-malic acid. In the literature it is reported that some wild-type Aspergillus strains produce high levels of malic acid under specific cultivation conditions. Concentrations up to 113 g/L malate (94% w/w from glucose) reported for A. flavus in fed-batch fermentations (Battat, et. al., 1991. Biotechnol. Bioeng. 37:1108-1116). The goal of our work was to improve malic acid production in the natural malic acid producing filamentous fungus Aspergillus oryzae NRRL3488 by overexpression of cloned genes and classical mutagenesis. More than 75 different recombinant strains were tested containing combinations of overexpression of genes as well as deletions. A high throughput screen was developed and used to screen mutagenized strains. Combined genetic engineering and mutagenesis/HTS was used to increase the malic acid production rate of A. oryzae NRRL3488 by 4-fold with final C4 acid totals of 340 g/l at 8 days in lab scale fermentations.


The yeast Saccharomyces cerevisiae is a powerful and versatile workhorse intensively exploited for a wide range of biotechnological applications. Besides the large scale production of endogenous products, such as the biofuel ethanol, S. cerevisiae has been genetically engineered to produce many heterologous compounds, including half of the worldwide insulin market. The past decade has been marked by the conversion of S. cerevisiae into a complex cell factory with remarkable new capabilities such as the production of the anti-malaria drug precursor artemisinic acid. The ever-increasing demand for cheap and sustainable production of complex molecules combined with its attractiveness as a host for pathway engineering will inevitably intensify the exploitation of S. cerevisiae as cell factory in the future. Even in the genetically accessible bakers yeast, expression of dozens of genes is still largely based on laborious classical techniques involving successive restrictions and ligations, complemented with the creative application of PCR. However, the increasing size and complexity of today’s constructs in metabolic engineering has made design and construction of plasmids by these classical techniques increasingly complicated and time consuming. Although uncovered nearly three decades ago, the high efficiency of S. cerevisiae homologous recombination is only beginning to reveal its full potential for the assembly of large DNA constructs (Gibson et al., 2008). In vivo assembly in yeast is predicted to have a large impact on laboratory practices, ranging from simple plasmid construction to engineering of complex pathways via automated high-throughput strain construction. Despite those promising prospects, in vivo assembly has not yet become a standard technique in most academic laboratories. This offers unique possibilities for standardization and, simultaneously, for further optimization. In the present work we describe an approach designed to improve the efficiency of in vivo assembly and to make a robust, versatile in vivo assembly strategy for multi-component plasmids. As a proof of principle, the method was used to assemble a 21 kb plasmid from 9 overlapping fragments, using only PCR and yeast transformation. Gibson D.G. et al. (2008), Science, 319, 1215-1220.

Analysis of the intracellular galactoglycome of Trichoderma reesei grown on lactose. Levente Karaffa, Leon Coulier, Erzsébet Fekete, Karin M. Overkamp, Irina S. Druzhinina, Marianna Mikus, Bernhard Seiboth, Levente Novák, Peter J. Punt, Christian P. Kubicek

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Lactose (1,4-0-b-D-galactopyranosyl-D-glucose) is used as a soluble carbon source for the production of cellulases and hemicellulases for - among other purposes - in the biofuel and biorefinery industries. However, the mechanism how lactose induces cellulase formation in T. reesei is still enigmatic. Previous results generated the hypothesis that intermediates from the two D-galactose catabolic pathway may give rise to the accumulation of intracellular oligogalactosides that could act as inducer. We have therefore used HPAEC-MS to study the intracellular galactoglycome of T. reesei during growth on lactose, in T. reesei mutants impaired in galactose catabolism, and in strains with different cellulase productivity. Lactose, allo-lactose and lactulose were detected in the highest amounts in all strains, and two trisaccharides (Gal-b-1,6-Gal-b-1,4-Glc/Fru; and Gal-b-1,4-Gal-b-1,4-Glc/Fru) also accumulated to significant levels. D-Glucose and D-galactose, as well as two further oligosaccharides (Gal-b-1,3/1,4-Gal; Gal-b-1,2/1,3-Glc) were only detected in minor amounts, In addition, one unknown disaccharide and four trisaccharides were also detected. The unknown hexose disaccharide to correlate with cellulase formation in the improved mutant strains as well as the galactose pathway mutants, and Gal-b-1,4-Gal-b-1,4-Glc also two other unknown hexose trisaccharides to correlate with cellulase production only in the pathway mutants, suggesting that these compounds could be involved in cellulase induction by lactose.

Among filamentous fungi Aspergillus sp. are well known production host for several organic acids. These acids, traditionally being food ingredients, more recently have gained attention as platform or building-block chemicals. These chemicals, currently mostly produced based on petrochemistry, are the starting point for the production of a wide variety of materials, such as resins, plastics, etc. Production of these compounds via biobased routes will be a major contribution towards a Biobased Economy. For the production of these bulk compounds robust host organisms are required, suitable for using low cost lignocellulose-based feedstocks, resistant against adverse conditions due to inhibitory feedstock compounds and capable of coping with high product concentrations. A. niger was shown to fulfill most of these prerequisites (Rumbold et al., 2009). Based on the extended molecular genetic toolkit systems biology approaches were developed for A. niger and other fungi (e.g. Braaksma et al., 2010). These approaches were followed towards production of these platform chemicals in A. niger, as demonstrated by the example of itaconic acid (Li et al., 2011, 2012). The recent development of novel high throughput sequence methods has led to new more efficient transcriptomics approaches such as RNAseq. Combination of these approaches with novel experimental design and statistical methods for target gene identification in metabolic pathway engineering will be illustrated. Rumbold, K., van Buijsen, H.J.J., Overkamp, K.M., van Groenestijn, J.W., Punt, P.J., Werf, M.J.V.D. (2009) Microbial production host selection for converting second-generation feedstocks into bioproducts. Microbial Cell Factories 8, art. no. 64 Braaksma, M., van den Berg, R.A., van der Werf, M.J., Punt, P.J. (2010) A Top-Down Systems Biology Approach for the Identification of Targets for Fungal Strain and Process Development. In: Cellular and Molecular Biology of Filamentous Fungi. Eds: K.A. Borkovich & D.J. Ebbole ASM Press, Washington DC. pp. 25-35 Li, A., van Luijk, N., ter Beek, M., Caspers, M., Punt, P., van der Werf, M. (2011) A clone-based transcriptomics approach for the identification of genes relevant for itaconic acid production in Aspergillus. Fungal Genetics and Biology 48 (6), pp. 602-611.

A new method for gene mining and enzyme discovery. Y. Huang 1,2,3, P. Busk1, M. Grell2, H. Zhao2,3, L. Lange4. 1) Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University Copenhagen, Denmark; 2) Environmental Microbiology Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, Sichuan 610041, PR China; 3) University of the Chinese Academy of Sciences, Beijing 100049, PR China.

Peptide pattern recognition (PPR) is a non-alignment based sequence analysis principle and methodological approach, which can simultaneously compare multiple sequences and find characteristic features. This method has improved the understanding of structure/function relationship for enzymes within the CAZY families, which would make it easier to predict the potential function of novel enzymes, creating bigger promises for industrial purposes. Mucor circinelloides, member of the former subdivision Zygomycota, can utilize complex polysaccharides such as wheat bran, corn cob, xylan, CMC and avicel as substrate to produce plant cell wall degrading enzymes. Although the genome of M. circinelloides has been sequenced, only few plant cell wall degrading enzymes are annotated in this species. In the present project, PPR was applied to analyze glycoside hydrolase families (GH family) and mining for new GH genes in M. circinelloides genome. We found 19 different genes encoding GH3, GH5, GH6, GH7, GH9, GH16, GH38, GH43, GH47 and GH125 in the genome. Of the three GH3 encoding genes found, one was predicted by PPR to encode a b-glucosidase. We expressed this gene in Pichia pastoris and found that the recombinant protein has high b-glucosidase activity (4884 U/mL). In this work, PPR provided targeted short cut to discovery of enzymes with a specific activity. Not only could PPR pinpoint genes belonging to different GH families but it did also predict the enzymatic function of the genes.

Increased production of fatty acids and triglycerides in Aspergillus oryzae by modifying fatty acid metabolism. Koichi Tamano1, Kenneth Bruno1, Tomoko Ishii1, Sue Karagiosis1, David Culley1, Shuang Deng2, James Collet2, Myco Umemura2, Hideaki Koike3, Scott Baker3, Masayuki Machida3. 1) National Institute of Advanced Industrial Science and Technology (AIST); 2) Pacific Northwest National Laboratory (PNNL).

Biofuels are attractive substitutes for petroleum based fuels. Biofuels are considered they do not contribute to global warming in the sense they are carbon-neutral and do not increase carbons on the globe. Hydrocarbons that are synthesized by microorganisms have potential of being used as biofuels or the source compounds. In the hydrocarbon compounds synthesized by A. oryzae, fatty acids and triglycerides are the source compounds of biodiesel that is fatty acid methyl ester. We have increased the production by modifying fatty acid metabolism with genetic engineering in A. oryzae. Firstly, enhanced-expression strategy was used for the increase. For four enzyme genes related to the synthesis of palmitic acid [C16:0-fatty acid], the individual enhanced-expression mutants were made. And the fatty acids and triglycerides in cytosol were assayed by enzyme assay kits, respectively. As a result, both fatty acids and triglycerides were most synthesized in the enhanced-expression mutant of fatty acid synthase gene at 2.1-fold and 2.2-fold more than the wild-type strain, respectively. Secondly, gene disruption strategy was used for the increase. Disruptants of several enzyme genes related to long-chain fatty acid synthesis were made individually. And one of them showed drastic increase in fatty acid synthesis. In the future, further increase in the synthesis is expected by utilizing genetic engineering in A. oryzae.

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Molecular biological basis for statin resistance in naturally statin-producing organisms. Ana Rems, Rasmus Frandsen. DTU Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark.

Secondary metabolites can be toxic to the organism producing them; therefore gene clusters for biosynthesis of secondary metabolites often include genes responsible for the organism's self-resistance to the toxic compounds. One such gene cluster is the compactin (ML-236B) cluster in Penicillium salitum. Compactin is an inhibitor of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, and is used as a precursor for production of the cholesterol-lowering drug pravastatin. The compactin gene cluster includes two genes encoding proteins that may confer the self-resistance to compactin and its secretion [1]. The mlcD gene encodes a putative 'HMG-CoA reductase-like protein', and mlcE encodes a putative efflux pump. However, the function of these two putative proteins has not yet been confirmed. We aim to elucidate the biological basis for compactin resistance in the compactin-producing organism. A codon-optimized version of the mlcD gene was inserted into the Saccharomyces cerevisiae genome. The constructed yeast strain was tested for sensitivity to lovastatin, a strain structurally similar to compactin, by growing the strain on media containing lovastatin. The strain showed an increased resistance to lovastatin compared to the wild-type strain. Furthermore, we investigated if MlcD confers the resistance by functional complementation of the endogenous HMG-CoA reductase genes in S. cerevisiae. There are two isozymes of HMG-CoA reductase in yeast, HMG1 and HMG2, both involved in the sterol biosynthetic pathway, which leads to the synthesis of ergosterol. Following deletion of HMG1 and HMG2 genes in S. cerevisiae, we inserted the mlcD gene into the knockout mutants, and tested the resulted strains for sensitivity to lovastatin. The HMG1 and HMG2 knockout mutants were unable to grow on minimal media and had an increased sensitivity to lovastatin on rich media. However, insertion of the mlcD gene restored the growth of the yeast mutants and increased their resistance to lovastatin. These results show that MlcD complements the activity of the deleted HMG-CoA reductases, enabling synthesis of ergosterol in yeast. In addition MlcD confers statin resistance by being insensitive to the inhibiting effects of statins. Reference: [1] Abe Y., Suzuki T., Ono C., Iwamoto K., Hosobuchi M., Yoshikawa H. Mol Genet Genomics 2002, 267, 5:636-46.

Engineering Cyclic Peptide Biosynthesis in Poisonous Mushrooms. Hong Luo, John S. Scott Craig, Robert M. Sgambelluri, Sung-Yong Hong, Jonathan D. Walton. Department of Energy Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824, United States.

Ninety percent of fatal mushroom poisonings are caused by alpha-amanitin and related bicyclic peptides found in some species of Amanita, Galerina, Lepiota, and Conocybe. We showed that the amatoxins (mainly amanitins) and related phallotoxins are synthesized on ribosomes in A. bisporigera and the unrelated mushroom G. marginata. The primary gene products are short (34-35 amino acid) proproteins that are initially processed by a dedicated prolyl oligopeptidase. A genome survey sequence of A. bisporigera suggested that it has a repertoire of over 40 cyclic peptides, all produced on a single biosynthetic scaffold. Members of this extended gene family are characterized by conserved upstream and downstream amino acid sequences, including two invariant proline residues, flanking a six to ten-amino acid “hypervariable” region that encodes the amino acids found in the mature toxins (or predicted toxins). The evidence indicates that A. bisporigera has evolved a combinatorial strategy that could in principle biosynthesize billions of small cyclic peptides. In order to study the other steps in amanitin biosynthesis, and to engineer novel cyclic peptides, we have developed a transformation strategy for the amanitin-producing mushroom G. marginata. This first transformation method uses Agrobacterium-mediated transformation followed by hygromycin selection. Taking advantage of this platform, we are introducing artificial toxin genes that are deliberately designed to provide insights into the pathway. The synthetic genes include those that encode the cyclic octapeptide beta-amanitin, the heptapeptides philloidin and phallacidin, examples of the toxin gene family known from A. bisporigera but not G. marginata, and randomly generated artificial sequences. Currently, thousands of transformants have been generated through an efficient pipeline and the transformants are being analyzed for production of the expected products. If successful, the novel peptides will be screened in a number of assays including RNA polymerase (the site of action of alpha-amanitin), membrane ion channels, pathogenic bacteria, and cancer cell lines.
**Chemically Induced Haploinsufficiency Screens to Identify Drug Mechanism of Action in Aspergillus Fumigatus.** D. A. Macdonald, A. E. Johns, M. Eberle, P. Bowyer, D. Denning, M. J. Bromley. 1) Institute of Inflammation and Repair, Respiratory & Allergy Centre, University of Manchester, Manchester, United Kingdom; 2) Applied Microbiology, Institute for Applied Life Sciences, University of Karlsruhe, Hertzstrasse 16, 76187 Karlsruhe, Germany.

Current drugs used to treat Aspergillus infections are limited and suffer from a variety of shortcomings including low efficacy, toxicity and increasing resistance. Despite the discovery of numerous promising drug targets, few lead compounds have been discovered by target based approaches. This can be explained, in part, by the ‘drugability’ of a target as some compounds which demonstrate promising activity against an enzyme are not active against the whole cell or are toxic to humans. Consequently most of the antimicrobials presently on the market were originally discovered by random screening of compounds against whole cell screens. A solution to this problem is to identify gene targets utilizing compounds that already show antifungal activity and have clean toxicity profiles.

Chemical genetic profiling aids identification of drug mechanism of action as a diploid strain lacking a single copy of a drug’s target is hypersensitive to that drug. Heterozygote *S. cerevisiae* and *C. albicans* libraries have been used to identify the mechanism of action of several promising compounds; however, this has been hindered in *A. fumigatus* by the complexity in generating an adequate set of heterozygous strains. A high-throughput targeted gene KO method for *A. fumigatus* has been established by employing fusion-PCR to generate targeted gene disruption cassettes, optimizing the common transformation protocol for *A. fumigatus* high-throughput gene disruption, and utilising a diploid *Ku80* mutant to facilitate more reliable homologous recombination. Preliminary efforts have produced 46 heterozygous KO strains and subsequently, the feasibility of chemical genetic haploinsufficiency studies in filamentous fungi has been demonstrated with several compounds. High-throughput methods of chemical genetic profiling by pooling multiple heterozygous KO strains into a single culture is currently being validated and preliminary data is promising. This will enable high-throughput methods for surveying the genome of *A. fumigatus* for new drug targets and supports unveiling the mechanisms of action of antifungal drugs.

**Inhibition of benzoate 4-monooxygenase (CYP53A15) from Cochliobolus lunatus by cinnamic acid derivatives.** Branka Korosec, Barbara Podobnik, Sabina Berne, Neja Zupanec, Metka Novak, Nada Krasevec, Samo Turk, Matej Sova, Ljerca Lah, Jure Stojan, Stanislav Gobec, Radovan Komel. 1) National Institute of Chemistry, Ljubljana, Slovenia; 2) Lek Pharmaceuticals d.d., Verovskova 57, SI-1000 Ljubljana, Slovenia; 3) Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, SI-1000 Ljubljana, Slovenia; 4) Chair of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Aškerceva cesta 7, SI-1000 Ljubljana, Slovenia.

Fungal infections cause huge economic losses in agriculture. Some of the major phytopathogens also cause serious, and very often lethal, infections in human and animals. Plants may be a good source of antifungals since they have to defend themselves by producing numerous secondary metabolites, such as sterols, terpenes, polyacanols and phenolic compounds. Successful development of antifungal compounds, based on natural defense molecules, could prove useful in combating infectious and toxic-producing fungi in both agriculture and medicine. In recent years several promising antifungal targets have been under exploration. One of such is also fungal CYP53, member of the family of highly conserved CYP proteins, involved in detoxification of benzoate, a key intermediate in metabolism of aromatic compounds in fungi. High specificity and absence of homologue in higher eukaryotes assign CYP53A15 from the filamentous fungus *Cochliobolus lunatus* as interesting drug target. In our latest research we explored chemical properties of isoeugenol for ligand-based similarity searching, and the homology model of CYP53A15 of *Cochliobolus lunatus*, for structure-based virtual screening of a composite chemical library. Two cinnamic acid derivatives were amongst the highest scoring compounds. In the past few years, several other reports about antifungal activity of cinnamic acid derivatives have been published. In order to investigate the potential inhibitory activity on benzoate 4-monooxygenase (CYP53A15) we analyzed antifungal activity of 9 commercially available, and 10 representative cinnamic acid derivatives from our library. Furthermore, to obtain more information about structure-activity relationship 26 additional cinnamic acid esters and amides were synthesized and included in our assays. Among 45 cinnamic acid derivatives tested, 7 compounds have shown antifungal activity against *C. lunatus*, *A. niger* and *P. ostreatus* in in vivo inhibition tests. Compounds with antifungal activity were further evaluated for inhibition of CYP53A15 activity with spectral binding titration assay and HPLC. The best two inhibitors of CYP53A15 activity showed 70% inhibition at 600 mM concentration and were selected for further optimization of new lead structures.
Secretome analysis of *Trichoderma harzianum* cultivated in the presence of *Fusarium solani* cell wall or glucose. Marcelo HS Ramada1,2, Andrei S Steindorff3, Carlos Bloch Jr.4, Cirano J Ulhoa5. 1) Brasilia University, Cell Biology Department, Brasilia, DF, Brazil; 2) Federal University of Goias, Biochemistry Department, Goiania, GO, Brazil; 3) EMBRAPA CENARGEN, Mass Spectrometry Laboratory, Brasilia, DF, Brazil. *Trichoderma harzianum* is a fungus well known for its potential as a biocontrol agent of many fungal phytopathogens. The aim of this study was to evaluate the potential of *T. harzianum* ALL42 to control *Fusarium solani*, a phytopathogen fungus that causes several losses in common bean and soy crops in Brazil and to evaluate the secreted proteins of *T. harzianum* ALL42 when its spores were inoculated and incubated in culture media supplemented (TLE) or not (MM) with nitrogen sources and in the presence or not of *F. solani* cell walls (FsCW). In the absence of FsCW, the media were supplemented with glucose (GLU). *T. harzianum* was able to control the phytopathogen growth and started to sporulate in its area after 7 days in a dual culture assay, indicating that it had successfully parasitized the host. *T. harzianum* was able to grow in TLE+FsCW, MM+FsCW, TLE+GLU, but unable to grow in MM+GLU. Protein quantification showed that TLE+FsCW and MM+FsCW had 45 and 30 fold, respectively, more proteins than TLE+GLU, and this difference was observed in the bidimensional gels, as the two supernatants from media supplemented with FsCW had around 200 spots and the one supplemented with glucose only had 18. TLE+FsCW and MM+FsCW had above 80% of spot similarity. A total of 100 proteins were excised from all three conditions and submitted to mass spectrometry analysis. 85 out of 100 proteins were identified. The only protein observed in all three conditions is a small protein, called ep1, involved in eliciting plant-response against phytopathogens. An aspartic protease, previously described as related to mycoparasitism, was only found when *T. harzianum* was grown with glucose. Gene expression was evaluated and confirmed the gel results. In the media supplemented with FsCW, different hydrolyases like chitinases, beta-1,3-gluconanases, glucosaminylases, alpha-1,3-gluconanases, and proteases were identified. Some proteins like a small cystein-rich, alpha-L-arabinofuranosidase and NPP1, with no known function in mycoparasitism were also identified. *T. harzianum* ALL42 is able to inhibit the growth and parasitize *F. solani* and showed a complex and diverse arsenal of proteins that are secreted in response to the presence of the cell walls, with novel proteins not previously described in mycoparasitism studies.

Metabolic adaptation of the oomycete *Phytophthora infestans* during colonization of plants and tubers. Carol E. Davis, Howard S. Judelson. Plant Pathology and Microbiology, University of California, Riverside, CA 92521. *Phytophthora infestans* is the causative agent of late blight and was responsible for the Irish famine in the 1840’s. Today it still continues to be a global problem and in the USA it has been reported that the economic loss on potato crops alone exceeds $6 billion per year. A successful phytopathogenic relationship depends on the ability of the organism to adapt its metabolism during infection on various nutritional substrates (e.g., plant versus tuber) and at different times throughout infection when nutrients may be limiting. Investigation of this metabolic adaptation is key to understanding how *P. infestans* succeeds as a pathogen. To do this, tomato plants and potato tubers were infected with zoospores using a “dipping” method. RNA was extracted at 3 dpi and 6 dpi and subsequently used in library preparation. Gene expression was evaluated and confirmed the gel results. The libraries were quality checked by analysis on a Bioanalyzer using a high sensitivity DNA chip. Using illumina technology (50 bp, paired-end reads) RNA Sequencing was performed. For each sample an average of 262 million reads was obtained. As a reference for the *in planta* data, RNAseq was also performed on defined and complex media. Mining of the data shows that the expression profiles of some pathways change, such as glycolysis and gluconeogenesis. Learning how metabolic adaptation occurs will prove useful in the development of novel control strategies for this plant pathogen.

The fungi strike back: multidrug resistance in *Aspergillus fumigatus* and agricultural use of fungicides. Paul E. Verweij. Medical Microbiology, UMC St Radboud, Nijmegen, Netherlands. *Aspergillus fumigatus* is a saprophytic mould that causes a range of diseases in humans. The spectrum of diseases includes allergic conditions, aspergillosis and acute and chronic invasive aspergillosis. Acute invasive aspergillosis occurs typically in patients with compromised host defenses such as those receiving treatment for leukemia. The management of invasive aspergillosis is very difficult as the diagnostic tools often lack sensitivity and the prevention of antifungal agents effective against the infection is limited. The azoles are the most important class of agents used for the treatment and prevention of invasive aspergillosis. Since 1998 resistance to medical triazoles has emerged in the Netherlands in clinical A. fumigatus isolates. These isolates commonly showed a multi-azole resistant phenotype and patients with azole-resistant aspergillosis failed to respond to azole therapy. The mortality rate of azole-resistant invasive aspergillosis was 88%. In more than 90% of resistant isolates a combination of changes in the target gene Cyp51A was found: a substitution at codon 98 and a 34 bp tandem repeat in the promoter region (TR34/L98H). As person-to-person transmission is highly unlikely in invasive aspergillosis, the dominance of a single resistance mechanism could not be explained by resistance development in azole-treated patients. Surveys in the environment showed that A. fumigatus isolates resistant to medical triazoles could be recovered from the environment, especially from cultivated soil. The resistance mechanisms in these isolates were identical to those found in clinical isolates. Molecule alignment studies identified 5 triazole fungicides that showed highly similar molecule structures to the medical triazoles. These 5 fungicides have been authorized between 1990 and 1996, thus preceding the isolation of the first resistant clinical A. fumigatus isolate. TR34/L98H is increasingly reported in European countries, India, China and Iran. Recently a new azole-resistance mechanism was found in Dutch patients and in the environment. The resistance mechanism again consisted of a combination of changes in the Cyp51A-gene: TR46/Y121F/T289A. This new resistance mechanism has spread in addition to TR34/L98H in the Netherlands, and has also been reported in the neighboring country Belgium. The selection of multiple azole resistance mechanisms in the environment poses a threat for the use of azole drugs in the management of *Aspergillus* diseases in humans. Research that investigates the selection of azole resistance in A. fumigatus in the environment is urgently warranted.
Effect of antifungal resistance on virulence of Candida spp. L. Vale-Silva, A. Lohberger, D. Sanglard. Inst Microbiology, Univ Lausanne and Univ Hosp Center, Lausanne, VD, Switzerland.

Our laboratory has been involved in the understanding of mechanisms of antifungal resistance in fungal pathogens. While resistance is beneficial to fungi in the presence of antifungals both in vitro and in vivo, resistance mechanisms have some impact on the ability of fungal pathogens to propagate in the host and cause disease. We have addressed this question with two pathogens, C. albicans (Ca) and C. glabrata (Cg). Our studies have focused on resistance to azoles, which are widely used compounds. In Cg, azole resistance in clinical isolates is almost exclusively mediated by ABC transporters via gain of function (GOF) mutations in the transcription factor (TF) CgPDR1. We observed that development of azole resistance in Cg was correlated with gain of virulence and fitness in animal models as compared to susceptible isolates. Recent results suggest that ABC transporters (especially CgCDR1) participate to this phenotype. In further studies on interactions of Cg with murine bone marrow-derived macrophages (BMDM), we found that the GOF mutant was able to evade phagocytosis as compared to a matched susceptible isolate. This effect was a consequence of impaired adherence. This suggests that the mechanism behind escape from phagocytosis was rather based on decreased recognition and/or adhesion by host macrophages. GOF mutations in CgPDR1 may thus allow Cg to evade the host’s innate immune response, which may in turn contribute to increased virulence. In Ca azole resistance is mediated by mutations in TF and in azole targets. GOF mutations in the three different activators including TAC1, MRR1 and UPC2 regulate specific subclasses of drug resistance genes belonging to ABC-transporters, major facilitators and azole targets, respectively. We have constructed sets of isogenic strains lacking or carrying each GOF mutations in these TF and addressed their effect on virulence and tissue colonization in animal models. The presence of GOF mutations in TAC1 and MRR1 had a neutral effect on virulence and ability to colonize host tissues. A UPC2 GOF mutation was negatively affecting virulence and tissue colonization, which suggests that UPC2 activity generates fitness costs in Ca. Our results therefore highlight that the costs of resistance mechanisms on virulence are depending on the fungal species and the type of resistance mechanism.

From enzyme to fungal development or how sdhB mutations impact respiration, fungicide resistance and fitness in the grey mold agent Botrytis cinerea. Anais Lalèved, Anne-Sophie Walker, Stephanie Gamet, Valerie Toquin, Daniele Debieu, Sabine Fillinger. 1) BIOGER, INRA, Thiverval-Grignon, France; 2) BAYER SAS, Bayer CropScience, Lyon, France.

Respiration inhibitor fungicides are widely used to control fungal diseases on multiple crops. The succinate dehydrogenase inhibitors (SDHIs) are among the latest introduced molecules against the grey mold agent Botrytis cinerea on grapevine. We have recently isolated and characterized B. cinerea field strains resistant to the SDHIs. Most of the strains harbor one single mutation in the succinate dehydrogenase subunit gene, sdhB affecting the ubiquinone-binding pocket. In this study we have introduced these mutations into a B. cinerea wild-type strain (BOS.10) in order to evaluate the impact of each mutation on SDH- and respiratory activity and inhibition by SDHIs from different chemistries. We also analyzed several parameters of B. cinerea’s life cycle to assess the fitness cost associated with the resistance mutation. Our results show a strict correlation between the sdhB mutation and the resistance spectra to SDHIs. These resistances can be fully explained by the affinities of the SDHIs to its modified target enzyme. Four out of the seven sdhB alleles led to significantly reduced SDH activity and, in three cases (H272L, N230I, P225L), to reduced respiration rates. Concerning the fungal biology, we tested mycelial growth and sclerotia production on different media and temperatures, conidia production and germination, resistance to oxidative stress and ROS production, as well as pathogenicity on tomato and bean leaves. All mutants were affected for at least one parameter. However, fitness parameters of mutants sdhBH272L and sdhBN225L showed the strongest modifications among all strains, e.g., reduced pathogenicity, strongly reduced conidia- and sclerotia-production (H272R). A clear correlation between fitness and respiration on one hand, fitness and allele frequency among natural populations on the other, is not yet obvious. However, our results in terms of resistance spectra and fitness parameters should help defining more efficient treatment strategies against grey mold.


Resistance to crop protection fungicides can lead to a rapid loss of efficacy in the field. This is a major threat for the sustainability of our products. An early assessment is required in order to define best recommendations in product usage but also guide our choice in developing novel active ingredients. In addition to monitoring the situation for marketed fungicides, we are conducting early assessments for new active ingredients which include resistance generation, mode of resistance identification and fitness penalty determination. High throughput sequencing has become key for a rapid identification of resistance mechanisms both for novel fungicides but also to holder chemistries for which the resistance mechanisms were so far not confirmed. A few examples will be discussed.
Parallels between Fungal Pathogens of Plants and Animals
Co-chairs: Barbara Howlett and Axel Brakhage

Emerging fungal (and Oomycete) threats to plant and ecosystem health. Sarah J. Gurr1, Daniel Bebber2, Matthew Fisher3. 1) Plant Sciences, Oxford University, Oxford, Oxfordshire; 2) Imperial College of Science, Technology and Medicine, London.

Fungal diseases have increased in severity and scale since the mid 20th Century and now pose a serious challenge to global food security and ecosystem health (Gurr et al., 2011 Fungal Biology Reviews 25 181-188 ). We have demonstrated recently that the threat to plants of fungal infection has now reached a level that outstrips that posed by bacterial and viral diseases combined (Fisher et al., 2012 Nature 484 185-194) I shall highlight some of the more notable fungal and oomycete plant diseases and will draw attention to the emergence of new pathotypes affecting crop yields and decimating our natural and managed landscapes. We have calculated that the losses due to persistent disease (that is, non-epidemic) caused by, for example rice blast, wheat stem rust, corn smut, soybean rust and potato late blight, if mitigated, would be sufficient to feed 8.5% of the global population (based on 2000 calories per day for 1 year). Moreover, tree losses due to fungal and oomycete diseases such as dutch elm, chestnut blight, sudden oak death, jarrah die-back and pine beetle/blue stain fungus, thus far, have been estimated to account for significant CO2 sequestration losses (Fisher et al., 2012). The spread of such organisms around the world is facilitated primarily by trade, but there is increasing concern that climate change may allow their establishment in regions hitherto deemed unsuitable. Increasing latitudinal ranges are anticipated under rising temperatures. However, the interactions between climate change, crop and natural enemies are complex, and the extent to which crop pests and pathogens have altered their latitudinal ranges in response to global warming is largely unknown. We can demonstrate, from thousands of observations of hundreds of pests and pathogens, their shift polewards since 1950, with a more rapid shift since 1990 (Bebber et al., under review)). This latitudinal shift is seen in both Hemispheres. Moreover, the rate of movement since 1950 is identical to that predicted by global climate data. This observed trend cannot be explained by latitudinal variation in technical capacity to detect and report pest incidences.


In fungi, melanins are often associated with the cell wall and also contribute to the structural rigidity of spores. In several plant and human pathogenic fungi, melanins contribute to pathogenicity. For example, pigmentless mutants of the plant pathogens Magnaporthe oryzae and Colletotrichum lagenarium, as well as the human-pathogenic fungi Cryptococcus neoformans and Aspergillus fumigatus are less virulent when compared to melanin-producing wild-type strains. In M. oryzae, it was shown that a 1,8-dihydroxynaphthalene (DHN) melanin layer between the cell wall and the cell membrane is essential for turgor generation. The melanin acts as a barrier to the efflux of solute from the appressorium, which occurs as pressure is generated. Cellular turgor is translated into mechanical force of infection hyphae, forcing it through the leaf cuticle21. In human-pathogenic fungi, high turgor pressure is not required for penetration of tissue. In these fungi, melanin displays other virulence attributes such as the scavenging of reactive oxygen species. In A. fumigatus, at least two types of melanin are produced: Pyomelanin by polymerization of homogentisic acid, and DHN melanin. Transcription of genes essential for pyomelanin and DHN-melanin biosynthesis is detected during infection of mice. However, pyomelanin seems to be dispensable for fungal virulence in the murine infection models tested22,23. DHN melanin is responsible for the grey-green color of A. fumigatus conidia. The biosynthesis enzymes of DHN melanin are encoded by six genes. Centrally is the polyketide synthase gene pksP, whose deletion results in a mutant strain with drastically attenuated virulence. Recent data of our laboratory showed that DHN melanin is essential not only for inhibition of apoptosis of phagocytes by interfering with the host PI3K/Akt signaling cascade but also for effective inhibition of acidification of conidia-containing phagolysosomes24,25. These features allow A. fumigatus to survive in phagocytes and thereby to escape from human immune effector cells and to become an aggressive pathogen. 1) Wilson RA & Talbot NJ (2009) Nat Rev Microbiol. 7: 185-195 2) Keller et al. (2011) PLoS One 6:e26604 3) Schmaler Ripcke et al. (2009) Appl Environ Microbiol. 75: 493 4) Thywiben et al. (2011) Front Microbiol. 2: 96 5) Volling et al. (2011) Cell Microbiol. 13: 1130.

Nutrient immunity and systemic readjustment of metal homeostasis modulate fungal iron availability during the development of renal infections. Joanna Potrykus1, David Stead2, Dagmar S Urgast3, Donna MacCallum4, Andrea Raab5, Jörg Feldmann6, Alistair JP Brown7. 1) Aberdeen Fungal Group, University of Aberdeen, Aberdeen, United Kingdom; 2) Aberdeen Proteomics, University of Aberdeen, Aberdeen, United Kingdom; 3) Trace Element Speciation Laboratory, University of Aberdeen, Aberdeen, United Kingdom.

Iron is a vital micronutrient that can limit the growth and virulence of many microbial pathogens. Here we show, that in the murine model of disseminated candidiasis, the dynamics of iron availability are driven by a complex interplay of localized and systemic events. As the infection progresses in the kidney, Candida albicans responds by broadening its repertoire of iron acquisition strategies from non-herm iron (FTR1-dependent) to heme-iron acquisition (HMX1-dependent), as demonstrated in situ by laser capture microdissection, RNA amplification and qRT-PCR. This suggested changes in iron availability in the vicinity of fungus. This was confirmed by 55Fe iron distribution mapping in infected tissues via laser ablation-ICP-MS, which revealed distinct iron exclusion zones around the lesions. These exclusion zones correlated with the immune infiltrates encircling the fungal mass, and were associated with elevated concentrations of murine heme oxygenase (HO-1) circumventing the lesions. Also, MALDI Imaging revealed an increase in heme and hemoglobin alpha levels in the infected tissue, with their distribution roughly corresponding to that of 55Fe. Paradoxically, whilst iron was excluded from lesions, there was a significant increase in the levels of iron in the kidneys of infected animals. This iron appeared tissue bound, was concentrated away from the fungal exclusion zones, and was accompanied by increased levels of ferritin and HO-2. This iron accumulation in the kidney correlated with defects in red pulp macrophage function and red blood cell recycling in the spleen, brought about by the fungal infection. Significantly, this effect could be replicated by selective chemical ablation of splenic red pulp macrophages by clodronate. Collectively, our data indicate that systemic events shape micronutrient availability within local tissue environments during fungal infection. The infection attenuates the functionality of splenic red pulp macrophages leading to elevated renal involvement in systemic iron homeostasis and increased renal iron loading. Simultaneously, localized nutrient immunity limits iron availability around foci of fungal infection in the kidney. In response, the fungus modulates its iron assimilation strategies.
Common strategies in plant and human “necrotrophic” pathogens: role of PCD. N. Shlezinger, H. Irme, G. Braus, A. Sharon. 1) Tel Aviv University, Tel Aviv, Israel; 2) Georg-August University Goettingen, 37077 Goettingen, Germany.

Botrytis cinerea is a model system to study pathogenicity of necrotrophic fungi. As inferred by the term “necrotrophic”, such pathogens must first kill host cells before they can use them as a source of nutrients. To achieve this, B. cinerea promotes apoptotic cell death in infected plants, which facilitates lesion spreading during late infection stage. How the fungus survives the first encounter with living plant tissue remained unclear. We found that hyphae of B. cinerea undergo massive PCD during early stages of infection, but fully recover upon transition to second phase of infection. Further studies using the fungal and plant mutants showed that survival, and hence pathogenicity of the fungus depended on anti-apoptotic machinery; fungal mutants bearing defects in the anti-apoptotic gene BcBIR1 had reduced virulence whereas strains over-expressing this gene were hypovirulent. A similar phenomenon was observed with another necrotrophic plant pathogen, but not with two semi-birotrophic pathogens. These results showed that the anti-PCD machinery is essential for pathogenic development necrotrophic plant pathogens. In order to extend these finding to other systems, we generated transgenic strains of the human pathogen Aspergillus fumigatus. This fungus shares common stages of infection with B. cinerea and hence might also need the anti-PCD machinery for infection. Indeed, when conidia of A. fumigatus are inoculated on a sensitive tissue, the developing hyphae undergo massive cell death, and similar to B. cinerea they fully recover later. Collectively, our results show that certain plant and human fungal pathogen share common strategies and mechanisms when infecting their host. One such mechanism is control of host-induced cell death, through conserved anti-apoptotic machinery.


Magnaporthe oryzae is the causal agent of rice blast, one of the most important serious economic problems affecting rice production. During plant infection, M. oryzae develops a differentiated infection structure called an appressorium. This unicellular, dome-shaped structure generates cellular turgor, that is translated into mechanical force to cause rupture of the rice cuticle and entry into plant tissue. Development of a functional appressorium requires completion of a single round of mitosis shortly after conidial germination, which also leads to initialisation of autophagic recycling of the contents of the fungal spore to the appressorium. We have recently shown that a hetero-oligomeric septin GTPase complex is necessary for re-organisation of a toroidal F-actin network at the base of the appressorium which allows re-establishment of polarised fungal growth. Septins scaffold F-actin, via the ezrin-radixin-moesin (ERM) protein, Tea1, and phosphatidylinositol interactions at the appressorium plasma membrane. The septin ring assembles in a Cdc42 and Chn1-dependent manner and forms a diffusion barrier to localize the inverse-Bin-Amphiphysin-RVS (I-BAR)-domain protein, Rvs167, and Wiskott-Aldrich Syndrome protein (WASP), Las17 at the point of penetration. This leads to formation of a penetration hyphae that breaches the host cuticle and leads to plant tissue colonization. We present evidence that septin-mediated plant infection is regulated by a specialised NADPH oxidase-tetraspanin complex necessary for control of F-actin dynamics. We also describe the potential operation of a pressure-mediated checkpoint pathway that leads to initial septin assembly and activation and the re-orientation of the cortical F-actin cytoskeleton to facilitate plant tissue invasion.


In the soilborne pathogen Fusarium oxysporum, a mitogen-activated protein kinase (MAPK) cascade homologous to the yeast filamentous growth pathway controls invasive growth and virulence on tomato plants. Full phosphorylation of Fmk1 requires the transmembrane protein Msb2, a member of the family of signalling mucins that have emerged as novel virulence factors in fungal plant pathogens. A yeast two-hybrid screen for proteins interacting with the Msb2 cytoplasmic tail identified UreG, a component of the urease enzymatic complex. UreG belongs to a set of accessory proteins needed to activate Apo-urease, which converts urea to yield ammonia and carbon dioxide. The F. oxysporum genome contains two structural urease genes, ure1 and ure2. Mutants in ureG or ure1 showed reduced growth on urea as the sole carbon and nitrogen source. Lack of urease activity in the mutants resulted in failure to secrete amonia and to increase the extracellular pH. The DureG mutants caused significantly reduced mortality on tomato plants and on the animal model host Galliera melanella, while Dure1 mutants only showed reduced virulence on tomato plants. Real-time qPCR analysis of key genes involved in nitrogen uptake and assimilation, as well as in the urea cycle, during infectious growth of F. oxysporum in G. melanella revealed increased transcript levels of arginase, which converts arginine to urea. Our results suggest a role for the urease accessory protein UreG in fungal virulence on plant and animal hosts.

The role of LysM effectors in fungal fitness. Anja Kombrink, Jason Rudd, Dirk-Jan Valkenburg, Bart Thomma. 1) Phytopathology, Wageningen University, Wageningen, Netherlands; 2) Department of Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Hertfordshire, United Kingdom.

LysM effector genes are found in the genomes of a wide range of fungal species. The encoded LysM effectors are secreted proteins that contain a varying number of LysM domains, which are carbohydrate-binding modules. EcP6, secreted by tomato leek mould fungus Cladosporium fulvum, is the first characterized LysM effector. We demonstrated that EcP6 specifically binds chitin, the major constituent of fungal cell walls that acts as a microbial-associated molecular pattern (MAMP) and triggers immune responses upon recognition by the host. EcP6 outcompetes plant receptors for chitin binding, and thus prevents the activation of immune responses. Many fungal genomes, including saprophytes, carry multiple LysM effector genes that share low sequence conservation and encode a varying number of LysM domains. We speculate that fungal LysM effectors might bind different carbohydrates and exert various functions in fungal fitness. In the fungal wheat pathogen Mycosphaerella graminicola, two LysM effectors were identified. Mg3LysM, but not Mg1LysM, suppresses chitin-induced immune responses in a similar fashion as EcP6. Interestingly, unlike EcP6, both Mg1LysM and Mg3LysM inhibit degradation of fungal hyphae by plant chitinases, revealing an additional function for LysM effectors in pathogen virulence. We recently observed that Mg3LysM binds to the bacterial cell wall constituent peptidoglycan. Similarly, a LysM effector from the saprophytic fungus Neurospora crassa showed peptidoglycan binding. We hypothesize that peptidoglycan binding by LysM effectors plays a role in the interaction of fungal species with bacterial competitors. The soil-borne fungal pathogen Verticillium dahliae contains seven LysM effectors genes of which one (Vd2LysM) is induced during tomato infection. Inoculation with two independent knock-out mutants revealed that Vd2LysM is required for full virulence of V. dahliae. However, Vd2LysM does not specifically bind chitin and does not function in a similar fashion as previous characterized LysM effectors. Thus, its function in virulence remains unclear.
Genes important for in vivo survival of the human pathogen Penicillium marneffei. Harshini C. Weerasinghe, Michael J. Payne, Hayley E. Bugeja, Alex Andrianopoulos. Genetics, The University of Melbourne, Parkville, Victoria, Australia.

Pathogenic fungi are having an increasing global impact in the areas of health, agriculture and the environment. As such it is essential to understand the mechanisms that fungi employ to survive and grow within a host. The emergence of many new “opportunistic fungal pathogens” has to a great extent altered the traditional view that pathogenicity was solely reliant on the inherent properties of the pathogen. In fact, the ability of a pathogen to cause disease in some hosts but not in others suggests that pathogenic determinants are complex and dynamic, and are largely dependent on specific pathogen-host relationships. Despite this there are conserved aspects of the interactions between host and pathogen. For example, hosts employ innate immune responses as an almost immediate recognition and attack mechanism against invading pathogens. Penicillium marneffei is a temperature dependent dimorphic fungus, growing in a hyphal form producing conidia at 25°C and as a yeast form at 37°C. Despite its importance as an opportunistic pathogen, little is known about the biology and mechanism of infection of P. marneffei. The infectious agents (conidia) are believed to be inhaled, reaching the alveoli of the lungs, where they are phagocytosed by alveolar macrophages for elimination. At this point that P. marneffei switches growth to a pathogenic yeast cell form, and is able to withstand macrophage cytotoxic attacks to cause infection. In order to understand how P. marneffei responds to the host, RNA-seq analysis was used to create a transcriptomic profile of P. marneffei, when infected in murine macrophages. These results were compared to RNA-seq data from hyphal (25°C) and yeast (37°C) cells grown in vitro in order to identify genes that are specifically upregulated during infection. Based on this analysis a group of genes of varying functions were chosen for gene deletion studies and tested for defects in pathogenicity. Among these is a group of Pep1-like aspartic endopeptidases which are a uniquely expanded family in P. marneffei and that show reduced virulence in a macrophage model.
Secondary Metabolism
Co-chairs: Gillian Turgeon and Bettina Tudzynski

Genomic profiles of secondary metabolism genes in Cochliobolus pathogens. B. Condon\textsuperscript{1}, D. Wu\textsuperscript{1}, K. Bushley\textsuperscript{2}, I. Grigoriev\textsuperscript{2}, C. Elliott\textsuperscript{4}, B. Howlett\textsuperscript{4}, B. G. Turgeon\textsuperscript{1}. 1) Plant Pathology & Plant-Microbiology, Cornell University, Ithaca, NY; 2) DOE Joint Genome Institute, Walnut Creek, CA; 3) Dept of Plant Pathology, Oregon State University, Corvallis OR; 4) School of Botany, University of Melbourne, Melbourne, AU.

The genomes of five Cochliobolus heterostrophus strains, two Cochliobolus sativus strains, and three additional Cochliobolus species (Cochliobolus victoriae, Cochliobolus carbonum, Cochliobolus miyabeanus) were sequenced at the Joint Genome Institute (JGI). These species are notable pathogens of economically important grasses and many produce a signature secondary metabolite host selective toxin, conferring high virulence to a host of a particular genotype. We catalogued the suites of secondary metabolism genes [focusing on those encoding backbone nonribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs)] in these genomes and performed comparative phylogenomic analyses within the Cochliobolus genus and in a kingdom-wide context. We found that NRPS and PKS genes were broadly conserved, disparately conserved, or species-unique. Distribution patterns for conserved or disparately conserved genes suggest an evolutionary mechanism involving rapid duplication, loss, and recombination of protein domains. Genes categorized as species unique within our dataset often had lone orthologs in phylogenetically distant species. Expanding genomic resources may reveal that ‘signature’ genes, formerly thought to be species-unique are present broadly, but sporadically, in other fungi. These results have strong implications for understanding evolution of genes for host selective toxins and associated virulence.

A biosynthetic gene cluster for the antifungal metabolite phomenoic acid in the plant pathogenic fungus, Leptosphaeria maculans. Candace Elliott\textsuperscript{1}, Damien Callahan\textsuperscript{1}, Daniel Schwenk\textsuperscript{3}, Markus Nett\textsuperscript{3}, Dirk Hoffmeister\textsuperscript{3}, Barbara Howlett\textsuperscript{3}. 1) School of Botany, University of Melbourne, Melbourne, Australia; 2) Metabolomics Australia, School of Botany, The University of Melbourne, Victoria 3010, Australia; 3) Friedrich-Schiller-Universität, Department Pharmaceutical Biology at the Hans-Knöll-Institute, Beutenbergstrasse 11a, 07745 Jena, Germany; 4) Leibniz Institute for Natural Product Research and Infection Biology e.V., Hans-Knöll-Institute, Beutenbergstrasse 11a, 07745 Jena, Germany.

Phomenoic acid, a long chain aliphatic carboxylic acid, is a major metabolite produced by Leptosphaeria maculans, a fungal pathogen of Brassica napus (canola). Early biosynthetic studies suggested that the methyl group derived from S-adenosylmethionine (SAM), whereas the incorporation pattern of [13C]acetate suggested a polyketidic origin of the linear portion of phomenoic acid (Devys et al., 1984). We have used domain modelling to predict a candidate polyketide synthase (PKS) for phomenoic acid biosynthesis. Of the 15 predicted polyketide synthases (PKS) in the L. maculans genome, seven were reducing with the appropriate domains (KS - keto-synthase; AT - acyltransferase; DH - dehydratase; MT - methyltransferase; ER - enoylreductase; KR - ketoreductase; ACP - acyl carrier protein) for the biosynthesis of phomenoic acid. The most highly expressed of these seven genes, PKS2, was silenced to 10% of that of wild type levels and the resultant mutant produced 25 times less phomenoic acid than the wild type did, indicating that PKS2 is involved in phomenoic acid biosynthesis. This gene is part of a cluster and nearby genes are co-regulated. A two-fold reduction in the expression of the adjacent transcriptional regulator C6TF, led to at least a 20-fold reduction in expression of PKS2, as well as of other genes in the cluster (P450, YogA, RTA1 and MFS), but not of the adjacent ChoK or a hypothetical gene (Hyp). This down-regulated mutant also showed a marked reduction in phomenoic acid production. Phomenoic acid is toxic towards another canola pathogen Leptosphaeria biglobosa ‘canadensis’, but L. maculans and to a lesser extent the wheat pathogen, Stagonospora nodorum are more tolerant. Phomenoic acid may play a role in allowing L. maculans to outcompete other fungi in its environmental niche.

Fusarin C biosynthesis in Fusarium fujikuroi: the fusarin C gene cluster, their function and regulation. Eva-Maria Niehaus\textsuperscript{1}, Karin Kleigrewe\textsuperscript{1}, Philipp Wiemann\textsuperscript{1}, Lena Studt\textsuperscript{1}, Hans-Ulrich Humpf\textsuperscript{1}, Bettina Tudzynski\textsuperscript{1}. 1) Institute of Plant Biology and Biotechnology, Schlossplatz 8, 48143 Muenster, Germany; 2) Institute of Food Chemistry, Corrensstr. 45, 48149 Muenster, Germany.

The filamentous fungus F. fujikuroi is known to produce a variety of structurally diverse secondary metabolites such as the plant hormones gibberellins, pigments and mycotoxins. In order to reduce the health risk of mycotoxins in food, feed and biotechnologically produced gibberellin preparations, identification of mycotoxin biosynthesis genes is of great importance. The recently sequenced genome of F. fujikuroi contains 17 polyketide synthases (PKS). So far only four of them can be linked to specific products: bikaverin, fusarins, fumonisins and fusarubins. The focus of this work is studying the biosynthesis and regulation of the mutagenic mycotoxin fusarin C by external signals, such as nitrogen availability and pH. Furthermore the involvement of potential transcription factors and global regulators such as AreA, AreB, G5, PaC and three members of the velvet-like complex (Vel1, Vel2, Lae1) were investigated. We show that all nine genes are co-expressed under nitrogen sufficient conditions. Chromatin immunoprecipitation (ChIP) experiments revealed a high level of H3K9 acetylation under these favorite conditions. By combination of gene deletion and overexpression of the cluster genes and co-cultivation of different mutants, we were able to identify the intermediates and finally unraveled the entire fusarin biosynthetic pathway which we are presenting in a model.
**CONCURRENT SESSION A**

**Cellular development integrating primary and induced secondary metabolism in the filamentous fungus *Fusarium graminearum*.** Jon Menke, Jakob Weber, Karen Broz, H. Corby Kistler. 1) Department of Plant Pathology, University of Minnesota, St. Paul, USA; 2) Molekulare Phytopathologie, Universität Hamburg, Germany; 3) USDA ARS Cereal Disease Laboratory, St. Paul, MN, USA.

Several species of the filamentous fungus *Fusarium* colonize plants and produce toxic small molecules that contaminate agricultural products, rendering them unsuitable for consumption. Among the most destructive of these species is *F. graminearum*, which causes disease in wheat and barley and often contaminates the grain with harmful trichothecene mycotoxins. Induction of these secondary metabolites occurs during plant infection or in culture in response to chemical signals. Here we report that trichothecene biosynthesis involves a complex developmental process that includes dynamic changes in cell morphology and the biogenesis of novel subcellular structures. Two cytochrome P-450 oxygenases (Tri4p and Tri1p) involved in early and late steps in trichothecene biosynthesis were tagged with fluorescent proteins and shown to co-localize to vesicles we call “toxisomes.” Toxisomes, the inferred site of trichothecene biosynthesis, dynamically interact with motile vesicles containing a predicted major facilitator superfamily protein (Tri12p) previously implicated in trichothecene export and tolerance. The immediate isoprenoid precursor of trichothecenes is the primary metabolite farnesyl pyrophosphate. When cultures are shifted from non-inducing to trichothecene inducing conditions, changes occur in the localization of the isoprenoid biosynthetic enzyme HMG CoA reductase. Initially localized in the cellular endomembrane system, HMG CoA reductase increasingly is targeted to toxisomes. Metabolic pathways of primary and secondary metabolism thus may be coordinated and co-localized under conditions when trichothecene synthesis occurs.

**LaeA sleuthing reveals cryptic gene clusters in pathogenic Aspergilli.** Nancy Keller, Wenbing Yin, Saori Amaike, Katharyn Affeld, JinWoo Bok, Daniel Schwenk, Dirk Hoffmeister, Joshua Baccile, Ry Forseth, Frank Schroeder. 1) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA; 2) Department of Plant Pathology, Department of Medical Microbiology and Immunology, and Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706, USA; 3) Department of Pharmaceutical Biology at the Hans-Knöll-Institute, Friedrich-Schiller-Universität, Beutenbergstrabe 11a, D7745 Jena, Germany.

The human and plant pathogenic Aspergilli, Aspergillus fumigatus and A. flavus, are known to produce a plethora of secondary metabolites. However, most of these metabolites are not yet characterized although their gene clusters are apparent from genomic sequence. In both species, the nuclear protein LaeA regulates the expression of many of these uncharacterized gene clusters. Following leads from laeA mutant microarray data, we created gene deletion and overexpression strains and used 2D NMR-based comparative metabolomic analyses to identify previously undescribed metabolites from both species. In A. fumigatus a tryptophan-derived iron(III)-complex, hexaethylene-astechrome (HAS), was found to be the major product of the cryptic has non-ribosomal peptide synthetase (NRPS) cluster. In A. flavus we show that two separate clusters encode enzymes that produce partially overlapping sets of novel piperazines, pyrazines, and morpholines. These L-tyrosine metabolites are activated by two NRPS-like proteins, LnaA and LnbA. Loss and overexpression of these metabolites impacted fungal development in these species.

**The KMT6 Histone H3 K27 Methyltransferase Regulates Expression of Secondary Metabolites and Development in *Fusarium graminearum*.** Kristina M. Smith, Lanelle R. Connolly, Michael Freitag. Department of Biochemistry and Biophysics, Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331.

The cereal pathogen *Fusarium graminearum* produces secondary metabolites toxic to humans and animals, yet coordinated transcriptional regulation of secondary metabolite gene clusters remains largely a mystery. By ChIP-sequencing we found that regions of the *F. graminearum* genome with secondary metabolite clusters are enriched for a histone modification, trimethylated histone H3 lysine 27 (H3K27me3), associated with gene silencing. This modification was found predominantly in regions that lack synteny with other *Fusarium* species, generally subtelomeric regions. H3K27me3 and di- or trimethylated H3K4 (H3K4me2/3), modifications associated with gene activity, are found in mutually exclusive regions of the genome. To better understand the role of H3K27me3, we deleted the gene for the putative H3K27 methyltransferase, KMT6, a homolog of *Drosophila* Enhancer of zeste, E(z). The kmt6 mutant lacks H3K27me3, as shown by western blot and ChIP-sequencing, displays growth defects, is sterile, and produces mycotoxins under conditions where they are not generated in wildtype (WT) strains. RNA-sequencing showed that genes modified by H3K27me3 are most often silent, as about 75% of the 4,449 silent genes are enriched for H3K27me3. Surprisingly, we found 22% of the 8,855 expressed genes enriched for H3K27me3. A subset of genes that were enriched for H3K27me3 in WT gained H3K4me2/3 in kmt6 (1,780 genes), and an overlapping set of genes showed increased expression. Almost 95% of the remaining 2,720 annotated silent genes showed no enrichment for either H3K27me3 or H3K4me2/3. In these cases absence of H3K27me3 is insufficient for expression, which suggests a requirement for additional factors for gene expression. Taken together, we show that absence of H3K27me3 allows expression of 14% of all annotated genes, resulting in derepression of predominantly secondary metabolite pathways and other species-specific functions, including potentially secreted pathogenicity factors. This study provides the framework for novel targeted strategies to control the "cryptic genome" and specifically secondary metabolite expression.
Secondary metabolism in *Botrytis cinerea*: the grey and pink sides of a pathogen. M. Viaud, H. Sghyer, J. Schumacher, A. Simon, B. Dalmais, J.M. Pradier. 1) INRA, BIOGER, Av. L. Brégnières, 78850 Grignon, France; 2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47906, USA.

Sequencing the genome of the grey mould fungus *Botrytis cinerea* revealed 40 secondary metabolism (SM) gene clusters corresponding to the biosynthesis of mostly unknown compounds including polyketides (21), terpenes (11) and non-ribosomal peptides (8). The two identified phytotoxic metabolites are the polyketide botcinic acid (BOA) and the sesquiterpene botrydial (BOT). Transcriptomic studies previously identified the corresponding BOA and BOT gene clusters. Deletion of the key enzyme BcBOA6 (PolyKetide Synthase) or BcBOT2 (SesquiTerpene Cyclase) encoding genes did not cause any defects, while bcboa6/bcbot2 double mutants were significantly impaired in necrotrophic development on several hosts suggesting that the two compounds have a redundant function. We are currently investigating how the BOT and BOA gene clusters are regulated: The BOA cluster contains a gene (bcboa13) encoding a C6H6 zinc finger transcription factor (TF). Surprisingly, while bcboa6 mutant has no virulence defect, bcboa13 mutant shows a drastic reduced virulence which is even more pronounced than that of the bcboa6/bcbot2 double mutant lacking both known toxins. A whole transcriptome analysis of bcboa13 mutant is underway to determine whether other genes apart from those of the BOA cluster are regulated by this TF. In opposite to the BOA cluster, the BOT cluster does not contain any TF-encoding gene. We therefore set up a Yeast-One-Hybrid library that contains the majority of *B. cinerea* TFs (393 out of 406) and screened it with the promoter of bcbot2. This led to the identification of a C2H2 TF called BcYOH1. Inactivation of bcyoh1 gene and expression analysis revealed that this TF acts as a global regulator of SM, regulating the expression of genes of the BOT, BOA, and 20 other SM clusters. As in other fungi, the Velvet complex takes part in regulation of light-dependent development and SM in *B. cinerea*. Gene inactivation of bcvell1, however, does not significantly modify the production of BOA and BOT. Instead, BcVEL1 plays a significant role in the regulation of oxalic acid (OA) formation and pigmentation: it regulates negatively the synthesis of melanin and positively the synthesis of OA and bikaverin (BIK) another polyketidic pigment that is only produced in rare pink strains of *B. cinerea*.

Is fungal secondary metabolism regulated by competing insects? Annika Regulin, Nancy Keller, Frank Kempken. 1) Department of Botany, Christian-Albrechts University, Kiel, Germany; 2) Department Medical Microbiology and Immunology, Dept of Bacteriology, UW-Madison, USA.

Fungi synthesize an astonishing variety of secondary metabolites, some of which belong to the most toxic compounds in the living world. Even though little is known about the benefit of these metabolites, the ability to regulate the secondary metabolism might be seen as an evolutionary adaptation. Presumably fungi regulate secondary metabolites (e.g. mycotoxin) in response to confrontation with natural competitors like insects to guarantee efficient exploitation of environmental resources (1-3). Admittedly it should be mentioned that secondary metabolites are not the only defence mechanisms of fungi (4). In order to enlighten the biological function of these secondary metabolites with reference to chemical defence reactions of insect-fungal interactions, we utilized complementary approaches of experimental ecology and functional genomic techniques. The vinegar fly *Drosophila melanogaster* and its natural antagonist *Aspergillus nidulans* are used as an ecology model system. To analyse fungal up- or down regulated target genes in the interaction of *A. nidulans* with *Drosophila* larvae microarray analysis was performed. This led to the identification of secondary metabolite genes up- or down-regulated under these conditions. Quantitative RT-PCR was employed to analyze secondary metabolite gene expression at different time points. Fungal single, double and triple mutations of identified up-regulated genes are currently analyzed in confrontation assays to identify potential modifications in gene expression and the survival rate of larvae concerning to chemical defense reaction of fungus-insect interaction compared to wild type. This could reveal insights about the biological function of secondary metabolite genes and clusters such as *stc* and *mdp*. (1.) Rohlfis, M., Albert, M., Keller, N.P., and Kempken, F. (2007) Biol Lett 3, 523-25. (2.) Kempken, F., and Rohlfis, M. (2010) Fungal Ecol 3, 107-14. (3.) Rohlfis, M., Trienens, M., Fohgrub, U., and Kempken, F. (2009) in “The Mycota XV. (Anke, T., Ed.), Springer Heidelberg, New York, Tokyo, pp. 131-51 (4.) Kempken, F. (2011) Mol Ecol 20, 2876-77.
Light Sensing and Circadian Rhythms
Co-chairs: Luis Larrondo and Reinhard Fischer

Circadian rhythms in gene expression in Aspergillus nidulans. Maria Olmedo1,2, Julio Rodriguez-Romo3,4, Julian Röhrig3, Martha W. Merr01,2, Reinhard Fischer5. 1) Institute for Medical Psychology, Ludwig-Maximilians-Universität-München, München, Bavaria, Germany; 2) Department of Molecular Chronobiology, University of Groningen, The Netherlands; 3) Institute for Applied Biosciences, Karlsruhe Institute of Technology, Germany; 4) Centre for Plant Biotechnology and Genomics Universidad Politécnica de Madrid(UPM) Campus de Montegancedo Autopista M-40 (Km 38) 28223-Pozuelo de Alarcón (Madrid).

The circadian clock is an endogenous timekeeper that allows organisms to predict cyclic changes in their environment derived from the rotational movement of the Earth. The fungus Neurospora crassa has substantially contributed to the understanding of the mechanism of the circadian clock, mainly due to the presence of an easily assayable rhythm in conidiation. Our understanding of circadian rhythms tells us that they are present in most organisms (from bacteria to humans), yet they have been difficult to detect in other fungal species, likely due to the absence of a clear readout. To circumvent this problem, we have adopted a tool developed for the study of the clock in Neurospora and other model organism, namely bioluminescent reporter proteins, to study circadian rhythms in Aspergillus nidulans, where a previous report has suggested a circadian clock (Greene et al. 2003). The characterization of the Aspergillus clock represents a further step in the understanding of the biology of this fungal genus, which is of great importance for medical, industrial and agricultural reasons. We have produced strains with the promoters of Neurospora clock controlled gene homologs con1, cgaA and pdaA fused to the (A. nidulans optimized) firefly luciferase ORF. We observe an oscillation in the bioluminescence signal in these strains in constant conditions, implying the presence of an endogenous oscillator. This free running rhythm is detectable in both constant light and darkness and is entrained by environmental signals. Using these reporters we are studying the contribution of the wc (white collar) homologs and other photoreceptors to the Aspergillus clock. The protein WC-1 is a central molecule of the best-studied Neurospora oscillator, which also comprises the protein FRQ (FREQUENCY). The absence of a FRQ homolog in Aspergillus implies that the characterization of its clockwork may unveil new components of additional Neurospora oscillators.

Circadian clock-gated cell division cycles in Neurospora crassa. C. Hong1, J. Zamborszky2, M. Baek3, K. Ju4, H. Lee5, L. Larrondo6, A. Goity7, A. Csikasz-Nagy8. 1) Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH; 2) Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile; 3) Randall Division of Cell and Molecular Biophysics, and Institute for Mathematical and Molecular Biomedicine, King’s College London, London, SE1 UL, UK.

Asynchronous nuclear divisions are readily observed in filamentous fungi such as Ashbya gossypii and Neurospora crassa. Our computational simulations, however, predict synchronous circadian clock-gated mitotic divisions if the division cycles of such multicellular organisms are coupled with circadian rhythms. Based on this hypothesis, we investigate the coupling between the cell cycle and the circadian clock in Neurospora crassa. First, we show WC-1-dependent light-induced expression of stk-29 mRNA (homolog of wee1), which suggests that there exists a conserved coupling between the clock and the cell cycle via STK-29 in Neurospora as in mammals. Second, we demonstrate that G1 and G2 cyclins, CLN-1 and CLB-1, respectively, show circadian oscillations with luciferase bioluminescence reporters. Moreover, clb-1 and stk-29 gene expressions show circadian clock-dependent light-induced phase shifts, which may alter the timing of divisions. Third, we show circadian clock-dependent synchronized nuclear divisions by tracking nuclear morphology with histone hH1-GFP reporter. Synchronized divisions occur late in the evening, and they are abolished in the absence of circadian rhythms (frq10). Our findings demonstrate the importance of circadian rhythms for synchronized mitotic cycles and establish Neurospora crassa as an ideal model system to investigate mechanisms that couple the cell cycle and the circadian clock.

Light regulates growth, stress resistance and metabolism in the fungal pathogen Aspergillus fumigatus. Kevin K. Fuller, Carol S. Ringleberg, Jennifer J. Loros, Jay C. Dunlap. Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH.

Light serves as an important environmental cue that influences developmental and metabolic pathways in a variety of fungi. Interestingly, orthologs of a conserved blue light receptor, WC-1, promote virulence in two divergently related pathogenic species, Cryptococcus neoformans and Fusarium oxysporum, suggesting that photosensory systems may be conservatively linked to fungal pathogenesis. Aspergillus fumigatus is the predominant mold pathogen of immunocompromised patients, but if and how the organism responds to light has not been described. In this report, we demonstrate that the fungus can indeed sense and distinctively respond to both blue and red portions of the visible spectrum. Included in the A. fumigatus photoreceptors is a reduction in conidial germination kinetics, increased hyphal pigmentation, enhanced resistance to acute ultra-violet and oxidative stresses, and an increased susceptibility to cell wall perturbation. Through gene deletion analysis we have found that the WC-1 ortholog, LreA, is a bone fide blue light receptor in A. fumigatus that is required for the photopigmentation response. However, the DlreA mutant retains several blue light mediated responses, including the germination and stress resistance phenotypes, suggesting other blue light receptors are operative in this fungus. We also show that the putative red light sensing phytochrome, FphA, is involved with some, but not all, blue light specific phenotypes, indicating a complex interaction between red and blue light photosystems in A. fumigatus. Finally, whole genome microarray analysis has revealed that A. fumigatus displays broad patterns of gene induction and repression upon exposure to light. Affected genes are largely metabolic and include those involved in lipid and sterol synthesis, respiration, carbohydrate catabolism, amino acid metabolism and metal ion homeostasis. Taken together, these data demonstrate the importance of the photic environment on the physiology of A. fumigatus and provide a foundation for future studies into an unexplored area of this important pathogen.
Shedding light on Botrytis biology: characterization of the WC1 photoreceptor and FRQ homologues in the necrotrophic plant pathogen Botrytis cinerea. Paulo Canessa1, Montserrat Hevia2, Julia Schumacher2, Paul Tudzynski2, Luis F Larrondo2. 1) Depto. Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Santiago, Chile; 2) Purdue University, USA; 3) West F Wilhelms Univ Muenster Germany.

The non-pathogenic fungus Neurospora crassa has been a premier model for the study of circadian clocks, but it is the only fungus where clocks have been molecularly characterized. In Neurospora the oscillator is based on the transcriptional control of *frq* expression by the White Collar complex (WCC) composed of WC-1, a blue-light photoreceptor and WC-2, its functional partner. The WCC activates *frq* expression until FRQ promotes the phosphorylation of the WCC, shutting down its transcriptional activity. Then FRQ is progressively phosphorylated and degraded setting the bases of circadian rhythmicity. While circadian clocks modulate defense in plants, little is known about its participation in fungal pathogenicity. In an effort to characterize light responses and the circadian clock in B. cinerea, we have generated KO of homologues of wc-1 and *frq* (bcwc1 and bcfrql). Our results indicate that light can modulate *B. cinerea* growth and activates transcriptional responses. Most -but not all of them- are mediated by BcWC1. Moreover, the obtained bcwc1 KO mutant strains exhibit enhanced conidiation patterns and susceptibility to hydrogen peroxide. Virulence assays show significantly reduced lesion formation under constant light conditions (LL) and light:dark cycles (LD), but not under constant darkness (DD). Further analysis of the bcwc1 KO strains indicates non-altered ROS patterns in planta but delayed penetration on onion epidermis. RT-qPCR experiments have confirmed daily oscillations in *bcfrql* levels under LD and DD conditions. Using a BcFRQL-luciferase translational reporter, we have further observed oscillatory levels of the BcFRQL protein under both temperature cycles and constant culture conditions. Both BcFRQl and *bcfrql* respond to light pulses, while under oscillatory culture conditions, BcFRQL anticipates cyclical-environmental changes, a key characteristic of circadian behavior. Finally, *bcfrql* KO mutants also exhibit impaired pathogenicity, with a drastic reduction in spore production. To our knowledge, these results provide the first evidence of functional light/circadian machineries in *B. cinerea*, showing that in this necrotroph, homologues of circadian clock components modulate the plant pathogen interaction from a fungal perspective. Fondecyt 1090513, postdoc 3110127, AT24121100, ICGEB CRPCHI0902.

The transcription factor FL is phosphorylated and interacts with a trehalose related protein in Neurospora crassa. Carmen Ruiger-Herreros1, Gencer Sancar2, Michael Brunner2, Luis M. Corrochano1. 1) Departamento de Genetica, Universidad de Sevilla, Spain; 2) BZH, Universidad Heidelberg, Germany.

Several environmental cues, including light, promote a developmental transition in Neurospora crassa that leads to the formation of conidia. Conidiation is controlled by FLUFFY (FL), a zinc finger transcription factor. Light activates the transcription of *fl* through the transient binding of the WC complex to the *fl* promoter. Light also activates the transcription of several conidiation genes in Aspergillus nidulans, and their Neurospora homologs have been identified in the Neurospora genome. We have assayed the activation by light of the Neurospora homologs of *A. nidulans* conidiation genes (*flba, flbc, flbd, medA* and *stuk*), and the Neurospora conidiation gene con-10 as a control. Unlike con-10, none of the Neurospora homologs of the *A. nidulans* conidiation genes were induced by light in vegetative mycelia. However, we found that deletion *fl* resulted in light-dependent mRNA accumulation for all the conidiation genes. This result indicated that the absence of FL allows the binding of the WC complex to the promoter of these genes to activate transcription in a light-dependent manner. We have assayed the amount of WC proteins in the Dfl and wild type strains but we did not find any difference between the two strains. We expect to identify additional genes deregulated by the absence of FL after massive sequencing of total RNA (RNAseq) using a Dfl strain and wild-type strain in dark and light conditions. We have investigated the role of FL during conidiation in Neurospora using a tagged version of FL. FL is present in vegetative mycelia but the amount increases after light exposure. We observed several forms of FL due to phosphorylation, and we have determined by mass spectrometry that FL is phosphorylated in several residues. We have immunoprecipitated FL that interacts with proteins that may interact with FL. We have found a protein that interacts with FL in different growth conditions. This protein has been described in other organisms and plays a role in the ability to grow in the presence of trehalose. Since FL is a transcription factor, we have used FL::3XFLAG strain to do ChIPseq in order to identify the putative binding sites of FL to the DNA. We expect that the results from these experiments will help us to understand in more detail the role of FL in the activation of gene transcription during development.

Regulation of gene expression in response to light in Trichoderma atroviride. Jose Cetz1, Nohemi Carreras-Villaseñor1, Monica Garcia-Esquibel2, Ulises Esquivel-Naranjo1, Jose M. Villalobos-Escobedo1, Cei Abreu-Goodger1, Alfredo H. Herrera-Estrella1. 1) National Laboratory of Genomics for Biodiversity, CINVESTAV-IPN, Irapuato, Irapuato, Mexico; 2) Facultad de Ciencias Naturales. Universidad Autónoma de Queretaro. Queretaro, Mexico.

*Trichoderma* is used as a photomorphogenetic model due to its ability to conidiate upon exposure to light. In total darkness, *T. atroviride* grows synchronously as a mycelium provided that nutrients are not limiting. However, a brief pulse of blue light given to a radially growing colony induces synchronous sporulation. Photococidiation in *Trichoderma* is controlled by *b1rl* and *b2rl*, orthologs of the *N. crassa* white-collar genes. We applied high-throughput sequencing technology to RNA samples from the wild type strain grown in the dark or after exposure to a pulse of white or blue-light, as well as from a photoreceptor mutant (Dbir1-1) exposed to white light. We identified 331 transcripts regulated by white light and 204 responsive specifically to blue light, both induced and repressed, the majority of them *b1rl* dependent. Among the genes identified there is a set of transcription factors. We have obtained gene disruption mutants of several of the transcription factors, and all potential light receptors. Using this strategy we have obtained mutants that do not conidiate in response to light, as well as mutants that do not require this stimulus to conidiate, and a connection between light and carbon metabolism. Further, transcriptional analysis in RNAi machinery mutants indicated that light induced conidiation is defective in the Ddc2r, Ddc1Ddcr2, Ddr2 and Dago2 mutants, and significant differences were found in the set of light responsive genes between the dicer mutants and the wild type. These data indicate that in *T. atroviride*, the RNAi machinery plays an important role in controlling development.
Shedding light on secondary metabolite cluster gene expression, sporulation, UV-damage repair and carotenogenesis in the rice pathogen *Fusarium fujikuroi*. Philipp Wiemann, Bettina Tudzynski. Institut für Biologie und Biotechnologie der Pflanzen Westfälische Wilhelms-Universität Münster Schlossplatz 8 48143 Münster Germany.

The rice pathogen *Fusarium fujikuroi* produces economically important secondary metabolites like gibberellic acids and carotenoids as well as mycotoxins like bikaverin and fusarin C. Their production is activated in response to environmental stimuli such as light, pH or nutrient availability. In this study, we evaluate the effects of light and different putative light receptors on growth and differentiation as well as secondary metabolism. Bimolecular fluorescence complementation proved that homologs of the *Neurospora crassa* White Collar proteins in *F. fujikuroi* (WcoA and WcoB) form a nuclear localized complex (WCC) that is needed for full functionality. Deletion and complementation of both genes revealed that the WCC represses bikaverin gene expression in constant light conditions and induces immediate light-dependent carotenoid gene expression as shown by northern blot analyses. Additionally the WCC represses conidiogenesis in response to light. The effects observed regarding bikaverin and carotenoid gene expressions as well as conidiogenesis are antagonistically to the ones observed in the velvet mutant, making a connection between the WCC and the velvet complex feasible, similarly to the situation in *Aspergillus nidulans*. Since carotenoid production was maintained in both wcoA and wcoB single as well as in wcoA/B double mutants in constant light conditions, we focused on characterization of additional putative light receptors in *F. fujikuroi*. Deletion of the phytochrome-like-encoding gene *ph1* did not show any significant phenotype. Deletion of *phl1*, coding for a cryptochrome/photolyase demonstrated impaired carotenoid biosynthesis gene expression upon exposure to light. Additionally, gene expression and HPLC analyses of these mutants demonstrated loss of fusarin C gene expression and concomitant production formation compared to the wild type, suggesting a distinct transcriptional activity for this barely characterized class of enzymes. Finally UV mutagenesis experiments and qRT-PCR demonstrate that WcoA, WcoB and Phl1 are involved in UV-damage repair most likely by transcriptionally activating *phr1*, encoding a CPD-photolyase. The data presented here allow us to draw a first model of how light receptors function in a signaling network in the rice pathogen *F. fujikuroi*. 

**CONCURRENT SESSION ABSTRACTS**


Departamento de Genética y Microbiología, Universidad de Murcia, 30100 Murcia, Spain.

Light regulates developmental and physiological processes in a wide range of fungi. Particularly, Zygomycete fungi have developed complex mechanisms to control the responses to light that await detailed characterization at molecular level. The zygomycete *Mucor circinelloides* is a good model for this purpose because its genome has been sequenced and several molecular tools are available. *Mucor*, like other Zygomycetes, has three *white collar-1* genes (*mcwc-1a*, *mcwc-1b* and *mcwc-1c*) that code for proteins which present characteristics of photoreceptors. Each *mcwc-1* gene controls a specific response to light. Thus, *mcwc-1a* and *mcwc-1c* control phototropism and photocarotenogenesis, respectively, whereas the *mcwc-1b* function in regulation by light has not been proved. In order to deepen in the regulation by light in *Mucor*, a systematic approach using microarrays was followed to characterize white light-inducible transcriptional changes in wild-type and knockout mutants for each *mcwc-1* gene. Analysis of microarray data revealed that light is mainly a positive signal for transcription in *Mucor*, as in other fungi, since 123 genes were up-regulated in the wild-type strain in response to light, whereas only 26 were down-regulated, considering a threshold of threefold change. Genes strongly induced by light included genes known to be up-regulated by light, like the carotenogenic gene *carB* (74-fold), cryptochrome (45-fold) and *mcwc-1c* (22-fold), supporting reliability of the microarray data. Although many of up-regulated genes code for proteins implicated in protection against light-induced damage, several of them code for protein involved in signal transduction that could be involved in light responses like phototropism. Transcriptomic analysis of *mcwc-1* mutants showed that induction of around 60% of the genes is mediated by *mcwc-1a*, whereas only 1% is mediated by *mcwc-1c* and none is mediated by *mcwc-1b*, suggesting that *mcwc-1a* is the main photoreceptor. Searching for cis-acting regulatory motifs upstream of genes regulated by *mcwc-1a* identified consensus sequences similar to those found in light regulated genes of *Neurospora crassa*. Moreover, the identification of a small group of genes regulated by the three *mcwc-1* genes points out that the three proteins form complexes to regulate gene expression. Funded by MINECO (BFU2012-32246), Spain.
CONCURRENT SESSION ABSTRACTS

Saturday, March 16  2:00 PM–5:00 PM
Fred Farr Forum

Fungal Evo-Devo
Co-chairs: Steve Harris and Brian Shaw

The Molecular Foundations of the Fungal Lifestyle. Antonis Rokas. Department of Biological Sciences, Vanderbilt University, Nashville, TN.

A defining characteristic of Fungi is that, in contrast to plants and animals, they are typically embedded in their food and digest it externally in the presence of competitors (think of the blue lines of mold in blue cheese). Thus, different fungi specialize in “eating” different foods (hence their diverse primary metabolism), and because digestion happens externally, fungi have also evolved potent food defense mechanisms (hence their diverse secondary or specialized metabolism). In many ways, the genes involved in fungal primary metabolism are their “teeth”, whereas the genes involved in secondary metabolism are their “horns”, “spines” and “claws”. I will use examples from our recent studies on fungal human pathogens, domesticated fungi and fungal genome evolution to argue that metabolism and more generally physiology is fundamental to the fungal lifestyle. In contrast to plants and animals, in which most phenotypic evolution proceeds largely through developmental changes affecting growth and morphological form, phenotypic evolution in the fungal kingdom occurs largely through changes in metabolism.


The regulatory pathway controlling conidiation in N. crassa consists of five genes, acon-4 (aconidiate), fl (fluffy), acon-3, csp-1 (conidial separation) and csp-2. The first three genes are required for the transition of aerial hyphae from filamentous growth to the budding pattern resulting in proconidial chain formation. Maturation of the proconidial chain to individual conidia relies on the activation of genes for two additional transcription factors, csp-2 and csp-2 that are required for proper interconidial septum formation and spore release. High throughput mRNA sequencing defined a set of genes induced during conidiation and allowed us to identify genes for cell wall degrading enzymes required for conidial separation. Based on expression patterns and epistasis among the regulators, we defined the order of gene action required for conidial morphogenesis from the initiation of budding to the release of mature conidia.

Comparative developmental morphology in lentinoid mushrooms: toward a new fungal evo-devo? David S. Hibbett. Biology, Clark University, Worcester, MA.

Fungi produce a mind-boggling diversity of complex forms, including mushrooms, puffballs, coral fungi, and others. Reconstructing patterns of morphological transformations has been a major focus of fungal molecular systematics, and developmental morphology has been a traditional source of characters for fungal taxonomy. However, few studies have explicitly compared developmental programs in a phylogenetic context, and research into the genetic bases of morphological evolution in fungi has lagged far behind that in plants and animals. In this talk, I will review three cases of naturally occurring developmental variation in the polyphyletic “lentinoid” fungi, which may suggest profitable avenues for studies in fungal evo-devo: 1) Normally agaricoid species of Lentinus, Neolentinus and Lentinellus may be induced to produce coralloid forms under conditions of light deprivation or low temperatures. These phenomena provide examples of phenotypic plasticity that may reflect evolvability of developmental programs. 2) Panus rudis has a short lateral stipe and fruits directly on wood, while the closely related P. fulvus has a dramatically elongated stipe and fruits immersed in leaf litter. The developmental transformation between forms suggests a case of hypermorphosis, with a delayed onset of pileus initiation in P. fulvus. Light-induced formation of pilei in P. rudis may provide clues to the mechanisms of offset in stipe elongation. 3) Lentinus tigrinus is a gilled mushroom that has a naturally occurring “secotioid” mutant that has an enclosed, puffball-like hymenophore. The secotioid morphology appears to be conferred by a recessive allele at a single locus. Resolving the gene(s) responsible for the secotioid phenotype may provide clues to the evolution of gastromycetes. Complete genome sequences have been (or are being) produced for species of Lentinus, Panus, Lentinellus and Neolentinus, which will provide opportunities to study the mechanisms underlying inter- and intraspecific developmental variants in lentinoid mushrooms.

The Cdc42 GTPase module and the evolution of conidiophore architecture in Aspergillus. Steven D. Hibbett. Center Plant Sci Innovation, Univ Nebraska, Lincoln, NE.

Conidiophores are reproductive structures that enable filamentous fungi to produce and disseminate large numbers of asexual spores. The diversity in conidiophore morphology is sufficiently large to serve as a basis for fungal systematics. Aspergillus and Penicillium species are members of the family Trichocomaceae that form conidiophores with characteristic architecture. Whereas the Penicillium conidiophore appears to be a modified branched hyphal structure, the Aspergillus conidiophore is seemingly more complex and includes additional cell types. Here, it is proposed that the “aspergillioid” conidiophore may have evolved from a “penicillioid” ancestor via changes in expression of key regulators of the GTPases Cdc42 and RacA. In particular, mutations that affect these regulators in A. nidulans dramatically alter conidiophore morphology by eliminating terminal vesicles and permitting formation of branched stalks. Because the transcriptional regulatory network that controls conidiophore development in Aspergillus is well characterized, further study of how this network links to regulators of Cdc42 should provide fundamental insight into the evolution of developmental morphogenesis in fungi (i.e., fungal evo-devo).

Cdc14 association with basal bodies in the oomycete Phytophthora infestans indicates potential new role for this protein phosphatase. Audrey M.V. Ah-Fong, Howard S. Judelson. Plant Pathology & Microbiology, University of California, Riverside, CA.

The dual-specificity phosphatase Cdc14 is best known as a regulator of cell cycle events such as mitosis and cytokinesis in yeast and animal cells. However, the diversity of processes affected by Cdc14 in different eukaryotes raises the question of whether its cell cycle functions are truly conserved between species. Analyzing Cdc14 in Phytophthora infestans should provide further insight into the role of Cdc14 since this organism does not exhibit a classical cell cycle. Prior study in this organism already revealed novel features of its Cdc14. For example, instead of being post-translationally regulated like its fungal and metazoan relatives, PiCdc14 appears to be mainly under transcriptional control. It is absent in vegetative hyphae where mitosis occurs...
and expressed only during the sporulation stages of the life cycle which are mitotically quiescent, in contrast to other systems where it is expressed constitutively. Since transformants overexpressing PiCdc14 exhibit normal nuclear behavior, the protein likely does not play a critical role in mitotic progression although PiCdc14 is known to complement a yeast Cdc14 mutation that normally arrests mitosis. Further investigation into the role of PiCdc14 uncovered a novel role. Subcellular localization studies based on fusions with fluorescent tags showed that PiCdc14 first appeared in nuclei during early sporulation. During the development of biflagellated zoospores from sporangia, PiCdc14 transits to basal bodies, which are the sites from which flagella emerge. A connection between Cdc14 and flagella is also supported by their phylogenetic distribution, suggesting an ancestral role of Cdc14 in basal bodies and/or flagellated cells. To help unravel the link between PiCdc14 and the flagella apparatus, searches for its interacting partners using both yeast two hybrid and affinity purification are underway. Together with co-localization studies involving known basal body/centrosome markers such as centrin and gamma-tubulin, the location and hence the likely roles of PiCdc14 will be revealed.

Molecular Determinants of Sporulation in Ashbya gossypii, Jurgen W. Wendland, Lisa Wasserstrom, Klaus Lengeler, Andrea Walther. Yeast Genetics, Carlsberg Laboratory, Copenhagen V, Kopenhagen V, Denmark.

Previously we have analysed the pheromone response MAPK signal transduction cascade in A. gossypii. The major findings were (i) deletion of both pheromone receptor genes STE2 and STE3 did not inhibit sporulation whereas (ii) deletion of the transcription factor STE12 resulted in hypersporulation (Wendland et al. 2011). Here we present our analysis of key A. gossypii homologs of Saccharomyces cerevisiae sporulation specific genes. We show that mutants in IME1, IME2, KARA, and NDT80 are blocked in sporulation. Mutants in IME4, KARA, and UME6 also confer a vegetative growth defect. IME4 expression was found during vegetative growth while IME2 was not detected under these conditions. We performed transcriptional profiling of non-sporulating strains and determined a core set of about 50 down-regulated sporulation specific genes in these mutants. Interestingly, this set of down-regulated genes is upregulated in the A. gossypii ste12 mutant providing regulatory evidence of the hyopersporulation phenotype of this mutant. Other genes identified in the RNAseq data indicated that during development of sporangia metabolic genes for nutrient uptake are active. Therefore we performed Return-To-Growth assays with mutants inhibited in the sporulation pathway. These strains were kept under conditions in which the wild type initiates sporulation. This lead to induction of sporangium formation, a stage at which these strains remained. Supply of new nutrients resulted in hyphal outgrowth in all mutants indicating that after initiation of the sporulation program A. gossypii can revert to vegetative growth at different stages. In addition we identified differential regulation of two endoglucanases encoded by ENGI and ENG2. While ENGI was not differentially regulated, ENG2 was down-regulated in e.g. ime1 but strongly up-regulated in ste12. Deletion analysis of ENG2 showed that Eng2 is required for hyphal fragmentation into individual sporangia. We can thus provide a detailed overview of the genetic regulation of sporulation in A. gossypii. A comparison with S. cerevisiae highlights the role of KAR4 in sporulation upstream of IME1. Finally, our study provides further evidence that the pheromone signaling response MAPK-cascade in A. gossypii has a regulatory control function over sporulation alongside regulation of sporulation by nutritional cues.

THE velvet regulators in Aspergilli. Heesoo Park, Jae-Hyuk Yu. Bacteriology, University of Wisconsin Madison, Madison, WI.

The velvet regulators are key players coordinating fungal growth, differentiation and secondary metabolism in response to various internal and external cues. All velvet family proteins contain the conserved velvet homology motif (~190 a.a.), and define a novel class of fungal specific transcription factors with the DNA binding ability. Some velvet regulators form time and/or cell type specific complexes with other velvet regulators or non-velvet proteins. These complexes play differential roles in regulating growth, development, sporogenesis and toxigenesis. Among the velvet complexes, the VelB-VosA hetero-complex acts as a functional unit conferring the completion of sporogenesis (focal trehalose biogenesis and spores wall completion), and the long-term viability of spore, and the attenuation of conidial germination in the model filamentous fungus Aspergillus nidulans. Both velB and vosA are activated by AbaA in developing cells, and the VelB-VosA complex plays a dual role in activating genes associated with spore maturation and in exerting negative feedback regulation of developmental genes. Interestingly, the VelB-VosA complex plays similar yet somewhat distinct roles in spore maturation, dormancy and germination in Aspergillus fumigatus and Aspergillus flavus. A comprehensive model depicting the roles of the velvet regulators in aspergilli is presented.

A network of HMG-box transcription factors regulates mating cycle in the fungus Podospora anserina. J. Ait-Benkhali1,2, E. Coppin1,2, S. Brun1,2,3, T. Martin1, C. Dixiel1,2, R. Debuchy2. 1) Univ Paris-Sud, Institut de Génétique et Microbiologie, Orsay, France; 2) CNRS, Institut de Génétique et Microbiologie, Orsay, France; 3) UFR des Sciences du Vivant, Université Paris-7 Diderot, Paris, France; 4) Department of Plant Biology and Forest Genetics, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Uppsala, Sweden.

High-mobility group B proteins are eukaryotic DNA-binding proteins characterized by the HMG-box functional motif. These transcription factors play a pivotal role in global genomic functions and in the control of genes involved in specific developmental or metabolic pathways. The filamentous ascomycete Podospora anserina contains 12 HMG-box genes. Of these, four have been previously characterized; three are mating-type genes that control fertilization and development of the fruiting-body, whereas the last one encodes a factor involved in mitochondrial DNA stability. Systematic deletion analysis of the eight remaining uncharacterized HMG-box genes indicated that none were essential for viability, but that seven were involved in the sexual cycle. Two HMG-box transcription factors display striking features. Pa_1_13940, an ortholog of SpSte11 from Schizosaccharomyces pombe, is a pivotal activator of mating-type genes in P. anserina, whereas Pa_7_7190 is a repressor of several phenomena specific to the stationary phase, most notably hyphal anastomoses. Constitutive expression of mating-type genes in a DPa_1_13940 strain did not restore fertility, indicating that Pa_1_13940 has additional functions related to sexual reproduction besides activating mating-type genes. RT-qPCR analyses of HMG-box genes in different HMG-box deletion strains indicated that Pa_1_13940 is at the hub of a network of several HMG-box factors that regulate the sexual cycle. Complementation experiments with a strain deleted for mating-type genes revealed that this network control fertility genes in addition to mating-type target genes. This study points to the critical role of the HMG-box members in sexual reproduction in fungi, as 11 out of 12 members were involved in the sexual cycle in P. anserina.

Pa_1_13940 and SpSte11 are conserved transcriptional regulators of mating-type genes, although P. anserina and S. pombe have diverged 1.1 billion years ago. Two HMG-box genes, SOK9 and its upstream regulator SRY, play also an important role in sex determination in mammals. The mating-type genes and their upstream regulatory factor form a module of HMG-box genes similar to the SRY/SOX9 module, suggesting it may be ancestral in Opisthokonta.
Microbial Responses to a Changing Climate: Implications for the Future Functioning of Terrestrial Ecosystems. Donald R. Zak, University of Michigan, Ann Arbor, MI.

Soil harbors a phylogenetically diverse community of microorganisms whose physiological activity mediates the biogeochemical cycling of carbon and nitrogen at local, regional, and global scales. These microbial communities are structured by the physical environment as well as the availability of growth-limiting resources (i.e., organic compounds in plant detritus). Presently, human activity is manipulating both the physical conditions and the availability of limiting resources to soil microbial communities at a global scale, but the implications of doing so for the future functioning of ecosystems is presently unclear. In this presentation, I will discuss the ways in which humans are manipulating the ecological constraints on microbial communities in soil, the compositional and functional responses that may result, and identify gaps in our knowledge that limit our ability to anticipate the response of microbial communities and ecosystem processes in a changing environment. Using an array of metagenomic approaches, I will provide evidence that rates of atmospheric N deposition expected in the near future can down regulate the transcription of fungal genes with lignocellulolytic function, thereby altering microbial community composition, slowing plant litter decay, and increasing soil C storage. This mechanism is not portrayed by any biogeochemical model simulating ecosystem response to atmospheric N deposition, and it demonstrates that microbial communities in soil may respond to a changing environment in ways that have unanticipated consequences for the future functioning of terrestrial ecosystems.

The Interaction of Mycoplasma-related Endobacteria with their Arbuscular Mycorrhizal Fungal Host. Mizue Naito, Teresa Pawlowska. 1) Dept. of Microbiology, Cornell University, Ithaca, NY; 2) Dept. of Plant Pathology & Plant-Microbe Biology, Cornell University, Ithaca, NY.

Arbuscular mycorrhizal fungi (AMF), comprising the monophyletic phylum Glomeromycota, are obligate biotrophs, and form symbiotic associations with 80% of terrestrial plants. AMF associate symbiotically with the roots of plants, and are specialized in the transfer of nutrients from the soil to the plant host. In return for increased nutrient uptake, the plants supply AMF with up to 20% of their photosynthetically derived carbohydrates. Thus, AMF symbiosis contributes significantly to global nutrient cycling and terrestrial ecosystems. AMF have been known to harbour two types of bacteria in their cytoplasm: (i) the Burkholderia-related Candidatus Glomeribacter gigasporarum and (ii) a Mycoplasma-related bacteria, which we refer to as Mycoplasma-related endobacteria (MRE). MRE live freely in the AMF cytoplasm, and have been found associated with all lineages of AMF worldwide. Virtually nothing is known about the MRE, such as their evolution, biological capabilities, and whether they are mutualists or parasites of their AMF hosts. In order to understand the nature of this symbiosis, and determine the role that the MRE play in arbuscular mycorrhiza, next generation sequencing (Roche 454 and Illumina) was performed on MRE isolated from 3 distinct AMF hosts, Claroideoglomus etunicatum, Funneliformis mosseae, and Racocetra verrucosa. Phylogenetic reconstruction and divergence dating using 22 conserved genes have revealed that MRE form a novel monophyletic clade within the Mycoplasmas and have diverged from their Mycoplasma relatives at least 400 million years ago, which may indicate the establishment of the MRE-AMF association to be quite ancient. Analysis of annotated genes have revealed novel proteins that are likely to play a role in interacting directly with the fungal host. Preliminary data suggest that MRE are important in enabling the completion of the life cycle of their AMF hosts.

Metagenomic analysis reveals hidden fungal diversity in grass rhizosphere and tree foliage. Ning Zhang, Stephen Miller, Shuang Zhao, Hayato Masuya. 1) Plant Biology and Pathology, Rutgers Univ, New Brunswick, NJ; 2) Dept Forest Microbiology, Forestry and Forest Products Research Institute, Matsunosoito 1, Tsukuba, Ibaraki 305-8687, JAPAN.

The diversity of microorganisms on earth remains poorly understood. Unculturable fungi inhabiting rhizosphere, phyllosphere, and other less studied niches are thought to represent a large fraction of the unknown diversity. In this study, we used both culture-dependent method and Illumina metagenomic sequencing approach to explore fungal diversity in two environments: grass (Poa pratensis, Kentucky bluegrass) rhizosphere and tree (Cornus spp., dogwood) foliage. For the grass rhizosphere sample, Illumina metagenomic analysis identified 1,192 fungal genera from 2 Million reads, while the culture-based method identified 21 genera. For the Cornus sample, metagenomic analysis identified 73 fungal genera from 6.6 million reads, while 22 genera were isolated from culture. From both cases, we found that metagenomic sequencing analysis revealed significantly higher fungal diversity than culture-based method, which will help us better understand the diversity and role of fungi in the ecosystem.

Host-to-pathogen gene transfer facilitated infection of insects by a pathogenic fungus. Weiguo Fang, Xiaoxuan Chen. College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, China.

Inspite being of great concern to human health and the management of plants and animals, the mechanisms facilitating host switching of eukaryotic pathogens remain largely unknown. The endophytic insect-pathogenic fungus Metarhizium robertsi evolved directly from endophytes and its entomopathogenicity is an evolutionarily acquired characteristic. We found that M. robertsi acquired a sterol carrier (MrNPC2a) from an insect by horizontal gene transfer (HGT). Mr-NPC2a increased the amount of ergosterol in hylphal bodies by capturing sterol from insect hemolymph, and thus maintained cell membrane integrity and improved fungal survival rate. On the other hand, the reduction in sterol (substrate for molting hormone synthesis) in insect hemolymph elongated larval stage, which allows the fungus to fully exploit host tissues and produce more conidia. This is first report of HGT from host to a eukaryotic pathogen, and the host gene ultimately improved the infectivity of the pathogen.


Fungi are a critical component of the diversity and function of terrestrial ecosystems. They regulate decomposition rates, facilitate plant nutrient uptake and have a profound impact on agriculture and economics. Understanding the forces that structure fungal communities thus has important theoretical and
practical implications. While ecologists have long recognized the importance of scale on ecological processes, fungal communities have primarily been studied at small-scales, focusing on deterministic processes. To rectify this knowledge gap we are using next generation sequencing techniques to survey soil fungi in North American pine forests with a sampling design that allows us to examine community structure from roots-to-biomes. Our results show that soil fungal communities in these ecosystems are highly diverse and are structured primarily by large-scale, macroecological processes, rather than small-scale deterministic processes. Our results also show that there is high functional redundancy in soil fungal communities. This work demonstrates that increasing the scale of observation is critical to a complete understanding of the ecological dynamics of soil fungi and the ecosystem processes they mediate.

### Genomic analysis of Mortierella elongata and its endosymbiotic bacterium


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Mortierella belong to a group of basal fungi (Mortierellomycotina) common to soils and the rhizosphere and endosphere of many plant species. Mortierella species are known for rapid growth and abundant lipid production. Mortierella elongata is one species commonly isolated from forest soils and healthy plant roots where it grows asymptotically as an endosymbiont. Mortierella elongata is a heterothallic species but can also reproduce asexually through chlamydomospores and sporangiospores. Recent reports indicate that some isolates of M. elongata host endosymbiotic bacteria, which may be transmitted vertically via spores. However, it is still unclear whether all Mortierella species host endosymbionts or whether these are lineage-specific associations. Given the geographically widespread distribution of Mortierella elongata and its ubiquitous presence in forest soils and plants we chose to sequence its genome through the JGI Forest Metatranscriptome CSP. We also sought to assemble the genome of the bacterial endosymbiont to address whether there are genomic signatures of co-adaptation or co-evolution in the genomes of Mortierella and its endosymbiotic bacterium, which may impact the function and growth of Mortierella elongata. The 50 Mb genome of M. elongata was sequenced to 112x coverage. The 220,113 putative proteins identified in M. elongata, 109,093 appear to be unique (e.g. only ~50% have orthologs in other fungal species having sequenced genomes). The M. elongata genome appears to be enriched in genes related to cryptophyten metabolism, siderophore group nonribosomal peptides, glucan 1,4-alpha glucosidases, and in lipid metabolism (e.g. sphingolipids, etherlipids, and glycerophospholipids) compared to genome sequences of other basal fungi. The endosymbiotic bacterium sequenced along with the M. elongata isolate is related to Glomeribacter (endosymbiont of Gigospora, Scutellospora, and other Glomeromyctota) within the Burkholderiales. The >2.6 Mb endosymbiont genome is larger than that of Glomeribacter but quite reduced compared to free-living isolates of Burkholderia. The reduced genome size of this bacterium, and the fact that it has thus far evaded pure culture isolation, supports the view that this is an ancient and obligate symbiosis.

### Integrative genomics of poplar-fungal pathogen interactions

**Richard C. Hamelin**, Forest Sciences, University of British Columbia, Vancouver, BC, Canada.

Poplar is an important tree, both from an ecosystem point of view as riparian species, and as a commercial agro-forestry crop for the production of wood and paper products and increasingly as a source of bioenergy. Fungi in the Urediales and the Dothideomycetes are responsible for the most important diseases of poplars. In most tree-fungal pathogen interactions, a few founder species are key determinants of the outcome. To better understand these interactions at the landscape level and predict their outcome, we are using a variety of genome-based approaches. We have applied DNA barcoding and multigene phylogeny to poplar pathologists to assess species diversity and host specificity both from environmental and herbarium-derived samples. We have found a high level of fidelity in host tracking of pathogens vis-à-vis their host. However, this fidelity tends to break down when poplar is grown in intensive plantations or when interspecific hybrids are planted. To further investigate and understand this pattern, we sequenced the genomes of 12 poplar pathogens with different host specificity. By comparing these genomes we identified core gene sets as well as genes that are unique to each species and are candidate determinants of the interaction outcomes. Annotation of these genome sequences with customized pipelines allowed us to assign putative functions to the candidate genes and detect effector-like sequence profiles. RNAseq profiling of the interaction of poplar rust on different hosts and their hybrids confirms the uniqueness of expression patterns in host-specific infections. In addition, we are using population genome re-sequencing approaches to detect selection patterns at the genome level. Several of the candidate genes identified in the upstream analyses encode secreted proteins and possess a signature of positive selection. Integration of this data can be used as predictive resources to determine the outcome of poplar-pathogen interactions in the environment.

### Fungal pathogen and endophyte genetics within the context of forest community dynamics

**M.S. Benitez**, M. H. Hersh, L. Becker, R. Vilgalys, J. S. Clark.

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Fungal pathogens play important roles in forest community dynamics, particularly through negative-density dependent regulation. Negative-density dependence regulation is hypothesized to be regulated by the presence of host-specific pathogens. Studies on forest pathogens, however, indicate the predominance of generalist seedling pathogens, capable of infecting more than one host species. To understand the mechanisms through which "generalist" pathogens contribute to forest-community dynamics we conducted extensive surveys of seedling pathogens in temperate hardwood forests of the eastern U.S.A. Species in the genera *Colletotrichum* and *Ilyonectria* were among the most commonly isolated and recovered amplicon sequence from seedlings of multiple host species showing disease symptoms. Further, co-infection by both *Colletotrichum* and *Ilyonectria* species decreases host survival, as quantified by posterior model probabilities. To investigate molecular mechanisms associated with multi-host generalism and co-infection, and to determine whether these "generalist" pathogens are distinct species or species-complexes, the genomes of three common species in our dataset (e.g. *C. floriniae*, *C. gloeosporioides* and *Ilyonectria europea*) were sequenced. The largest genome of the three belonged to *Ilyonectria* at 63.66 Mb, which also contained the highest number (22,250) of genes. The smallest genome belonged to *C. floriniae* with 50.04 Mb and 15,777 genes. Genome size and number of predicted genes appears expanded, confirming their role as seedling pathogens. For instance, three out of four polysaccharide lyase (PL) enzyme domains found in fungal genomes, are enriched in these three species. PL enzymes are relevant in plant pathogenicity since they may contribute to initial stages of host penetration. The genome sequence of these fungal groups will serve as a reference set for population level studies to address host-specificity and local adaptation within our isolate database.
**Dimorphic Transitions**

**Co-chairs: Anne Dranginis and Alex Andrianopoulos**

**Epigenetic Switching Regulates the Yeast-Hyphal Transition in Candida albicans.** Haoyu Si1, Allison Porman2, Matthew Hirakawa3, Stephen Jones1, Aaron Hernday2, Alexander Johnson2, Richard Bennett1. 1) Mol Microbiol & Immunology, Brown University, Providence, RI; 2) Mol Microbiol & Immunology, UCSF, San Francisco, CA.

*Candida albicans* is a dimorphic yeast that is normally found as a commensal organism in the mammalian gastrointestinal tract. It is also a prevalent opportunistic pathogen able to infect multiple mucosal and internal sites in the human body. A principle feature of *C. albicans* biology is its ability to grow in multiple phenotypic states, including both yeast and filamentous forms. Phenotypic plasticity is also exemplified by the “white-opaque switch”, in which cells can reversibly transition between the white and opaque states. White and opaque forms differ in multiple aspects including their shape, their interaction with host immune cells, their mating competency, and their pathogenesis. Furthermore, white cells are induced to form hyphal filaments when grown at 37°C, neutral pH, or in the presence of serum, whereas opaque cells do not form filaments in response to these conditions, and this difference could explain the decreased virulence of opaque cells in models of systemic infection. In this study, we show that opaque cells can undergo the yeast–filament transition in response to environmental cues, but that these cues are distinct from those that induce the transition in white cells. For example, growth on low phosphate medium or medium containing the sugar sorbitol induced efficient filamentous growth in opaque cells, while these conditions did not induce filamentous growth in white cells. Genetic dissection of the regulation of opaque cell filamentation showed extensive overlap with the regulation of filamentation in white cells, including roles for the established transcriptional regulators Ume6, Efg1, and Tup1. However, genes induced by filamentous growth in opaque cells showed only limited overlap with those induced during white cell filamentation. Together, these studies indicate that *C. albicans* white and opaque cells are both capable of undergoing filamentation but do so in response to different environmental signals and generate distinct transcriptional profiles, reflecting intrinsic differences in the programming of the two phenotypic states.

**Extracellular and intracellular signaling orchestrates morphotype-transition and virulence in human pathogen Cryptococcus neoformans.** Linqi Wang, Xiuyun Tian, Rachana Gyawali, Xiaorong Lin. Biology, College Station, TX.

Interactions with the environment and divergent species drive the evolution of microbes. To sense and rapidly respond to these dynamic interactions, “simple” microbes developed bet-hedging social behaviors, including the construction of heterogeneous biofilm communities and transition between different morphotypes. The human fungal pathogen *Cryptococcus neoformans* can undergo morphotype transition between the yeast and the filamentous form. Most recently, we demonstrated that the zinc-finger regulator Znf2 bridges the bi-direction yeast-hypha transition and virulence in this pathogen. One of Znf2 downstream targets is extracellular protein Cfl1. Cfl1 is a cell-wall bound adhesin and a signaling molecule when it is released. This matrix protein Cfl1 plays a similar but less prominent role than Znf2 in orchestrating morphogenesis and virulence in *C. neoformans*. Through transcriptome analyses and screening Znf2 downstream targets by overexpression, we identified an additional player in the control of morphogenesis and biofilm formation. This factor is an intracellular RNA-binding protein Pum1. As expected, Pum1 affects filamentation in a Znf2 dependent manner. However, the effect of Pum1 on morphogenesis is independent of Cfl1. The *pum1Δ cfl1Δ* double mutant shows a more severe defect in filamentation than either of the single mutant, indicating that Pum1 and Cfl1 act in two parallel pathways. Two of Pum1’s targets, Fad1 and Fad2, form a *Cryptococcus*-specific adhesin family. Like Cfl1, these two extracellular adhesins show regulatory roles in conducting morphogenesis and virulence in *C. neoformans* and thus may be involved in extracellular signaling transduction. Our results indicate that complex regulatory cascades composed of extracellular and intracellular circuits may be responsible for mediating morphological transition in response to the cues in the environments and the host.

**Histoplasma strain variations and differences in pathogenic-phase transcriptomes.** Jessica A. Edwards1, Chenxi Chen2, Megan M. Kemskey1, Thomas K. Mitchell2, Chad A. Rappeleye3. 1) Microbiology, Ohio State University, Columbus, OH; 2) Plant Pathology, Ohio State University, Columbus, OH.

The morphological dimorphism of *Histoplasma capsulatum* reflects an underlying change in gene expression that is essential for pathogenesis. In the yeast-phase, *Histoplasma* infects the mammalian lung and proliferates within phagocytic cells. Geographically distinct strains of *Histoplasma* exhibit differences in their relative virulence and in their pathogenic mechanisms. The close similarity in the genome sequences of these diverse strains suggests that phenotypic variations result from gene expression differences rather than gene content. To better understand how the transcriptional program translates into morphological and pathogenic differences between strains, we profiled the yeast-phase transcriptomes of two *Histoplasma* strains by RNAseq methodology. For both strains, about 50% of sequence reads align to the genome providing evidence for approximately 9000 genes. Quantitative comparisons reveal about 200 genes are at least 10-fold differentially expressed between strains, and these include genes related to *Histoplasma* pathogenesis (SOD3, YPS3, AGS1). The genes encoding the secreted calcium-binding protein (CBP1), histone proteins (H2B, H3, and H4) and an ammonium transporter are among the most highly expressed genes overall. Using GFP-transcriptional fusions and their introduction into both strain backgrounds, we demonstrate that dissimilarity in the transcriptional activity of individual genes reflects variations in the trans-acting factors between strains rather than the sequence of the promotors, themselves. These studies lay an essential foundation to facilitate discovery of the factors that contribute to strain-specific virulence differences of *Histoplasma*. 
The C2H2 transcription factor HgrA promotes hyphal growth in the dimorphic pathogen *Penicillium marneffei*. Hayley E. Bugeja, Michael J. Hynes, Alex Andrianopoulos. Department of Genetics, University of Melbourne, Parkville, VIC, Australia.

*Penicillium marneffei* (recently renamed *Talaromyces marneffei*) is well placed as a model experimental system for investigating fungal growth processes and their contribution to pathogenicity. An opportunistic pathogen of humans, *P. marneffei* is a dimorphic fungus that displays multicellular hyphal growth and asexual development (conidiation) in the environment at 25°C and unicellular fission yeast growth in macrophages at 37°C. We have characterised the transcription factor hgrA (hyphal growth regulator), which contains a C2H2 DNA binding domain closely related to that of the stress-response regulators Msn2/4 of *Saccharomyces cerevisiae*. HgrA is not required for controlling yeast growth in response to the host environment, nor does it appear to have a key role in response to stress agents, but is both necessary and sufficient to drive the hyphal growth program. *hgrA* expression is specific to hyphal growth and its deletion affects multiple aspects of hyphal morphogenesis and the dimorphic transition from yeast cells to hyphae. Loss of HgrA also causes cell wall defects, reduced expression of cell wall biosynthetic enzymes and increased sensitivity to cell wall, oxidative, but not osmotic stress agents. As well as causing apical hyperbranching during hyphal growth, overexpression of *hgrA* prevents condiation and yeast growth, even in the presence of inductive cues. HgrA is a strong inducer of hyphal growth and its activity must be appropriately regulated to allow alternative developmental programs to occur in this dimorphic pathogen.

N-acetylglucosamine (GlcNAc) Triggers a Morphogenetic Program in Systemic Dimorphic Fungi. Sarah A. Gilmore1, Shamoon Naseem2, James B. Konopka1, Anita Sil1. 1) Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, CA; 2) Department of Molecular Genetics and Microbiology, Stony Brook University, Stony Brook, NY.

Cellular differentiation is an essential process for the development and growth of multicellular eukaryotic organisms. Similarly, many unicellular organisms undergo a program of cellular differentiation to produce a new cell type specialized for survival in a distinct environmental niche. Systemic dimorphic fungal pathogens, such as *Histoplasma capsulatum* (Hc) and *Blastomyces dermatitidis* (Bd), can switch between a unicellular parasitic yeast form adapted for growth within mammals and an infectious soil-growing filamentous form as part of their natural life cycles. Temperature is thought to be the predominant environmental cue that promotes cellular differentiation of systemic dimorphic fungi; however, work with other fungi indicates that additional environmental cues including CO2, light, and nutrient availability can influence how an organism responds to its environment. Recent work suggests that the ubiquitous monosaccharide N-acetylglucosamine (GlcNAc) can play a role in cell signaling in fungi. We identified GlcNAc as a potent inducer of the yeast-to-filament transition in Hc and Bd. Micromolar concentrations of exogenous GlcNAc were sufficient to induce a robust morphological transition of *Hc* yeast cells to filamentous cells at room temperature, indicating that dimorphic fungal cells may be sensing GlcNAc, or one of its catabolic byproducts, to promote filamentation. Using GlcNAc as a tool to induce a robust and more synchronous phase transition of *Hc* yeast cells to filaments, we examined the temporal regulation of the *Hc* transcriptome during morphogenesis to reveal candidate genes involved in establishing the filamentous growth program. Two genes we identified during transcriptome analysis included *NGT1* and *NGT2*, which encode GlcNAc major facilitator superfamily transporters. RNAi depletion of *NGT1* or *NGT2* rendered *Hc* cells unable to respond to exogenous GlcNAc. Furthermore, wild type levels of *NGT1* and *NGT2* transcripts were important for efficient *Hc* yeast-to-filament conversion even in the absence of exogenously added GlcNAc. These data suggest that Ngt1 and Ngt2 may monitor endogenous GlcNAc as part of an autoregulatory system that allows *Hc* to regulate its filamentous growth.


The thermally dimorphic fungi infect several million people each year including those with normal immune defenses. These fungi grow as mold in the soil (22°C) where they produce infectious conidia. Upon soil disruption, aerosolized conidia are inhaled into the lungs (37°C) where they convert into yeast. This reversible, temperature-dependent phase transition defines the lifestyle of the dimorphic fungi. In *Blastomyces dermatitidis*, we discovered *SREB* (siderophore biosynthesis repressor in Blastomyces), which encodes a GATA transcription factor that promotes the conversion to mold at 22°C and also regulates iron homeostasis. To begin to dissect how *SREB* affects the transcriptional response to temperature, we used gene expression microarrays and chromatin immunoprecipitation with quantitative real-time PCR (ChIP-qPCR). For microarrays, RNA was isolated from *SREB* and wild type (WT) isolates at baseline (yeast) and 6, 24, and 48-hours after a drop in temperature to 22°C. LIMMA and EArrays were used to identify differentially expressed (DE) genes. For ChIP, we engineered *SREB* to contain an in-frame, C-terminal 3x-HA tag. SREB-3xHA was cross-linked to its DNA binding targets in vivo using 1% formaldehyde at 37°C and 48-hours at 22°C. Following chromatin shearing and reversal of cross-links, enrichment for SREB-3xHA binding (vs. isogenic control) at GATA motifs was assessed by qPCR. Gene expression microarray analyses indicated that *SREB* was a global regulator of transcription at 37°C and 22°C. Gene Ontology enrichment demonstrated *SREB* was involved with diverse processes including iron ion binding, amino acid transport, metabolic
process, and fatty acid biosynthesis. Complementary analysis using weighted gene co-expression network analysis identified modules with DE genes enriched for transmembrane transport, metabolic process, transcription factor activity, translation, and fatty acid biosynthesis. To identify candidate genes for ChIP-qPCR, we integrated gene expression microarray data with genome-wide in silico analysis of GATA transcription factor binding motifs. Using this approach, we identified a subset of genes bound and regulated by SREB including SIDA, MIRB, WD, HAPX, and PDH at 37°C and 22°C. In contrast, enrichment for SREB-3xHA binding of ECI1 occurred at 22°C, but not 37°C. In conclusion, integration of microarray analysis, in silico GATA motifs, GO enrichment, and ChIP-qPCR indicates that SREB affects pleiotropic events in *B. dermatitidis*.

**Functional Analysis of Genes in Regions of Introgression in Coccidioides.** Bridget M. Barker. Immunology & Infectious Diseases, Montana State Univ, Bozeman, MT.

Coccidioides immitis and *C. posadasii* are dimorphic fungi endemic to the Americas. Genomic analysis of sequenced strains of *C. posadasii* and *C. immitis* reveals insights into the population biology of these organisms. There is strong evidence for hybridization and introgression, such that for many of the *C. immitis* strains, there are several regions that have a closer match to *C. posadasii*, but few regions within *C. posadasii* matching *C. immitis*. Multiple hybridization regions were located in several genomes analyzed, and at least one region containing ten genes exhibits a pattern consistent with introgression in *C. immitis*. This conserved region was further evaluated in a larger collection of isolates. Approximately half of the *C. immitis* isolates contain the *C. posadasii* fragment, and the majority of those are from the southern California and Mexico populations. The region of introgression represents a unique opportunity to functionally assess genes that are likely to be relevant for species-specific virulence and adaptation to mammalian hosts or the environment. This region has a shared recombination point flanking a metalloproteinase, Mep4; genes that are highly expressed in the parasitic phase; and genes of unknown function. Importantly, evolutionary selection has preserved this region in multiple strains of *C. immitis* further emphasizing the possible role in virulence of these genes. Variation among strains for virulence in murine models of coccidioidomycosis has been observed, but has not been tested in the context of the newly discovered species or with a targeted underlying genetic mechanism hypothesis to test. Gene deletion mutants are being generated for three genes in the conserved introgression region to determine effects on in vitro growth and morphological change under host relevant conditions.
Role of the cell fusion gene idcA in fungal mutualism. Carla J. Eaton1,2, Cornelia Staerkel1, Barry Scott1,2. 1) Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand. 2) Bio-Protection Research Centre, Massey University, Palmerston North, New Zealand.

Maintenance of the mutualistic association between the fungal endophyte Epichloë festucae and perennial ryegrass relies on a number of important signalling pathways including ROS signalling by the NADPH oxidase (Nox) complex, and the cell integrity- and stress-activated MAP kinase pathways. Perturbation of these signalling pathways leads to a dramatic switch from mutualistic to pathogenic-like association with perennial ryegrass. Interestingly, all E. festucae ‘symbiotic regulator’ genes identified to date are involved in vegetative cell fusion in other fungi, suggesting hyphal fusion may play an important role in maintenance of the mutualistic association. To investigate this putative link, the role of the cell fusion gene IDC1 (ham-5) was examined. IDC1 is of particular interest as in addition to its role in cell fusion it is also linked to Nox signalling in Podospora anserina. Disruption of E. festucae idcA leads to a dramatic symbiotic switch from mutualistic to pathogenic-like association with perennial ryegrass. Infected plants are severely stunted and display precocious senescence. Biomass of the DldcA mutant in planta is significantly increased relative to the wild-type strain, and hyphae extensively colonise host vascular tissues. Formation of intra-hyphal hyphae by the DldcA mutant in planta is also abundant, possibly due to defective hyphal fusion or defects in septation. The importance of idcA for maintenance of mutualistic association with perennial ryegrass supports the hypothesis that hyphal fusion is required for establishment of an interconnected hyphal network essential for mutualism.


The molecular details of membrane fusion during yeast mating are poorly understood. The tetranspanner protein Prm1 is one of the few known components that acts at the step of bilayer fusion. In its absence, mutant mating pairs lyse or arrest in the mating reaction with tightly apposed plasma membranes. The absence of another tetranspanner, Fig1p, which controls pheromone-induced Ca\textsuperscript{2+} influx, yields similar cell fusion defects. Although extracellular Ca\textsuperscript{2+} is required for efficient cell fusion of wild-type cells, cell fusion in prm1 mutant mating pairs is dramatically reduced when Ca\textsuperscript{2+} is removed. A genetic screen was conducted to uncover genes that promote mating-dependent lysis in the absence of extracellular Ca\textsuperscript{2+}. The role of different candidates in relation to Prm1p will be reviewed here.

The role of calcium and calmodulin during cell fusion and colony initiation in Neurospora crassa. Chia-Chen Chang, Nick Read. Fungal Cell Biology Group, Institute of Cell Biology, University of Edinburgh, Edinburgh EH9 3JH.

Calcium is an ubiquitous signalling molecule which regulates many important processes in filamentous fungi including spore germination, hyphal growth, mechanosensing, stress responses, circadian rhythms, and virulence.Transient increases in cytosolic free calcium ([Ca\textsuperscript{2+}]) act as intracellular signals. As the primary intracellular Ca\textsuperscript{2+} receptor, calmodulin (CaM) converts these Ca\textsuperscript{2+} signals into responses by regulating the activity of numerous target proteins. We have found that both Ca\textsuperscript{2+}-free medium and two CaM antagonists (calmidazolium and trifluoperazine) selectively inhibit a form of cell fusion called conidial anastomosis tube (CAT) fusion that occurs during colony initiation in the fungal model Neurospora crassa. GFP labelled CaM localized as dynamic particles within the cytoplasm of both germ tubes and CATs. In particular, CaM showed a dynamic accumulation at two growing tips of CATs that exhibit chemoattraction towards each other. CaM also localized at developing septa in germ tubes. The b-tubulin inhibitor, benomyl, reduced the movement of CaM in the cytoplasm. Moreover, the absence of extracellular Ca\textsuperscript{2+} inhibited the recruitment of CaM to CAT tips as well as inhibiting CAT chemoattraction. The deletion of the myosin-5 (myo-5) gene caused the mis-localization of CaM in tips of growing germ tube and CATs. This suggests that the movement of cytoplasmic CaM involves transport along microtubules, and the recruitment of CaM to tips involves myosin-5 along F-actin and is dependent on extracellular Ca\textsuperscript{2+}.

LFD-1 is a component of the membrane merger machinery during cell-cell fusion in Neurospora crassa. Javier Palma-Guerrero, N. Louise Glass. Plant and Microbial Biology Department, UC Berkeley, Berkeley, CA.

Cell-cell fusion is an essential part of the development of most eukaryotic organisms, playing an important role both during sexual development and vegetative growth. In the filamentous fungus Neurospora crassa, cell fusion events occur during all stages of the life cycle. This, together with the sequenced and annotated genome and the genetic tools available, makes this fungus a good model system for dissecting cell fusion. Plasma membrane merger is the last step in the cell-cell fusion process, occurring after cell wall remodeling, and it is the definitive event that allows for cytoplasm mixing between the fusing cells. Although molecular mechanisms associated with intracellular membrane fusion are well characterized, the molecular mechanisms of plasma membrane merger between cells are poorly understood. Only one gene encoding a protein involved in this last step of cell fusion had been previously identified in N. crassa: Prm-1, a deletion of which results in strains that show a >50% reduction in vegetative and sexual cell fusion. We have identified a second gene, ifd-1, which is also involved in plasma membrane merger in N. crassa. LFD-1 is a plasma membrane protein only present in ascomycete filamentous fungi. N. crassa strains carrying a deletion of ifd-1 results in a reduction in both vegetative and sexual cell fusion, and having a similar, but less severe, phenotype than a Prm-1 deletion strain. Strains carrying both Prm-1 and Ifd-1 deletions indicate that LFD-1 acts independently of PRM-1. Strains carrying Prm-1 or Ifd-1 mutations result in increased cell lysis during cell-cell fusion, a phenotype that was enhanced by reducing extracellular calcium concentration. These results suggest that the lysis phenotype associated with cell fusion events is due to membrane damage caused by defects during membrane merger, and which may be repaired in a calcium dependent process. Our results indicate that both PRM-1 and LFD-1 are important, but non-essential components of the cell fusion membrane merger machinery.
Specific Structural Features of Sterols Affect Cell-Cell Signaling and Fusion in Neurospora crassa. Martin Weichert1, Ewald Priegnitz2, Raphael Brandt2, Thorben Nawrath3, Stefan Schulz4, André Fleissner4. 1) Institut für Genetik, Technische Universität Braunschweig, Spielmannstrasse 7, 38106 Braunschweig, Germany; 2) Institut für Organische Chemie, Technische Universität Braunschweig, Hagenring 30, 38106 Braunschweig, Germany.

Sterols are major constituents in the plasma membrane of eukaryotic cells. They modulate the physical properties of the lipid bilayer, e.g. fluidity. By interacting with certain lipids and proteins in the plasma membrane, sterols cluster into microdomains which might act as platforms for many biological functions, such as signal transduction. In the early stages of colony formation in Neurospora crassa, germinating spores direct their growth towards each other, establish physical contact, and fuse. Cell-to-cell signaling requires the coordinated dynamic recruitment of the MAP kinase MAK-2 and the cytoplasmic protein SO to the tips of interacting cells. Subsequent plasma membrane fusion is facilitated by the transmembrane protein PRM1. Here, we report that mutants affected in the biosynthesis of ergosterol, the major sterol in most fungal species, show distinct defects during germling fusion. Deletion of erg-2, which encodes an enzyme mediating the last step in the pathway, strongly impairs both directed growth and cell fusion. Interestingly, both MAK-2 and SO mislocalize at the tips of interacting Derg-2 germlings. In contrast, the absence of ERG-10a and ERG-10b, two enzymes with redundant function that act upstream of ERG-2, does not affect cell-to-cell communication. However, Derg-10a Derg-10b germling pairs show DPrm1-like deficiencies in plasma membrane merger. By relating the sterol composition and fusion competence of several erg mutants, we find that not the absence of ergosterol but the accumulation of sterol intermediates specifically impairs distinct steps of germling fusion. While the presence of two double bonds in the sterol side chain provokes Derg-2-like deficiencies, an altered double bond arrangement in the sterol ring system causes DPrm1-like defects. During sexual development, cell fusion precedes the fertilization of fruiting bodies. Unlike the defects during germling fusion, female and male mating partners of Derg-2 and Derg-10a Derg-10b efficiently fuse, suggesting that alterations in the sterol composition specifically impair signaling mechanisms mediating vegetative cell fusion. These data suggest that specific structural features of sterols differentially affect membrane properties and functions, such as the membrane recruitment of proteins, the assembly of signaling complexes, and plasma membrane fusion.


Fungal hyphae explore the surrounding environment in search of nutrient sources, mating partners or host organisms by sensing gradients of tropically active cues. Chemotropism is crucial for fungal development and virulence, but the underlying mechanisms are poorly understood. Here we followed a genetic approach to dissect chemotropism in the soilborne pathogen Fusarium oxysporum. A plate assay was used to measure directed growth of germ tubes towards different classes of compounds, including carbon and nitrogen sources, sex pheromones, plant secondary metabolites and tomato root exudate. F. oxysporum mutants lacking the mitogen activated protein kinase (MAPK) Fmk1 or the transcription factor Ste12, two components of the conserved Pathogenicity MAPK cascade, were impaired in chemotropism towards nutrients, but fully responsive to a pheromone and root exudate. By contrast, Rho1 and Mpk1, two components of the cell integrity MAPK cascade, were specifically required for directed growth towards a pheromone and root exudate. Deletion of the seven transmembrane G protein coupled receptor Ste2 abolished the chemotropic response to a pheromone and, unexpectedly, also to tomato root exudate. Our results provide evidence for co-option of a cognate sex pheromone receptor and a conserved MAPK signalling pathway for chemotropism of F. oxysporum towards plant host compounds.

Characterization of new STRIPAK complex interaction partners in the filamentous ascomycete Sordaria macrospora. Britta Herzog, Yasmine Bernhards, Berit Habing, Eva Reschka, Sabine Riedel, Stefanie Pöggeler. Institute of Microbiology and Genetics, Department of Genetics of Eukaryotic Microorganisms, Georg-August-University Göttingen, Germany.

Using Sordaria macrospora as model organism we investigate the complex process of fruiting-body development and involved proteins in this filamentous ascomycete. This differentiation process is regulated by more than 100 developmental genes. Recently, we have shown that a homologue of the human STRIPAK (striatin-interacting phosphatase and kinase) complex engages a crucial role in sexual development in fungi. The S. macrospora striatin homologue PRO11 and its interaction partner SmMOB3 are key components of this complex (Bloeemendal et al., 2012). PRO11 contains a conserved WD40 repeat domain and is supposed to function as scaffolding protein linking signaling and eukaryotic endocytosis (Pöggeler and Kück, 2004). SmMOB3 (phocine) is a member of the MOB family (Bernhards and Pöggeler, 2011). Beside their important role in multicellular development and hyphal fusion both proteins seem to be involved in vesicular trafficking and endocytosis.

By means of yeast two-hybrid screens and GFP-Trap analysis we identified several new interaction partners of PRO11 and SmMOB3. Similar to PRO11 and SmMOB3, a multitude of them are predicted to be involved in vesicular trafficking and are localized to the ER or to the Golgi. Here, we show the results of a detailed analysis of the new STRIPAK complex interaction partners. Initially, we isolated the cDNA of the genes and confirmed the interaction by yeast two-hybrid. For further characterization and to get knowledge about their cellular functions we created knock-out strains and analyzed their morphological phenotypes. For localization and expression studies we constructed EGFP-tagged fusion proteins and expressed them in S. macrospora.

Bernhards and Pöggeler, 2011; Curr Genet 57 (2): 133-49.
Bloeemendal et al., 2012; Mol Microbiol 84 (2): 310-23.
Characterisation of contact-dependant tip re-orientation in *Candida albicans* hyphae. Darren Thomson, Silvia Wehmeier, Alex Brand. Aberdeen Fungal Group, Aberdeen University, Aberdeen, United Kingdom.

*Candida albicans* is a pleiomorphic fungus that lives as a commensal yeast in the human body but can become pathogenic in susceptible patient groups. Virulence is strongly linked with the production of penetrative hyphae that can adhere to and invade a wide range of substrates, including blood vessels, organ tissue, keratinised finger-nails and even soft medical plastics. Using live-cell imaging and nanofabricated surfaces, we are characterising the spatio-temporal dynamics of contact-induced hyphal tip behaviour (thigmotropism). To test whether tip re-orientation responses positively correlate with levels of hyphal adhesion, we generated substrates with increasing adhesive force. Hyphal tip re-orientation was absent in poorly-immobilised hyphae and a threshold adhesive force was required sub-apically to generate the hyphal tip pressure required for re-orientation. Interestingly, sub-threshold adhesion resulted in sub-apical hyphal bending. Localization of fluorescent protein markers for the Spitzenkörper and the Polarisome (Mlc1-YFP and Spa2-YFP, respectively) showed that *C. albicans* hyphal tips grow in an asymmetric, ‘nose-down’ manner on a surface. Additionally, hyphal tips can detect surface stiffness and show a distinct preference for nose-down growth on the softer of two substrates. Localisation of fluorescent cell-cycle reporter proteins over time revealed that hyphal tip contact slowed the cell-cycle, suggesting that tip-contact perturbs cell-cycle mechanics. Finally, we examined the role of cytoskeleton regulators in thigmotropism and determined the force that can be generated by the hyphal tip. Our results suggest that *C. albicans* hyphae can exert sufficient force to penetrate human epithelial tissue without the need for secreted enzyme activity. This is consistent with the observed hyphal penetration of medical-grade silicone, which has a similar Young’s modulus to human cartilage.
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Biochemistry and Metabolism

1. Heme regulation in Aspergillus fumigatus. Nicola Beckmann1, Ernst R. Werner2, Hubertus Haas2. 1) Division of Molecular Biology, Biocenter, Innsbruck Medical University, Austria; 2) Division of Biological Chemistry, Biocenter, Innsbruck Medical University, Austria.

Sufficient iron supply is indispensable for survival of almost all organisms. However, an excess of iron is potentially toxic. In the opportunistic human-pathogenic fungus Aspergillus fumigatus the ability to adapt to iron limitation represents a crucial virulence factor. Iron regulation is tightly interconnected with heme metabolism, as iron-containing heme is an essential cofactor of a variety of cellular processes, e.g. respiration, sterol biosynthesis, oxidative stress detoxification and also reductive iron assimilation. Most knowledge on fungal heme regulation derives from studies in Saccharomyces cerevisiae. A. fumigatus, as well as most other fungal species, lack homologs of key heme regulators found in S. cerevisiae. The goal of this study is to elucidate heme-dependent regulation in A. fumigatus (wt). As a first step, we generated a mutant strain (DhemA) lacking the gene encoding aminolevulinic acid synthase (HemA), which catalyzes the committed step in heme biosynthesis. This mutation offers the possibility to control the cellular heme content by supplementation with aminolevulinic acid (ALA). Growth of DhemA was blocked at ALA concentrations below 20 mM, but fully restored by addition of 200 mM on solid as well as in liquid media. Supplementation with protoporphyrin IX (PpIX), the iron free heme precursor, and hemin (chloroproporphyrin IX iron(III)) supported growth of DhemA, proving that A. fumigatus is able to utilize exogenous porphyrins. Nevertheless, A. fumigatus in contrast to several other fungal species is not able to utilize hemin as iron source. Under iron starvation, ALA supplementation led to a tremendous accumulation of PpIX in both DhemA and wt, which indicates that HemA represents the major rate limiting step in heme biosynthesis when iron is scarce. In DhemA, ALA restriction transcriptionally increased the heme-biosynthetic coproporphyrinogen(III)oxidase and the putative heme receptor CFEM3. Additionally, ALA limitation decreased the resistance of DhemA to oxidative stress and the triazole antifungal drug posaconazole, which underlines the crucial role of heme in detoxification and sterol biosynthesis. This work was supported by the Austrian Science Foundation grant FWF P21643-B11 to HH.

2. Key Steps in the Biosynthesis of the Fungal Virulence Factor Gliotoxin. Pranatchareya Chankhomjorn1, Daniel H. Scharf2, Kirstin Scherlach1, Nicole Remme1, Andreas Habel1, Thorsten Heinek1, Martin Roth1, Axel A. Brakhage1, Christian Hertweck1. 1) Biomolecular Chemistry, HKI, Jena, Germany; 2) Bio Pilot Plant, HKI, Jena, Germany.

The prototype of epipolythiodioxopiperazine (ETP) family, gliotoxin, is an infamous virulence factor of the human pathogen Aspergillus fumigatus, notably the leading cause of invasive aspergillosis in the immunocompromised patients. Its toxicity has been attributed to the unusual intramolecular disulfide bridge, which is the functional motif of all ETPs. A number of studies showed that the diketopiperazine core of gliotoxin is assembled by a non-ribosomal peptide synthetase. However, downstream pathway steps have remained elusive, mainly because of the scarcity and instability of pathway intermediates. Here we present the critical role of a specialized glutathione S-transferase, GliG, in the enzymatic sulphuration and the key step of edithiol formation by an unprecedented twin carbon-sulfur lyase, GliL. Our studies not only unveil the understanding of key steps in the biosynthesis pathway of an important microbial virulence factor, but also outline a new function of microbial GSTDs and gain insights into the formation of organosulfur compounds.

3. Identification of a gene cluster mediating the biosynthesis of the Aspergillus fumigatus cell wall and secreted polysaccharide, galactosaminogalactan. Fabrice N. Gravelat1, Mark J. Lee2, Alexander Geller3, Dan Chen3, Anne Beauvais1, Hong Liu1, William C. Nierman1, Jean-Paul Latgé1, Thierry Fontaine2, Scott G. Filler1, Donald C. Shedd3. 1) Microbiology & Immunology Department, McGill University, Montréal, QC, Canada; 2) J. Craig Venter Institute, Rockville, Maryland, USA; 3) Aspergillus Unit, Institut Pasteur, Paris, France; 4) Division of Infectious Diseases, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California, USA.

Aspergillus fumigatus is the most common cause of invasive mold disease in humans. Although adherence of fungal hyphae to host constituents is a critical early step in the pathogenesis of invasive aspergillosis, the molecular mechanisms underlying this process have not been elucidated. Using a forward genetic approach, we identified a glucose epimerase, Uge3, which is required for adherence of hyphae to a wide variety of substrates. Biochemical analyses confirmed that Uge3 is required for the synthesis of the secreted glycan galactosaminogalactan (GAG), which in turn functions as the predominant adhesin of A. fumigatus hyphae and is required for virulence. However, the biochemical and regulatory pathways governing GAG synthesis remain unknown. Using comparative transcriptome analysis, we found that uge3 is found within a cluster of 5 co-regulated genes on chromosome 3. Interestingly, 3 of the 5 proteins (Uge3, Gbt3 and Ega3) encoded by these genes are predicted to contain conserved domains involved in polysaccharide metabolism. The 2 other proteins (Sph3 and Er3) have no homologs in other organisms. We hypothesized that this cluster of genes may be required for GAG biosynthesis. To test this hypothesis, we constructed deletion mutants of two of the cluster genes: sph3, encoding a cell surface spheronulin 4-like protein; and er3, encoding an extracellular serin-rich protein. Phenotypic analysis of both the Desr3 and Dsph3 mutant strains confirmed that deletion of these genes resulted in both impaired GAG production and impaired adherence, similar to the phenotype of the Duge3 mutant strain. Gene deletion for the 2 remaining genes is ongoing. Collectively, these data suggest that the 5 gene cluster identified on chromosome three is likely to control carbohydrate biosynthetic cluster required for the synthesis of GAG. Importantly, this is the first description of a gene cluster for the biosynthesis of a cell wall polysaccharide in A. fumigatus, and suggests the possibility that other similar gene clusters may govern the synthesis of glycans in this fungus. The discovery of this cluster, and the subsequent characterization of the role of each of the component elements, may provide insight into the synthesis and function of GAG.

4. A fasciclin-like protein in Aspergillus fumigatus. Thomas Hartmann1, Lei Sun2, Cheng Jin1. 1) Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; 2) Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

The saprophytic filamentous fungus Aspergillus fumigatus has been gaining importance as an opportunistic human pathogen over the past decades, as advances in modern medicine have created a growing group of patients susceptible to potentially deadly invasive aspergillosis. The role of fungal adhesion during infection progression is still poorly understood and this work aims to focus on this neglected aspect of the infection process. Fasciclin-like proteins can be found in a wide variety of organisms, where they often perform functions related to surface adhesion. Here we describe a fasciclin-like protein in A. fumigatus. The Fas protein was first discovered as differentially expressed in an O-mannosyltransferase Dpm1 deletion strain, which indicates that it may be substantially glycosylated. We generated Dfas deletion strains in two different A. fumigatus strain backgrounds. The Dfas deletion strains grew normally on plates and in solution and neither bright field microscopy, nor TEM revealed phenotypical differences to the wildtype strains. Interestingly, the Dfas deletion strains showed reduced adhesion to hydrophobic plastic surfaces, but adhered normally to glass slides. Whether these altered adhesive properties have an effect on the strains’ virulence in mammalian hosts such as mice remains to be investigated.
5. Characterization of fumiquinazoline biosynthesis in *Aspergillus fumigatus*. Fang Yun Lim, Brian Ames, Christopher Walsh, Nancy Keller. 1) Medical microbiology and Immunology, University of Wisconsin-Madison, Madison, WI; 2) Biological chemistry and molecular pharmacology, Harvard Medical School, Boston, MA.

The fumiquinazolines (FQs) comprise a related, sequentially generated family of bioactive peptidyl alkaloids that are signature metabolites of *Aspergillus fumigatus*. The FQ framework is built by nonribosomal peptide synthetase (NRPS) machinery with anthranilate as a key non-proteinogenic amino acid building block. Despite being prevalent across the species, its gene cluster has not been characterized. Prior bioinformatic analysis coupled with heterologous expression of the putative *A. fumigatus* proteins termed here FmqA -FmqD led to the identification of a four-enzymatic process that builds increasingly complex FQ scaffolds. Briefly, FmqA, a trimodular NRPS condenses alanine, tryptophan, and anthranilic acid to form fumiquinazoline F (FQF). The tandem action of a flavoprotein (FmqB) and a monomodular NRPS (FmqC) converts FQF to fumiquinazoline A (FQA). Finally, FmqD, a FAD-dependent oxidoreductase converts FQA to the heptacyclic fumiquazoline C (FQC). Interestingly, FmqD contains an N-terminus signal peptide predicted for extracellular transport. This study is aimed at providing in vivo validation to the FQ biosynthetic framework and characterizing how cellular localization of FmqD affects production of FQC in *A. fumigatus*. We found that the conidial metabolite, FQC, is the predominant FQ moiety in two wild type isolates and is selectively accumulated in the conidia. Targeted single gene deletions of FmqA through FmqD coupled with metabolic profiling of the single biosynthetic gene mutants supported previous biochemical prediction of FQ biosynthesis. Fluorescent microscopy of mutants bearing a C-terminal FmqD-GFP fusion showed that FmqD is localized to the cell wall of the fungus and this localization is abolished when the signal peptide is removed. Future studies will elucidate if cell wall localization of FmqD is crucial for FQC production.


Biosynthetic pathways of secondary metabolites from fungi are currently subject to an intense effort to elucidate the genetic basis for these compounds due to their large potential within pharmaceutics and synthetic biochemistry. The preferred method is methodological gene deletions to identify supporting enzymes for key synthases one cluster at a time. In earlier work we presented a method for using a gene expression compendium to accurately predict co-regulated gene clusters in general, and in particular the members of gene clusters for secondary metabolism. A benchmarking of the method in *Aspergillus nidulans* by comparison to previous gene deletion studies showed the method to be accurate in 13 out of 16 known clusters and nearly accurate for the remaining three. In this work, we have expanded the algorithm to identify cross-chromosome between physically separate gene clusters (super clusters), and validate this both with legacy data and experimentally by prediction and verification of a new supercluster consisting of the non-ribosomal peptide synthetase (NRPS) AN1242 (on chr VIII) and the prenyltransferase AN1108 (on chromosome V) as well as identification of the shared product compound nidulanin A.

We also propose further implications of the gene clustering, as our analysis shows that approximately 10% of the genes seem to be non-randomly (p<0.05) co-regulated with more than two neighboring genes. We have employed *A. nidulans* for our method development and validation due to the wealth of available biochemical data, but the method can be applied to any fungus with a sequenced and assembled genome, thus supporting further secondary metabolite pathway elucidation in the fungal kingdom. We furthermore present the preliminary analysis of the application of the method to *A. niger*.

7. N-glycan profiling of *Aspergillus nidulans* using solid-phase glycan extraction and mass spectrometry. Diana Anyaogu, Shuang Yang, Jakob B. Nielsen, Hui Zhang, Michael Betenbaugh, Uffe Hasbro Mortensen. 1) Department of Systems Biology, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark; 2) Department of Pathology, Johns Hopkins University, Baltimore, Maryland 21287, United States; 3) Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, Maryland 21218, United States.

Filamentous fungi from the *Aspergillus* species are widely used as cell factories for the production of chemicals and enzymes, especially *Aspergillus niger* and *Aspergillus oryzae* as used as protein producers. Fungi have a high secretion capacity in comparison to other eukaryotic expression systems as algae, yeast and insect cells. The majority of the secreted proteins are glycosylated, thus glycosylation plays an important role in the secretory pathway. Glycosylation is also important in the production of therapeutic proteins as it is involved in protein stability, ligand binding, immunogenicity and serum half-life. Furthermore the efficacy of many therapeutic proteins depends on correct glycosylation. Thus, understanding the glycosylation will enable the directed glycoengineering in *Aspergillus* to improve protein production. In the present study, the Solid-Phase Glycan Extraction (SPEG) method was used to isolate and purify N-glycans from the secretome and whole cell lysates from *Aspergillus nidulans*. The mass of the glycans was determined using a MALDI-TOF MS. In addition, *A. nidulans* strains with mutations in the glycosylation pathway were analyzed and compared to the reference strain. This study shows that some of the mutations had an effect on the N-glycan profile, which shifted the profile towards glycans with a lower mass. The method presented here is thus very efficient for extracting N-glycans and for quantifying the relative abundance of different N-glycans in the secretome and whole cell lysate.


*Aspergillus nidulans* produces a great variety of secondary metabolites such as mycotoxins and antibiotics. The biosynthesis requires often a large number of enzymes, and the corresponding genes are located in clusters. Each cluster normally contains a specific transcription factor, whose expression induces the coordinated expression of all other genes located in the cluster. In addition to the coordinated expression, the sequential biosynthesis of a given secondary metabolite depends on the concerted action of all enzymes. Each enzyme produces the substrate for the next enzyme in the pathway and thus the spatial distribution of the enzymes and the diffusion of the low-molecular weight intermediates could be rate-limiting factors. In order to test the hypothesis whether the production of a certain metabolite could be increased by bringing some enzymes closer together, we studied penicillin biosynthesis in *Aspergillus nidulans*. The pathway comprises only three enzymes encoded by acva, ipna and the peroxosomal aoxA. In order to test whether peroxisomes could serve as production organelles, we tried to target all three enzymes to these organelles. Targeting Ipna to peroxisomes caused a complete inhibition of penicillin formation. However, in the case of AcvA peroxosomal targeting, a 4-fold increase of the penicillin yield was obtained. Currently, we are trying to further increase the penicillin yield by elevating peroxisome numbers through overexpression of penK. Recently, it was shown that peroxisomes may serve novel metabolic functions in fungi, since several glycolytic enzymes appear to be partially localized in peroxisomes. These results suggest that peroxisomes may also be used as production containers for other metabolites. (1)Kiel JA, van der Kie IJ, van den Berg MA, Bovenberg RA & Veenhuis M (2005). Fungal Genet Biol 42: 154-164. (2)Freitag J, Ast J & Bolker M (2012). Nature 485: 522-525.
9. Characterization of the 3-methyl orsellinic acid gene cluster in Aspergillus nidulans. Jakob B. Nielsen1, Marie L. Kleijnstrup1, Paiman K. Jamal2, Dorte K. Holm3, Michael L. Nielsen1, Anna M. Kabat1, Charlotte H. Götfredsen1, Thomas O. Larsen3, Uffe H. Mortensen1. 1) Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark; 2) Center for Systems Microbiology, Department of Systems Biology, Technical University of Denmark; 3) Department of Chemistry, Technical University of Denmark.

With the aim of mapping the polyketome of Aspergillus nidulans we have made a library of strains, which individually overexpress PKS genes from an ectopic locus. A screen of this collection on different media demonstrated that overexpression of AN6448 (pkbA) leads to increased production of 3-methyl orsellinic acid. An inspection of the DNA sequence surrounding this gene uncovered a putative gene cluster including a gene, AN6446 (pkbB), with homology to transcription factors. Based on this observation, we decided to overexpress pkbB. A qRT-PCR analysis of this strain was used to delineate the borders of the gene cluster as well as to stimulate formation of dichiorine, dichiorinic acid, nidulol and a novel cichonidulol dimer, just to name a few of the products that we have linked to this gene cluster. Subsequent deletion of all genes in the cluster has allowed us to propose a comprehensive model for the biosynthetic pathway of this cluster.


The purpose of this study was to induce sclerotium production in Aspergillus niger and other black Aspergillus. Some species in Aspergillus section Nigri are known for their production of sclerotia, especially A. carbonarius, A. tubingenis (few isolates), A. sclerotiorenger, A. sclerotiarbonarius, A. costaricaensis, A. piperi, A. japonicus, and A. aculeatus. A heteromorphus was reported in 1955 to produce sclerotia, but this could not be confirmed in later studies. There are also un-confirmed data on sclerotium production in Aspergillus niger, but often isolates reported to produce sclerotia were not A. niger anyway. Induction of sclerotium production in Aspergillus niger is important, since this may help in inducing the perfect state in this important Industrial fungus. By screening several media, we were able to develop some media and use some growth conditions that induced sclerotium production in Aspergillus niger and other species. Here, we report on the production of sclerotia in several media, including aerobic media, liquid media, and induced media.

11. Analyzing the impact of compartmentalization on organic acid production in Aspergillus niger. Matthias G. Steiger1,2, Charlotte H. Götfredsen1, Thomas Larsen3, 1) Austrian Centre of Industrial Biotechnology (ACIB GmbH), Muthgasse 11, 1190 Vienna, Austria; 2) University of Natural Resources and Life Sciences, Department of Biotechnology, Muthgasse 18, 1190 Vienna, Austria; 3) University of Applied Sciences FH-Campus Vienna, School of Bioengineering, Muthgasse 86, 1190 Vienna, Austria.

Aspergillus niger is a well-established host organism for the production of carboxylic acids. Acids like citric, gluconic and oxalic acids can already be produced by A. niger and high titers are obtained. The formation of carboxylic acids involves the shuttling of intermediate metabolites between different intracellular compartments and utilizes different enzymatic capabilities of the respective compartment. The knowledge about the involved shuttling mechanisms and the localization of the necessary enzymes is still fragmentary. Using fluorescence microscopy, it is possible to characterize the intracellular localization of GFP tagged proteins and hence mitochondrial leader sequences can be functionally tested. In order to analyze the influence of the compartmentalization on the organic acid production, we have chosen itaconic acid as a target substance. Iaconic acid, which was selected by the US Department of Energy as one of the 12 building block chemicals for the industrial biotechnology, is currently produced by A. terreus. Heterologous expression of the A. terreus cadA gene also enables the formation of itaconic acid in A. niger although only low titers are obtained. We set out to characterize the influence of the compartmentalization on the productivity and re-engineered the enzymatic cascade by flipping the enzymatic activities of the cis-aconic acid decarboxylase and aconitase between the mitochondrion and the cytosol. We will present new leader sequences for mitochondrial targeting in A. niger alongside with results about the positive impact of the enzymatic re-localization on the itaconic acid production.


In recent years, a lot of biosynthesis gene clusters involving in secondary metabolite biosynthesis from filamentous fungi have been revealed, and thus the attempts to produce these valuable metabolites at high yield have been actively made. To this end, Aspergillus oryzae is an attractive host for heterologous secondary metabolism production because of its less productivity for own secondary metabolites, which leads to the production for the metabolite of interest at a highly pure grade. Actually, the number of report has been increasing recently, in which biosynthetic genes involved in fungal secondary metabolism biosynthesis were heterologously overexpressed in A. oryzae. On the other hand, it would be necessary to consider the cellular compartments where the target secondary metabolite is synthesized in filamentous fungi to produce it efficiently in the heterologous host, A. oryzae. However, currently there is very little knowledge about the spatial distribution of the biosynthesis enzymes of the secondary metabolite in fungi. Therefore, in this study, we examined the subcellular localization of the enzyme proteins encoded by a gene cluster involved in aphidicolin biosynthesis from Phoma betae, which were expressed as GFP-fusion proteins in A. oryzae. The gene cluster of aphidicolin in P. betae contains 4 genes encoding biosynthesis enzymes (geranylgeranyl diphasphate synthase [GGS], aphidicolan-16b-ol synthase [ACS], cytochrome P450 monoxygenase 1 [P450-1], and P450-2), a gene for transporter (TP), and a gene for transcription factor. We constructed 4 biosynthesis enzymes and the transporter that each was fused to GFP under the a-amylase gene promoter, and introduced into A. oryzae. Similarly, the organelle marker proteins fused to RFP were also constructed and expressed simultaneously with GFP-fusion proteins to identify the organelle where the biosynthesis enzyme was localized. Fluorescent microscopy revealed that GGS and ACS were distributed in the cytoplasm and P450-1 was located in endoplasmic reticulum (ER). Interestingly, all of GFP-fused P450-2 was not observed in ER when only P450-2 was expressed, but mostly localized in ER when coexpressed with P450-1. In addition, TP fused to GFP was localized mainly on the plasma membrane and also rarely observed on other organelles such as vacuole.
13. Increased production of fatty acids and triglycerides in Aspergillus oryzae by modifying fatty acid metabolism. Koichi Tamano¹, Kenneth Bruno², Tomoko Ishii³, Sue Karagiosis³, David Culley³, Shuang Deng³, James Collet³, Myco Umemura³, Hideaki Koike³, Scott Baker³, Masayuki Machida³. 1) National Institute of Advanced Industrial Science and Technology (AIST); 2) Pacific Northwest National Laboratory (PNNL).

Biofuels are attractive substitutes for petroleum based fuels. Biofuels are considered they do not contribute to global warming in the sense they are carbon-neutral and do not increase carbon on the globe. Hydrocarbons that are synthesized by microorganisms have potential of being used as biofuels or the source compounds. In the hydrocarbon compounds synthesized by A. oryzae, fatty acids and triglycerides are the source compounds of biodiesel that is fatty acid methyl ester. We have increased the production by modifying fatty acid metabolism with genetic engineering in A. oryzae. Firstly, enhanced-expression strategy was used for the increase. For four enzyme genes related to the synthesis of palmitic acid ([C16:0-fatty acid], the individual enhanced-expression mutants were made. And the fatty acids and triglycerides in cytosol were assayed by enzyme assay kits, respectively. As a result, both fatty acids and triglycerides were most synthesized in the enhanced-expression mutant of fatty acid synthase gene at 2.1-fold and 2.2-fold more than the wild-type strain, respectively. Secondly, gene disruption strategy was used for the increase. Disruptants of several enzyme genes related to long-chain fatty acid synthesis were made individually. And one of them showed drastic increase in fatty acid synthesis. In the future, further increase in the synthesis is expected by utilizing genetic engineering in A. oryzae.

14. Improved Properties of Thermostable Cellobiohydrolase in a Treatment of Cellulosic Material. Taiga Leinonen¹, Susanna Mäkinen¹, Kari Juntunen¹, Merja Niemi², Juha Rouvinen², Jari Vehmaanperä³, Terhi Puranen³. 1) Roal Oy, Rajamäki, Finland; 2) University of Eastern Finland, Department of Chemistry, Joensuu, Finland.

Production of biofuels i.e. bioethanol from lignocellulosic material is a promising alternative technology for using biomass as a renewable and clean source of energy instead of consuming limited natural resources e.g. fossil fuels, and releasing increasing amounts of CO₂. Enzymatic hydrolysis is considered to be the most promising technology for converting cellulosic biomass into fermentable sugars. Enzymatic total hydrolysis of ligno-cellulosic substrates requires at least cellobiohydrolases, endoglucanases and beta-glucosidases. Previously cloned thermostable glycoside hydrolase family 7 cellobiohydrolase (CBHI) from Acromonium thermophilum was expressed in Trichoderma reesei. The purified A. thermophilum CBHI was crystallized, and the structure of the catalytic domain of the protein was determined at a resolution of 1.8Å revealing the overall structure of the catalytic core of the enzyme to be similar with the previously determined structures of glycosidases family 7 cellobiohydrolases. In the biomass hydrolysis experiments the A. thermophilum CBHI, as part of the enzyme mixture, was shown to have enhancing effect on hydrolysis yield as compared to the Trichoderma reesei enzyme. To achieve even further improvements in thermal stability and hydrolysis performance, several single and combined amino acid mutations were designed based on the resolved 3D-structure. The data obtained from the mutants demonstrates that thermal stability and hydrolysis performance of the A. thermophilum CBHI protein can be increased by introducing single mutations as well as mutation combinations to the molecule.

15. The phytopathogenic fungus Botrytis pseudocinerea is resistant to the fungicide fenhexamid due to detoxification by a cytochrome P450 monooxygenase Cyp684. Saad Azeddine, Alexis Billard, Jocelyne Bach, Catherine Lanen, Anne-Sophie Walker, Sabine Fillingen, Danièle Debieuc, INRA UR1290 BIOGER CCP, avenue Lucien Brétignières F78850 Thiverval-Grignon, France.

The Botrytis species complex responsible for the grey mould disease found on grapevines is composed of two species: Botrytis cinerea, to a large extent (roughly 90%), and Botrytis pseudocinerea. Despite their genetic polymorphism, these species cannot be morphologically distinguished. However, they do differ in their response to several fungicides, especially to the sterol biosynthesis inhibitor fenhexamid. While B. cinerea is sensitive to this hydroxylated fungicide, B. pseudocinerea is naturally resistant. Because a strong synergism was found on B. pseudocinerea between fenhexamid and sterol 14α-demethylation inhibitors (DMIs) known to inhibit Cyp51, a cytochrome P450 monooxygenase, it was hypothesized that the detoxification of fenhexamid by a cytochrome P450 monooxygenase similar to Cyp51 is involved in the resistance B. pseudocinerea displays. To test this, we sought the gene overexpressed in the presence of fenhexamid with the highest similarity to cyp51. Taking into account the Cyp P450 classification based on homology and phylogenetic criteria, this gene, whose function remains unknown, belongs to the Cyp684 family. It was then deleted in a B. pseudocinerea strain. Cyp684 knockout mutants exhibit a loss of fenhexamid resistance and synergism between DMIs and fenhexamid, showing that the Cyp684, cytochrome P450 protein is responsible for B. pseudocinerea’s natural resistance to fenhexamid and is involved in fenhexamid detoxification. Although cyp684 is also present in B. cinerea, which is sensitive to fenhexamid, a polymorphism was observed between the two species: in B. pseudocinerea the cyp684 promoter shows a deletion of 25 bp. We are currently establishing the cyp684 expression profiles in both species in order to analyze the impact of the promoter deletion on its expression. Metabolization studies are also being conducted to identify metabolites that would help in understanding the enzymatic functions of Cyp684 and to determine to what extent Botrytis sp. is sensitive to these metabolites.

16. Evolutionary rewiring of ubiquitination targets in Candida albicans promotes efficient carbon assimilation in host niches. Alistair J P Brown, Doblin Sandai, Zhiheng Yin, Laura Selway, David Stead, Janet Walker, Michelle D Leach, Iryna Bohovych, Juliana V Ene, Stavroula Kastora, Susan Budge, Carol A Munro, Frank C Odds, Neil A R Gow. School of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom.

Pathogens must assimilate carbon to grow and infect their host. Interesting questions remain about the regulation of carbon assimilation in Candida albicans despite the wealth of knowledge about this major fungal pathogen of humans. C. albicans is classified as a Crabtree-negative yeast because it continues to respire in the presence glucose. [J Med Vet Mycol 26, 195]. How then can C. albicans be exquisitely sensitive to sugars, down-regulating transcripts involved in the utilization of alternative carbon sources following exposure to 0.01% glucose [Molec Biol Cell 20, 4815]? We have now shown that there is a significant dislocation between the transcriptome and proteome in C. albicans: glucose triggers the decay of key transcripts but the enzymes they encode are retained. This allows the simultaneous assimilation of alternative carbon sources such as fatty acids, carboxylic acids and sugars by C. albicans. This contrasts with the situation in Saccharomyces cerevisiae where simultaneous carbon assimilation is prevented by catabolite inactivation [Arch Micro 134, 187; Arch Micro 147, 231]. We show that C. albicans has retained the molecular apparatus that mediates ubiquitin-mediated, glucose-accelerated protein degradation. For example, S. cerevisiae isocitrate lyase (Sac11) is degraded rapidly when expressed in C. albicans. However, C. albicans isocitrate lyase (Calcl1) lacks critical ubiquitination sites that mediate this catabolite inactivation. Furthermore, other C. albicans enzymes involved in gluconeogenesis and the glyoxylate cycle appear to lack such sites, whereas glycolytic enzymes are ubiquitinated (e.g. Fba1, Pkg1, Enol1). Therefore there has been significant rewiring of ubiquitination targets in C. albicans compared to S. cerevisiae. This metabolic flexibility probably enhances efficient colonisation of host niches that contain complex mixtures of nutrients.
17. Can-Hsp31 is important for Candida albicans growth and survival. S. Hasin, N. Ahmad hussin, K. Nickerson. Biological Science, University of Nebraska Lincoln, Lincoln, NE.

Candida albicans is an opportunistic pathogen that can grow as budding yeast, pseudohyphae, and hyphae. A key feature of C. albicans is its ability to grow in diverse microenvironments and develop complex and highly efficient responses in order to survive within the host environment. The C. albicans Hsp31 (ORF19.251) gene encodes a protein that belongs to the DJ1/Ppi family with close homology to other fungal Hsp31-like proteins. Despite intensive study, the function of these fungal Hsp31 proteins is unknown. The crystal structure of Can-Hsp31 was solved to 1.6 Å resolution. Its structure is similar to those of the E.coli and S. cerevisiae Hsp31 proteins except that Can-Hsp31 is a monomer in the crystal while all other known homologues are dimers. In this report, we show that the C. albicans Hsp31 is important for growth and survival under various stress conditions.

18. Influence of N-glycans on α-b-(1,3)-glucanase and α-(1,4)-amylase from Paracoccidioides brasiliensis yeast cells. Fausto Bruno Dos Reis Almeida, Valdirene Neves Monteiro, Roberto Nascimento Silva, Maria Cristina Roque-Barreira. 1) Cellular and Molecular Biology, University of Sao Paulo, Ribeirao Preto, Brazil; 2) University of Goias, Anapolis, Brazil; 3) Biochemistry and Immunology, University of Sao Paulo, Ribeirao Preto, Brazil.

Paracoccidioides brasiliensis (PB) is a temperature-dependent dimorphic fungus and the causative agent of paracoccidioidomycosis, the most prevalent systemic mycosis in Latin America. The cell wall (CW) of PB is a network of glycoproteins and polysaccharides, such as chitin, glucan and N-glycosylated proteins, that may perform several functions. N-glycans are involved in glycoprotein folding, intracellular transport, secretion, and protection from proteolytic degradation. Our group has been describing the role of N-acetylglucosaminidase (NAGase) in fungal growth, exerted through participation in chitin metabolism and CW remodeling. In addition, by assessing yeast cells cultured with tunicamycin (TM), we determined that N-glycans play important roles in growth and morphogenesis of PB yeasts and are required for the fungal NAGase function. In this study, we verify the influence of TM-mediated inhibition of N-linked glycosylation on α- and β-(1,3)-glucanase, as well as the α-(1,4)-amylase, produced by PB yeast cells. The treatment of PB with 15 mg TM/ml did not interfere with α- and β-(1,3)-glucanase production, secretion or on enzyme structure. The absence of N-glycans did not affect pH optimum (5.5) or temperature optimum (45 °C). Moreover, the fully and under-glycosylated forms of the enzymes had similar Km and Vmax values. On the other hand, α-(1,4)-amylase demonstrated lower enzymatic activity when underglycosylated, although no difference was detected between the pH and temperature optimums of the two forms. Our results corroborates with the recent observation that α-(1,4) amylase from PB plays important roles on the fungal CW α-(1,3)-glucan biosynthesis. However, interestingly the Pbglucanase gene, that encode to α-(1,3)-glucanase, had its expression increased by 2.5-fold in Pb cells treated with TM when evaluated by qRT-PCR, suggesting an indirect influence of TM on CW glucan synthesis. Genes encoding to UPR (Unfolding Protein Response) and CW synthesis showed their expression increased, corroborating with our data. Analyses investigating the effect of N-glycans in mycelium cells are under way in our laboratory. Our results suggest that N-glycans do not play direct effect on α- and β-(1,3)-glucanase activity produced by yeast cells but indirect effect by affecting α-(1,4)-amylase.


Cell wall polysaccharides play a central role in vital processes like the morphogenesis and growth of eukaryotic micro-organisms. Thus, the enzymes responsible for their biosynthesis represent potential targets of drugs that can be used to control diseases provoked by pathogenic species. One of the most important features that distinguish oomycetes from true fungi is their specific cell wall composition. The cell wall of oomycetes essentially consists of (1,3)-b-glucans, (1,6)-b-glucans and cellulose whereas chitin, a key cell wall component of fungi, occurs in minute amounts in the walls of some oomycete species only. Thus, the cell walls of oomycetes share structural features with both plants [cellulose; (1,3)-b-glucans] and true fungi [(1,3)-b-glucans, (1,6)-b-glucans and chitin in some cases]. However, as opposed to the fungal and plant carbohydrate synthases, the oomycete enzymes exhibit specific domain compositions that may reflect polyfunctionality. In addition to summarizing the major structural differences between oomycete and fungal cell walls, this presentation will compare the specific properties of the oomycete carbohydrate synthases with the properties of their fungal and plant counterparts, with particular emphasis on chitin, cellulose and (1,3)-b-glucan synthases. The significance of the association of these carbohydrate synthases with membrane microdomains similar to lipid rafts in animal cells will be discussed. In addition, distinguishing structural features within the oomycete class will be highlighted with the description of our recent classification of oomycete cell walls in three different major types. Genomic and proteomic analyses of selected oomycete and fungal species will be correlated with their cell wall structural features and the corresponding biosynthetic pathways.


The oomycete Phytophthora infestans is a plant pathogen that causes potato late blight, a devastating disease associated with tremendous economic losses. In contrast to true fungi, oomycetes are traditionally described as cellulose micro-organisms. Indeed, in addition to other b-glucans, cellulose is a major polysaccharide in the mycelial cell wall of P. infestans while chitin and other N-acetylglucosamine (GlcNAc)-based carbohydrates are absent from hyphal walls. However, a putative chitin synthase gene (chs) is present in the genome. Bioinformatic analysis identified the C-terminal region of the predicted protein to be highly similar to glycosyltransferase family 2 proteins, such as fungal chitin synthases, while the N-terminal domain is more divergent. Orthologous putative chs genes are present in all sequenced oomycete genomes and phylogenetic analysis shows the oomycete gene products form a new clade separate from the fungal lineage. The P. infestans chs transcript is highly abundant in older mycelium. However, no chitin synthase activity was detectable in microsomal fractions assayed with radiolabeled UDP-GlcNAc, the natural substrate of chitin synthase. Surprisingly, hyphal growth was severely retarded in the presence of low micromolar concentrations of the chitin synthase inhibitor nikkomycin Z, a structural analogue of UDP-GlcNAc. Microscopic analysis of nikkomycin Z-treated hyphae revealed frequent tip swelling and bursting. Similarly, transient RNA-mediated silencing of the chs gene resulted in severely reduced growth, and hyphae showed a hyper-branch morphology with swollen tips. As a first step to determine the precise function of the P. infestans chs gene, we have cloned and expressed it in Saccharomyces cerevisiae.


Some oomycete species are severe pathogens of economically important animals or plants. Proteins involved in cell wall metabolism represent excellent targets for disease control. The objective of our work was to determine the fine cell wall polysaccharide composition of selected species and identify the corresponding membrane-bound biosynthetic enzymes and other proteins involved in cell wall remodeling. In the first instance, we performed a detailed carbohydrate analysis of the mycelial cell walls of 11 oomycete species from 2 major orders, the Saprolegniaceae and Peronosporales. We then selected the fish pathogen Saprolegnia parasitica for in-depth proteomics analysis. Our results indicate the existence of 3 clearly different cell wall types. This
biochemical distinction is in agreement with the taxonomic grouping based on molecular markers of the species studied. The 3 cell wall types are distinguishable by their cellulose content and the fine structure of their 1,3-b-glucans. Furthermore, unique features were found in each case. Type I cell walls (e.g. Phytophthora) are devoid of N-acetylglucosamine (GlcNAc) but contain glucuronic acid and mannose; type II (e.g. Achlya, Dictyuchus, Leptogonia and Saprolegnia) contain up to 5% GlcNAc and residues indicative of cross-links between cellulose and 1,3-b-glucans; type III (e.g. Aphanozymes) are characterized by the highest GlcNAc content (> 5%) and the occurrence of unusual carbohydrates that consist of 1,6-linked GlcNAc residues. Analysis of the recently sequenced genome of S. parasitica was combined with quantitative mass spectrometry-based proteomics (label-free and iTRAQ) to characterize the plasma membrane proteome of hyphal cells. This strategy allowed us to experimentally identify a total of 677 plasma membrane proteins, including several key wall polysaccharide synthases, e.g. cellulose, 1,3-b-glucan and chitin synthases, some of which are specifically enriched in plasma membrane microdomains similar to lipid rafts in animal cells.

22. Identification and characterization of the chitin synthase genes in the fish pathogen Saprolegnia parasitica. Elżbieta Rzeszutek, Sara Diaz, Vincent Bulone. School of Biotechnology, Division of Glycoscience, Royal Institute of Technology (KTH), Stockholm, Sweden.

The oomycete Saprolegnia parasitica is a fungus-like microorganism responsible for fish diseases and huge losses in aquaculture. The analysis of the cell wall composition of the microorganism and the characterization of key enzymes involved in cell wall biosynthesis may facilitate the identification of new target proteins for disease control. The cell wall of hyphal cells of S. parasitica consists mainly of cellulose, b-(1\(^\beta\)-3)- and b-(1\(^\beta\)-6) glucans, whereas chitin is present in minute amounts only. The main objective of this work was to test the effect of nikkomycin Z, a competitive inhibitor of chitin synthase (CHS), on the growth of S. parasitica. Genome mining allowed the identification of six different putative chs genes whose actual occurrence in the genomic DNA of the microorganism was confirmed by Southern blot analysis. The expression of the chs genes in the mycelium was analyzed using Real-Time PCR. The results revealed a higher expression level of four of the six genes while the two others exhibited undetectable levels of expression in the mycelium. This suggests that the latter genes are mostly likely primarily involved in chitin formation at a different developmental stage. The presence of nikkomycin Z increased the expression level of one of the genes, chs3, suggesting that the corresponding product is involved in forming the abnormal branching structures in the hyphae exposed to the inhibitor. The capacity of the mycelium to synthesize chitin was demonstrated by performing in vitro synthesis reactions using cell-free extracts. CHS activity was measured in intact cell membranes as well as in detergent-extract of membranes. The polysaccharide synthesized in vitro was characterized by enzymatic hydrolysis with a specific chitinase. Our data demonstrate that CHS represent promising targets of anti-oomycete drugs, even though the amount of chitin in the cell wall of S. parasitica does not exceed a few percent.

23. Role of Ccr4-mediated mRNA turnover in nucleotide/deoxyribonucleotide homeostasis and Amphotericin B susceptibility in Cryptococcus neoformans. D. Banerjee, J. Panepinto. Department of Microbiology and Immunology, University at Buffalo, SUNY, Buffalo, NY.

Ccr4 mediated deadenylation is the first and rate limiting step in eukaryotic mRNA decay. The end products of mRNA degradation are nucleoside monophosphates (NMPs) which are then converted to nucleotides (NDPs and NTPs) and deoxyribonucleotides (dTTPs) in downstream reactions. A C. neoformans degradation deficient ccr4D mutant exhibits replication stress sensitivity and stabilizes ribosomal protein (RP) transcripts during carbon starvation, suggesting that ccr4D mutant is deficient in intracellular nucleotide stores. Analysis of gene expression showed an up-regulation of the nucleotide synthesis machinery in ccr4D mutant even under unstressed conditions consistent with our hypothesis. Time-kill assays in the presence of mycophenolic acid (MPA), an inhibitor of guanine nucleotide de novo synthesis, showed a reduction in the viability of ccr4D mutant that was rescued by the addition of exogenous guanine, suggesting that the salvage pathway is indeed functional. These results suggest that the degradation of mRNA transcripts lead to the production of NMPs that replenish NTP/dNTP pools in C. neoformans during starvation stress. The fungicidal efficacy of Amphotericin B (AmB) is enhanced by the use of Flucytosine, a pyrimidine analog, suggesting a synergy between AmB and nucleotide deficiency for cryptococcosis treatment. We compared the sensitivity of wild type (H99), ccr4D mutant and H99-FOA strain (de novo mutant of pyrimidine synthesis) to a combination of AmB and NTP/dTNP inhibitors. Both mutants exhibited higher sensitivity to AmB which was unaltered by additional stressors. H99 exhibited an increased sensitivity to the combination of AmB with both NTP and dTNP inhibitors, compared to AmB alone. Taken together, these data suggest that nucleotide depletion, either by a pharmacologic agent or a mutation predisposes the cells to enhanced AmB mediated cell death. Thus our overall hypothesis is that Ccr4 mediated mRNA turnover results in the maintenance of intracellular NTP/dTNP pools to promote growth, virulence, stress tolerance and also modulates AmB susceptibility in C. neoformans. Results from these studies will identify a novel role of mRNA degradation machinery in C. neoformans pathogenesis and stress tolerance and also aid in the identification of new anti-cryptococcal drug targets.

24. WITHDRAWN

25. Blue light induce Cordyceps militaris fruiting body formation and cordycepin production. Chun-Hsiang Yang\(^{1,}\), Shun-Kuo Sun\(^{2,}\), Su-Der Chen\(^{2,}\).

Cordyceps militaris is a very important fungal medicine in Chinese. The fruiting body of Cordyceps militaris has been described by many researchers containing biological activities, such as being able to inhibit cell proliferation, provide anti-ageing activity, inhibit protein synthesis and lowering cardiovascular risk. Cordyceps militaris has been grown and harvested by many Chinese people, and were able to obtain its fruiting body with orange collar and bar shape. Solid cultivation as carried out 3 to 4 days after mycelium seeding from liquid culture, then fruiting body formation can be induced by light(12 hours per day). In this research, the light-inducing mechanism of fruiting body formation was studied. The results showed the fruiting body was induced by blue light but not red light. Cordycepin, the most important compound with medical potential of Cordyceps militaris, is mainly stored in fruiting body, rather than in mycelium from liquid culture. Cordycepin production depends on various factors, including: wave length of light and culture in solid or liquid. The results also showed the relationship between cordycepin production and blue light sensor in Cordyceps militaris, which might contain LOV domain.

26. Insight into alkaloid diversity of the epilobeae, protective symbionts of grasses. Carolyn A. Young\(^{1,}\), Nikki D. Charlton\(^{1,}\), Johanna E. Takah\(^{1,}\), Ginger A. Swoboda\(^{1,}\), Bradley A. Hall\(^{2,}\), Kelly D. Craven\(^{2,}\), Christopher L. Schardl\(^{1,}\).

The epilobeae consist of both sexual (nonhybrid) and asexual (hybrid and nonhybrid) species that can produce the bioactive anti-herbivore compounds, ergotalkoids, indole-diterpenes, lollines and peramine. Epilobeae can exhibit considerable chemotypic diversity within the pathways of these four alkaloid classes as well as the combination of alkaloids that can be produced in an individual, and as such, may equate to fitness benefits for the host. The current genome sequencing efforts, whereby at least 10 epilobeae have been sequenced, now allows us to develop simple
approaches to rapidly screen large cool-season grass populations to identify endophyte diversity. Molecular analyses of endophyte genetic traits from among and between host populations allow us to explore resident endophyte incidence and diversity present in single host species. PCR with genomic DNA extracted from individual plants (seeds or tillers) is used to determine endophyte incidence within a line and to predict alkaloid chemotypes at the EAS (ergot alkaloids), LOL (loelines), IDT/LTM (indole-diterpenes) and PER (peramine) loci. The presence or absence of genes at each locus can be used to predict the likely pathway end product for a given endophyte-infected plant line. Phylogenetic analyses of housekeeping and mating-type genes are used to infer hybrid versus nonhybrid origins as well as hybrid ancestral progenitors. Sequence analyses of alkaloid genes encoding key pathway steps provide allele copy number and can be used to determine progenitor origins to further support the phylogenetic relationships. Grass collections across multiple host tribes have recently been evaluated and considerable endophyte chemotypic diversity was identified. Multiple endophyte species were able to independently associate with some grass host species and often both hybrid and nonhybrid endophytes could be found within a population. In many cases, chemotypic diversity of the hybrids may have arisen from independent hybridization events and as such, this alkaloid diversity likely translates into differences in fitness and persistence of the host.


In the temperate forests of North America and Europe, basidiomycetes such as Agaricus bisporus are renowned for their ecological significance in the cycling of carbon from dead plant matter. A. bisporus is also the most widely produced mushroom in the world and has been cultivated for centuries. However, little is known about the interaction between A. bisporus and its most preferred substrate, composted plant matter. In this study, a wide array of extracellular polysaccharide degrading enzymes was studied under semi-sterile conditions to understand the carbon nutritive needs of the fungi. Various time points were sampled from filling of the beds, vegetative growth and development and maturation of fruiting bodies. Clear correlations in the enzymatic activities were observed from different stages of development of A. bisporus between compost, casing layer and fruiting bodies. This could suggest that vegetative mycelia and the fruiting body divide their metabolic roles as vegetative mycelium of A. bisporus provides nutrients for the growth of fruiting bodies, while fruiting bodies aids on reproduction. This was also confirmed by identification of the expression of genes encoding plant and fungal polysaccharide modifying enzymes in compost, casing layer and fruiting bodies.

28. Reconstruction of the rubrofusarin biosynthetic pathway in Saccharomyces cerevisiae. Rasmus J N Frandsen¹, Peter Rugbjerg¹, Michael Naesby², Uffe H Mortensen¹. 1) Systems Biology - CMB, Technical University of Denmark, Kgs. Lyngby, Denmark; 2) Evolva SA, Duggingerstrasse 23. CH-4153 Reinach, Switzerland.

The aromatic heptaketide rubrofusarin is a common core substructure of several fungal pigments, including rubrofusarin B, aurofusarin, nigerone, nigerasperone A, chaetochromin, ustilaginoidin and parasperone A. Compounds that are produced by a wide variety of different filamentous fungi such as Fusarium graminearum, Aspergillus niger, Aspergillus parasticus, Chaetomium gracile and Ustilaginoidea virens. Previous reverse genetics analysis of the aurofusarin biosynthetic pathway, by targeted gene replacement in F. graminearum (Fg), has resulted in the formulation of a six step biosynthetic pathway that includes rubrofusarin as an intermediate. In the current study we have used heterologous expression in Saccharomyces cerevisiae to test whether all the enzymes required for biosynthesis of rubrofusarin have been identified. Successful reconstruction of the rubrofusarin pathway is dependent on the heterologous co-expression of four genes: the Fg polyketide synthase PKS12, the Fg dehydratase aurZ, the Fg O-methyltransferase aurJ and the Aspergillus fumigatus phosphopantetheine transferase npgA. To eliminate potential problems with intron splicing of the fungal genes in S. cerevisiae the required coding sequences were de novo synthetized in codon optimized versions. The four genes were expressed individually from four different single copy plasmids, each with a unique auxotrophic marker. Co-expression of the codon optimized version of PKS12 with npgA did not result in production of any new metabolites. However, surprisingly co-expression of a CdNA version of PKS12, assembled from gDNA by USER-fusion, resulted in production of the expected product YWA1. Additional co-expression of the codon optimized dehydratase encoding aurZ gene lead to production of nor-rubrofusarin, and subsequent introduction of the O-methyltransferase gene aurJ yielded rubrofusarin. These results support the previously proposed biosynthetic route for the formation of rubrofusarin in F. graminearum. The utilized bottom-up approach shows that formation of rubrofusarin is dependent only on the combined action of PKS12, AurZ and AurJ in F. graminearum, and likely also in other fungal species that produce compounds with a rubrofusarin core. The latter is further supported by sequence homology searches in the available relevant fungal genome sequences.


Recombinant protein production is a fast growing market area. The need for novel production platforms is growing together with the number of new applications for recombinant proteins. The ascomycete T. reesei is an excellent producer of hydrolytic enzymes. However, heterologous protein production in T. reesei is often suffering from low product yields due to protease degradation and inefficiency in heterologous protein secretion. We have previously demonstrated a novel recombinant protein production system for T. reesei using GFP as a model protein. This system uses hydrophobin, a small and amphipathic fungal protein, as a fusion tag for purification and ER retention signal for targeting the produced protein to intracellular protein bodies. The GFP-HFBI fusion protein can be extracted from total protein lysate by aqueous two-phase separation system. We have now further optimised the expression for GFP-HFBI fusion and demonstrated the applicability of this production concept for two additional proteins, glucose oxidase (GOX) and tissue plasminogen activator (tPA). Effect of C- and N-terminal hydrophobin fusion on productivity and extraction in two-phase separation system will be discussed. The new production concept is aiming at widening the spectrum of recombinant proteins that can be produced efficiently in T. reesei.

Nutrient acquisition and metabolic adaptation to host-derived nutrients is an important aspect of pathogen biology. An understanding of the metabolic adaptations made by Phytophthora infestans, an important pathogen of potato and tomato, to optimize nutrient uptake from diverse host tissues and within the microenvironments of the host will lead to a better understanding of host-pathogen relationships. In order to study metabolic adaptations of P. infestans, transcriptional profiling and live cell imaging using promoter-fluorescent protein fusions will be used. Preliminary results demonstrated the differential gene expression of many metabolic genes of P. infestans grown on different natural hosts and that grown on rich media. The next step involves answering the question: Are some metabolic genes expressed in a stage-specific or time-dependent manner? In addition, the role of enzymes involved in energy homeostasis and metabolite channeling are being studied. The roles of two such genes encoding putative creatine kinases are being elucidated using subcellular localization, substrate utilization and loss of function studies.

31. Platforms for secondary metabolite analysis in filamentous fungi. Uffe H. Mortensen1, Jakob B. Nielsen1, Diana C. Anyaogu1, Dorte K. Holm1, Lene M. Petersen2, Morten T. Nielsen1, Mikael S. Joergensen1,2, Kristian F. Nielsen1, Pia F. Johannessen1, Dominique A. Skovlund1, Thomas O. Larsen1. 1) DTU Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark; 2) Department 463, Fungal Gene Technology, Novozymes, Denmark.

We are developing versatile methods that allow for rapid and simple genetic manipulation of filamentous fungi. Currently, we use our methods for elucidation of pathways for secondary metabolite production in a number of different species. The platform includes simple systems for gene targeting and defined expression platforms for pathway reconstitution. Alternatively, if few or no genetic tools are available for the fungus, we use AMA1 based plasmids for transformation. All DNA handling prior to fungal transformation is based on assembly by efficient USER cloning that allows for many DNA fragments to be merged in a single cloning step. Examples of pathway reconstitution will be presented including functional transfer of the entire geodin producing gene cluster from Aspergillus terreus into A. nidulans. In an attempt to map the first intermediates of polyketide pathways in a fungal species, we have individually expressed all PKS genes from A. niger as a starting point for pathway elucidation. Using this approach we identified a PKS gene responsible for production of 6-MSA. Next, we individually deleted all genes in the corresponding gene cluster in A. niger to further map the pathway. These analyses suggest that 6-MSA is a precursor of Yanuthone D/E. In a similar study, we have identified a related PKS gene in A. aculeatus that also produces 6-MSA when expressed in A. nidulans. The corresponding gene cluster in A. aculeatus contains a gene encoding a transcription factor. Using our AMA1 based expression system, this gene has been overexpressed in A. aculeatus. As a result a new 6-MSA based compound has been identified. Lastly, using a knock-in/knock-out platform in Trichoderma reesei we use the same principles to uncover a gene cluster that is responsible for a very complex family of sorbicillinoids.

32. Transcriptional analysis of oxalate degradation in the white rot basidiomycete Dichomitus squalens. Mia R. Makelä, Johanna Rytila, Outi-Maarja Sietio, Sari Timonen, Annele Hatakka, Kristiina Hildén. Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland.

Basidiomycetes white rot fungi are the most efficient degraders of lignocellulose with a unique ability to mineralize the recalcitrant lignin polymer. Lignocellulose decay involves a complex enzymatic system, but is also suggested to be promoted by the fungal secretion of oxalic acid. White rot fungi synthesize oxalate as a metabolic waste compound and typically secrete it to their environment in millimolar quantities. As oxalate is a toxic compound, regulation of its intra- and extracellular concentration is extremely crucial for fungi and also for lignocellulose degradation since high oxalate levels are shown to inhibit the decomposition reactions. Therefore, specific oxalate-converting enzymes, namely oxalate decarboxylases (ODCs) that work in conjunction with formate-degrading formate dehydrogenases (FDHs), are recognized as key fungal enzymes in lignocellulose decay. Dichomitus squalens is a white rot fungus that degrades effectively all the wood polymers, i.e. cellulose, hemicelluloses and lignin, and secretes oxalic acid during its growth on wood. The genome of D. squalens harbours 5 putative ODC and 3 putative FDH encoding genes, while these numbers differ in other fungi based on comparative genomics. In order to enlighten the roles of the multiple oxalic acid catalysing enzymes of D. squalens, the expression of the odc and fdh genes was followed with quantitative real-time RT-PCR when the fungus was grown on its natural substrate, i.e. Norway spruce (Picea abies) wood. In addition, the effect of organic acid (oxalic acid) and inorganic acid (HCl) supplementation on the relative transcript levels of the oxalate-catalyzing genes was examined in the submerged liquid cultures of D. squalens. The results show for the first time the sequential action of ODC and FDH at the transcript level in a white rot fungal species. The constitutive expression of odc1 suggests the pivotal role of the corresponding enzyme during the growth of D. squalens on wood. In addition, the strong upregulation of the transcription of odc2 in oxalic-acid amended cultures indicates the distinct roles of individual ODC isoenzymes.


Filamentous fungi have been used extensively in industry for decades, most prominently for the purposes of protein expression. An emerging technology is the use of fungi in the production of ecologically-friendly materials used in packaging and insulation, materials presently manufactured using non-renewable petroleum-based technologies. The growth characteristics of the mycelia used in these manufacturing processes play a pivotal role in the properties of the final product. We decided to utilize the classical approach of UV mutagenesis to create new strains of a basidiomycete fungus that would potentially have growth characteristics more suited to the wild type to particular industrial applications. One example of this would be the production of fungal strains with a wider temperature spectrum for growth, a factor applicable to the industrial reality of the fluctuating ambient temperature found in non-laboratory conditions. Protoplasts from our wild-type strain were subjected to 70,000 microjoules/cm2 of UV irradiation at a wavelength of 254 nm. Survivors were allowed to recover overnight at 28°C, and then plated at a selective temperature of 40°C. Out of an estimated 6400 protoplasts irradiated, we observed 48 colonies of various sizes after 11 days growth at the selective temperature of 40°C. This temperature was chosen because it approaches the maximum permissible growth temperature for this fungus. We further characterized 22 of these presumed mutants and were able to sort them into broad categories based upon their growth rates over the range of 28°C to 37°C. The categories were: (1) strains that grew well at the normal laboratory temperature (28°C), but less well at elevated temperatures (33.5°C and 37°C), (2) strains that grew best at elevated temperature (33.5°C), and (3) strains that grew at relatively similar rates at all three temperatures. The morphology of the hyphae (density, branching pattern) was also found to differ between the growth mutants. Selected mutants will be analyzed by both RNA seq and genomic sequencing approaches in an effort to identify any common genes that may have been altered as a result of the mutagenesis regime.
34. Functional Analysis of a Novel Diaminopimelate Decarboxylase from the Oomycete Saprolegnia parasitica. Lingxiao ge, Josie Hug, Stan Ome, Paul Morris. Biological Science, Bowling Green State Univ, Bowling Green, OH; 2 Plant-Microbe Interactions, Utrecht University, The Netherlands.

In bacteria and plants, the lysine precursor L-DAP is produced by de novo synthesis. In contrast, DAP is converted to lysine in a DAP decarboxylase. Comparative analysis of seven sequenced oomycete genomes, revealed that only Saprolegnia parasitica contains a predicted epimerase. Sequence homology in all of the predicted DAP decarboxylases in oomycetes is strongly conserved, suggesting that these proteins have similar biochemical activity. The oomycete DAP gene appears to have been acquired by horizontal transfer from Archaea sp. Notably, these particular Archaea sp. have all the genes needed to synthesize lysine, except for epimerase. Thus we postulated that the oomycete DAP might be a novel enzyme capable of converting L-DAP directly to lysine. To test this hypothesis, we codon-optimized the DAP gene from S. parasitica and expressed it in an E. coli DAP mutant. Complementation assays of the mutant expressing the S. parasitica gene in lysine-minus media indicate that the gene functions as a DAP decarboxylase. To determine the substrate specificities of the S. parasitica DAP gene, we have developed an HPLC method to separate the D, L, and meso isomers of chemically synthesized DAP. Authentic L-DAP has also been purified from the culture filtrate of an E. coli epimerase mutant. Functional assays of the affinity-purified protein will enable us to characterize the substrate specificities of the oomycete enzyme. If the S. parasitica DAP enzyme can utilize L-DAP as a substrate, then the retention of epimerase in this genome may indicate that meso-DAP is incorporated into the cell wall of this group of organisms.

35. Living on Air?: Ustilago maydis cells grow without being provided nitrogen in their growth media. Michael H Perlin, Michael Cooper. Dept Biol and Program on Disease Evolution, Univ Louisville, Louisville, KY, USA.

Nitrogen is an essential nutrient for all living creatures. Ammonium is one of the most efficiently used nitrogen sources. As with other dimorphic fungi, yeast-like cells of Ustilago maydis, the fungal pathogen of maize, switch to filamentous growth when starved for nitrogen/ammonium. U. maydis carries two genes, ump1 and ump2, encoding ammonium transporters that facilitate both uptake of ammonium and the filamentous response to its absence. While no obvious phenotype is observed when ump1 is deleted, cells without ump2 are unable to filament in response to low ammonium, although they can still grow. Surprisingly, ump1ump2 double mutants can also grow on low ammonium. More amazing still, both wild type and mutant cells continue to grow, even after strenuous efforts were made to remove all nitrogen sources from their growth media. To investigate these unusual observations further, we grew wild type and mutant cells in the absence or presence of added nitrogen, as ammonium or supplied as NH. Septum bottles with rich, low ammonium and no ammonium media were inoculated with rinsed overnight wild type and mutant cells, infected with 0.1% N gas. Septum bottles with rich, low ammonium and no ammonium media were inoculated with rinsed wild type and mutant cells, and were then incubated for seven days. The resulting biomass was sampled for microscopic examination, collected by filtration, dried and loaded into tin sample capsules for d analysis by the Stable Isotope Research Unit at Oregon State University. The wild type cells under rich, minimal and no ammonium conditions had mean d15N ratios of 0.7, 10.8 and 45.2, respectively, while the mutant cells had mean d15N ratios of 3.29, 49.5 and 134.8, respectively, for these growth conditions. This indicated significant incorporation of the 15N tracer from the injected gas into the cellular biomass. We are currently investigating additional candidate genes that may play a role in this novel capability by a fungus.

36. Saprotrophic metabolism of the White-Nose Syndrome fungus Geomyces destructans in bat hibernacula. Hannah Reynolds, Tom Ingersoll, Hazel Barton. 1) Department of Biology University of Akron Akron, OH 44325; 2) National Institute for Mathematical and Biological Synthesis (NIMBioS) University of Tennessee Knoxville, TN 37996.

Geomyces destructans (Mycotrichaceae, Leotiomycetes), an emerging epizootic disease of hibernating bats in North America has arisen from a predominately saprotrophic genus. We have isolated multiple, non-infectious Geomyces species from cave surfaces and healthy bats for physiological and genetic comparison with G. destructans to better understand its disease ecology. In particular, we are interested in 1) whether G. destructans retains saprotrophic ability, acting as a facultative rather than an obligate pathogen and 2) identifying the microhabitats that support Geomyces and presumably G. destructans growth. Identifying an environmental niche for G. destructans would aid in understanding future disease ecology. Comparative genomics indicates the presence of multiple enzymes involved in saprotrophic metabolism, including endogucanases, b-glucosidas and chitinases, while in vitro saprotrophic assays demonstrate similar cellulase and lipase functions in both pathogenic and non-pathogenic Geomyces. To understand the native microbial habitats that might inhibit or promote G. destructans growth we used molecular phylogenetic analyses of environmental fungal ITS sequences to examine both the overall fungal diversity and the diversity of Geomyces in multiple cave microhabitats. Knowledge of the specific habitat of G. destructans will allow us to determine the likelihood for saprotrophic growth within caves and estimate the role that subsidies can play in disease ecology. Indeed, disease modeling indicates that an environmental subsidy for the growth of G. destructans increases the likelihood of bat host extinction events.


Plant biomass is a diverse raw material that has great potential to be exploited e.g. in second generation biorefinery applications. In order to overcome the economic and technological thresholds in biomass utilization, novel cellulose attacking enzymes and optimal enzyme mixtures are needed. The synergistic effect of cellulose hydrolyzing enzymes, namely endoglucanases (E.C. 3.2.1.4), cellobiohydrolases (CBH, E.C. 3.2.1.91) and b-glucosidases (E.C. 3.2.1.21), during cellulose degradation is a well-defined phenomenon, which has also been reported for cellulose oxidizing enzymes. The fungal produced oxidative enzymes related to cellulose degradation include cellulose dehydrogenases (CDH, E.C. 1.1.99.18) and the proteins of glycolate hydrolyase (GH) family 61 (www.cazy.org).

Basidiomycete white-rot fungi are able to efficiently degrade all the wood polymers, i.e. cellulose, hemicelluloses and lignin. Their lignin-modifying oxidoreductases (peroxidases and laccases) are rather well characterized, whereas their cellulose acting enzymes (CAZymes) have so far gained less attention. In the large screening of hydrolytic enzymes of basidiomycetous fungi from the Fungal Biotechnology Culture Collection (FBBCC, University of Helsinki), the white-rot fungus Dichomitus squalens was found to produce high cellulosic activity and appeared as a promising source of novel CAZymes.

In this work, the expression of selected hydrolytic and oxidative CAZyme encoding genes (cdh, four cbhs, five putative gh61s) was followed with quantitative real-time RT-PCR during the growth of D. squalens on solid Norway spruce (Picea abies) wood and in semi-solid microcrystalline cellulose (Avicel) -peptone liquid medium. The enzymatic activities of cellulases and xylanase as well as lignin-modifying oxidoreductases were measured from the semi-solid cultures. In addition, CBH and CDH enzymes of D. squalens were purified and characterized.
38. Metabolomics of growth and type B trichothecenes production in *Fusarium graminearum*. L. Legoaeć1, V. Atanasova-Penichon1, N. Ponts1, C. Deborde1,2, M. Maucourt1,2, S. Bernillon3, A. Moing1,2, F. Richard-Forget1. 1) 1NIRA, UR1264 MycSA, 71 avenue Edouard Bourlais, BP81, F-33140 Villenave d’Ornon, France; 2) INRA, UMR1332 Fruit Biology and Pathology, 71 avenue Edouard Bourlais, BP81, F-33140 Villenave d’Ornon, France; 3) Metabolome Facility of Bordeaux Functional Genomics Center, IBVM Centre INRA de Bordeaux, F-33140 Villenave d’Ornon, France; 4) Univ. Bordeaux, UMR1332 Fruit Biology and Pathology, Centre INRA de Bordeaux, F-33140 Villenave d’Ornon, France.

The plant fungal pathogen *Fusarium graminearum* can produce type B trichothecenes, a family of sesquiterpene molecules with toxic properties upon human or animal ingestion. Deoxynivalenol, or DON, and its acetylated forms belong to this family of secondary metabolites and are frequent contaminants of cereals worldwide. The biogenesis of trichotheces initiates with the condensation of two molecules of farnesyl pyrophosphate, at the end of the mevalonate pathway in *Fusarium*, and is under the control of various factors such as the redox parameters of the environment or the carbon source. For example, supplementing liquid submerged cultures of *F. graminearum* with caffeic acid, a phenolic acid with known antioxidant properties, reduces the accumulation of DON and its acetylated forms in the DON. Such a result, however, gives a partial glimpse of the effect of phenolic acids, from the trichothecene production point of view only. The present study analyzes *F. graminearum* metabolome in conditions when DON and its acetylated forms are produced. Liquid chromatography coupled with mass spectrometry and proton nuclear magnetic resonance were used to characterize the metabolites produced by the fungus, secreted in the culture medium or not, over the course of 14 days. Fifty-two polar and semi-polar metabolites were identified in the culture medium, i.e., the exo-metabolites, and/or in the mycelium, i.e., the endo-metabolites, comprising amino acids and derivatives, sugars, polyketides, and terpenes including trichothecenes and DON precursors. Sample composition varied over time in terms of primary metabolites as well as secondary metabolites. Data analysis further revealed correlations, positive or negative, between metabolic pathways. In the presence of caffeic acid, metabolomic profiles were modified, counting those resulting from primary metabolism even though fungal biomass production was not affected by the treatment. Several metabolites affected by the treatment were identified for both the exo- and endo-metabolome, in particular DON and its precursors. For the first time, these results expose a unique outlook of a hidden aspect of *Fusarium*’s response to antioxidant treatment.


Telomeres are specialized DNA-protein complexes that cap chromosome ends. Telomeric DNA is composed of repetitive short sequences synthesized by telomerase, an RNA-containing DNA polymerase. The integral telomerase RNA (TER) contains telomerase provides a short template for telomeric DNA synthesis and two highly conserved structural elements essential for enzymatic function. Fungal telomerase from budding and fission yeasts has been studied extensively. We have recently developed *Neurospora crassa* as a new fungal model organism for telomere and telomerase studies, and have identified TER structural domains highly conserved in vertebrate and Pezizomycotina, but not in budding yeasts (Qi et al. 2012). N. crassa telomerase processively synthesizes the (TTAGGG)ₙ telomere repeats, an attribute conserved in vertebrate but not yeast telomeres. In contrast, both budding and fission yeast telomerase synthesize irregular telomere repeats non-processively. Two structural elements of TER, the template-pseudoknot and the three-way junction (TWJ) domain, are conserved in vertebrates, Pezizomycotina as well as Taphrinomycotina. Both of these elements are necessary for telomerase activity in vitro for Pezizomycotina and Taphrinomycotina telomerases, while the TWJ is dispensable for budding yeast telomerase. Furthermore, spliceosome-mediated TER 3’-end processing is conserved in Pezizomycotina and Taphrinomycotina, but not in budding yeasts. In comparison, the budding yeast (e.g. S. cerevisiae) TER employs a nucleoside-mediated mechanism for the 3’-end processing. Our results indicate that Pezizomycotina telomerase preserved ancestral features that budding and fission yeast species lost during evolution and supports N. crassa as an excellent model for the study of telomere and telomerase. (Reference: Qi, X., Y. Li, S. Honda, S. Hoffmann, M. Marz, A. Mosig, J.D. Podlevsky, P.F. Stadler, E. Selker and J.J.-L. Chen (2012) The common ancestral core of vertebrate and fungal telomerase RNAs. Nucleic Acids Research 40: doi:10.1093/nar/gks980.).

40. Characterizing a putative three-step formaldehyde oxidation pathway in *Neurospora crassa*. Ethan Addicott1, Kolea Zimmerman2, Anne Pringle1. 1) Faculty of Arts and Sciences, Harvard College, Harvard University, Cambridge, MA; 2) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

Using bioinformatic analyses, we identified 13 Neurospora genes that code for putative secreted-proteins. One of these proteins, NCU01056 - a proposed S-(hydroxymethyl)glutathione synthase, is implicated in a highly conserved formaldehyde oxidation pathway involving two other genes, NCU06652 - an NAD and GSH dependent formaldehyde dehydrogenase and NCU0173 - an S-formylglutathione hydratase. Knockout strains for the three genes in this pathway were obtained from the FGSC and confirmed by PCR. We conducted standard phenotypic assays on the three knock-outs and WT controls, including growth morphology, growth rate, and mating ability. Additionally, growth in the presence of methanol, the compound just upstream of formaldehyde in the pathway, was tested by biomass and flow cytometry. Two key observations were made: (1) NCU06652 knockout strains showed significant growth defects compared to the WT (2) Knockouts for NCU01056 (hypothesized to be upstream of the critical enzyme) showed increased pigmentation on SC media (3) NCU06652 knockouts germinated significantly slower than other strains in the presence of methanol compared to a control treatment. The data suggest NCU06652 is involved in the critical oxidation step of the pathway and that the absence of NCU01056 may induce stress, which points to its role in the formation of a formaldehyde-glutathione complex, immediately upstream of NCU06652. The fact that NCU01056 codes a secreted protein may suggest that N. crassa may detoxify formaldehyde extracellularly or in membrane-bound vesicles. Further exploration will involve determining a dose-response curve for formaldehyde, confirming the localization of the proteins, and investigating the GSH balance in each of the strains.


Nitrogen is one of the essential components for a variety of cellular elements. Regulation of nitrogen assimilation can be critical for the evolutionary advantage of an organism and it has been extensively studied in filamentous fungi *Neurospora crassa*. Nitrate is an important source of inorganic nitrogen for *N. crassa*, but it is not utilized unless favored nitrogen sources such as ammonium, glutamine or glutamate are absent in the environment. It was shown that nitrate is transported into the cell by high affinity transporter, NIT10, where it is stepwise reduced, first, by nitrate reductase, NIT3 to nitrite, and then by nitrite reductase, NIT6 to ammonia, which is then converted to organic nitrogen in a form of glutamate, making it available for further utilization by the cell.

Although biochemical pathways of nitrate assimilation have been extensively studied, there is a certain disagreement in literature about the requirement of functional nitrate reductase activity for nitrate uptake. In the paper by Scholemer and Garrett, 1974, it was shown that nitrate transport is not dependent upon nitrate reduction. However, later Unkles et. al., 2004, concluded that functional nitrate reductase is required for the nitrate accumulation in *Neurospora crassa*.

The goal of this work was to investigate nitrate assimilation and involvement of nitrate reductase in this process in *N. crassa*. Nitrate disappearance from
the media and weight of the mycelium were measured in nit-10 (KO) and nit-3 (KO) strains in comparison to a wild type strain. The nit-10 (KO) mutant was not able to grow on nitrate as a sole nitrogen source, and no nitrate depletion from the media was observed. Therefore, it was concluded that NIT10 is the only active transporter. The accumulation of nitrate in the mycelium was measured and it was found that in the nit-3 (KO) mutant it was 9 times higher and in the nit-10 (KO) mutant 3 times higher than in the wild type strain. Therefore, we concluded that nitrate reductase is not required for the nitrate transport.

42. Protein kinases affecting glycogen accumulation and likely regulating the glycogen synthase phosphorylation status in Neurospora crassa. T. Candido, A. P. Felício, R. Gonçalves, F. Cupertino, F. Freitas, M. C. Bertolini. 1) UNESP - IQ - Araquara, Araquara, SP, Brazil; 2) Departamento de Genética e Evolução, USFSCar, São Carlos, SP, Brazil.

The ability to sense and respond appropriately to environmental changes is required for all living organisms and reversible phosphorylation of proteins mediated by protein kinases plays a key role in this process. In this work we describe the results of a screen aimed to identify protein kinases regulating glycogen metabolism in Neurospora crassa. The glycogen synthase (GS) and glycogen phosphorylase (GP), the regulatory enzymes in glycogen synthesis and degradation processes, respectively, are highly regulated by phosphorylation, however the protein quinases that phosphorylate them in N. crassa have not been identified. In this work, a set of mutant strains individually knocked-out in genes encoding proteins kinases was used. The glycogen levels were quantified under normal growth temperature (30°C) and under heat stress (45°C). From 84 mutant strains, 37 strains presented glycogen accumulation profile different from the wild-type strain suggesting that the missing protein kinase is implicated in glycogen metabolism control. Among the protein kinases selected most are Ser/Thr protein kinases, and it is important to mention proteins already characterized as regulators of glycogen metabolism, such as the Saccharomyces cerevisiae Pho85 and Snf1 proteins. The GS oscillation was quantified in the selected strains grown under normal temperature (30°C) and under heat stress (45°C) in the presence and absence of the allosteric activator glucose-6-phosphate (G6P). The ratio -/+/G6P is considered as an index of phosphorylation, lower levels correlating with higher phosphorylation. Some protein kinases were implicated in glycogen metabolism control by likely influencing the GS phosphorylation status. Some protein kinases showed phosphorylation profile different from the wild-type strain and the results revealed putative proteins kinases not yet described as able to phosphorylate GSN. The expression of glycogen synthase (gpn) and glycogen phosphorylase (gpp) genes was analyzed by qRT-PCR in the mutant strains and the results showed that some protein kinases regulate the expression of both genes. Supported by FAPESP and CNPq.

43. Endogenous ergothioneine is required for wild type levels of Neurospora crassa conidiogenesis and conidial survival, but does not protect against uv-induced kill or mutagenesis. Lynn Epstein, Marco Bello, John Mogannam. Plant Pathology, University of California, Davis, CA. 95616-8860.

Ergothioneine (EGT) is a histidine derivative that apparently is only synthesized by fungi (except in the Saccharomycotina), and by some bacteria in the Cyanophyta and Actinomycetales. Although plants and animals do not synthesize EGT, they acquire it from the environment; EGT is concentrated in animal cells with an EGT-specific transporter. Bello et al. (2012, Fungal Genet Biol 49:160) showed that the concentration of EGT is 5x greater in Neurospora crassa conidia than in mycelia, and that growth of strain NcEgt-1 with a knockout in gene NCU04343 is indistinguishable from the wild type. To investigate the function of EGT, wild type (Egt+) and NcEgt-1 were crossed and six Egt+ and six Egt- sibs were analyzed. Compared to the Egt+ sibs, Egt- sibs had a highly significant reduction (59 ± 0.2 days whereas Egt- sibs had a highly significantly lower LE = 58 ± 0.2 days, an 18 ± 3% reduction. We tested the hypothesis that EGT protects against uv-induced kill or mutagenesis. There were no significant differences between the germinability of Egt+ and Egt- sibs after exposure to 0 to 400 Joules/m² of 254 nm light. However, we concluded that nitrate reductase is not required for the nit rate accumulation profile during irradiation. Seven-day-old conidia were stored at 30 °C at 97% and 51% relative humidity (RH) for a time course to either 17 or 88 days, respectively. Life expectancies (LE) were calculated from logistic curves fitted to percentage germination as a function of days in storage in two trials. At 97% RH, Egt- sibs had a LE = 11.0 ± 0.2 days whereas Egt- sibs had a highly significantly lower LE = 8.4 ± 0.2 days, a 23 ± 8% reduction. At 51% RH, Egt- sibs had a LE = 71 ± 1 days whereas Egt- sibs had a highly significantly lower LE = 58 ± 1 days, an 18 ± 3% reduction. We tested the hypothesis that EGT protects against uv-induced kill or mutagenesis. There were no significant differences between the germinability of Egt+ and Egt- sibs after exposure to 0 to 400 Joules/m² of 254 nm light. Consequently, our in vivo analysis indicates that EGT does not protect against uv-induced kill or mutagenesis.

44. Thiolutin inhibits protein turnover in Neurospora and yeast. Linda Lauinger, Michael Brunner, Axel Dierrfellner. BZI, Heidelberg, Germany.

Proteasome inhibitors are a powerful tool for the characterization of proteins in vivo. In yeast as well as in filamentous fungi, however, the available proteasome inhibitors, like e.g. MG132 do not function due to the barrier posed by the cell wall of the organisms and an efficient evacuation of the molecules out of the cells. The dithiole thiolutin has been shown to be a potent inhibitor of RNA polymerases in prokaryotes and fungi. In the filamentous fungus Neurospora crassa, thiolutine efficiently suppresses transcription, indicating that the drug is cell permeable and not subject to a significant efflux by the multidrug resistance system. Our data indicate that thiolutine also significantly inhibits protein turnover. Comconitant with the increase in protein stability after treatment with thiolutin, we observe an accumulation of ubiquitinated protein species. Thus, our findings suggest that thiolutine may be a pleiotropic inhibitor suppressing both, RNA polymerase as well as the proteasomal activity.

45. Characterization of a Phanerochaete chrysosporium glutathione transferase reveals a novel structural and functional class with ligandin properties for wood extractive molecules. Yann Mathieu, Pascalita Prosper, Marc Buée, Stéphane Dumarçay, Frédérique Favier, Eric Gelhaye, Philippe Gérardin, Luc Harvengt, Jean-Pierre Jacquot, Tiphaine Lamant, Edgar Meuch, Sandrine Mathiot, Claude Didierjean, Melanie Morel, 1) Université de Lorraine, IAM, UMR 1136, IFR 110 EFABA, Vandoeuvre-lès-Nancy, F-54506, France; 2) INRA, IAM, UMR 1136, Vandoeuvre-lès-Nancy, F-54506, France; 3) Université de Lorraine, CRML, UMR 7036, Vandoeuvre-lès-Nancy, F-54506, France; 4) CNRS, CRML, UMR 7036, Vandoeuvre-lès-Nancy, F-54506, France; 5) Université de Lorraine, LERMAE, EA 1093, Vandoeuvre-lès-Nancy, F-54506, France; 6) Laboratoire de biotechnologie, Pole Biotechnologie et Sylviculture Avancée, FCBA, Campus Foret-Bois de Pierroton, 33610 Cestas, France.

Glutathione transferases (GSTs) form a superfamily of multifunctional proteins with essential roles in cellular detoxification processes. A new fungal specific class of GST has been identified by genomic approaches. The biochemical and structural characterization of one isoform of this class in Phanerochaete chrysosporium revealed original properties. The three-dimensional structure showed a new dimerization mode and specific features by comparison with the canonical GST structure. An additional b-hairpin motif in the N-terminal domain prevents the formation of the regular GST dimer and acts as a lid, which closes upon glutathione binding. Moreover, this isoform is the first described GST that contains all secondary structural elements, including helix 4’ in the C-terminal domain, of the presumed common ancestor of cytosolic GSTs, i.e. glutaredoxin 2. A sulfate binding site has been
identified close to the glutathione binding site and allows the binding of 8-anilino-1-naphtalene sulfonic acid (ANS). Competition experiments between ANS, which has fluorescent properties, and various molecules, showed that this GST binds glutathionylated and sulfated compounds, but also wood extractive molecules such as vanillin, chloro nitrobenzoic acid, hydroxycetophenone, catechins and aldehydes in the glutathione pocket. This enzyme could thus function as a classical GST through the addition of glutathione mainly to phenethyl isothiocyanate but alternatively and in a competitive way, it could also act as a ligand of wood extractive compounds. These new structural and functional properties, lead us to propose that this GST belongs to a new class that we name GSTFaA for Fungal specific GST class A.

46. Secretome analysis of Trichoderma harzianum cultivated in the presence of Fusarium solani cell wall or glucose. Marcelo HS Ramada$^{1,2}$, Andrei S Steindorf$^1$, Carlos Bloch Jr.$^1$, Cirano J Uhloa$^1$. 1) Brasilia University, Cell Biology Department, Brasilia, DF, Brazil; 2) Federal University of Goias, Biochemistry Department, Goiania, GO, Brazil. 3) EMBRAPA CENARGEN, Mass Spectrometry Laboratory, Brasilia, DF, Brazil.

Trichoderma harzianum is a fungus well known for its potential as a biocontrol agent of many fungal phytopathogens. The aim of this study was to evaluate the potential of T. harzianum ALL42 to control Fusarium solani, a phytopathogen fungus that causes several losses in common bean and soy crops in Brazil and to evaluate the secreted proteins of T. harzianum ALL42 when its spores were inoculated and incubated in culture media supplemented (TLE) or not (MM) with nitrogen sources and in the presence or not of F. solani cell walls (FsCW). In the absence of FsCW, the media were supplemented with glucose (GLU). T. harzianum was able to control the phytopathogen growth and started to sporulate in its area after 7 days in a dual culture assay, indicating that it had successfully parasitized the host. T. harzianum was able to grow in TLE+FsCW, MM+FsCW, TLE+GLU, but unable to grow in MM+GLU. Protein quantification showed that TLE+FsCW and MM+FsCW had 45 and 30 fold, respectively, more proteins than TLE+GLU, and this difference was observed in the bidimensional gels, as the two supernatants from media supplemented with FsCW had around 200 spots and the one supplemented with glucose only had 18. TLE+FsCW and MM+FsCW had above 80% of spot similarity. A total of 100 proteins were excised from all three conditions and submitted to mass spectrometry analysis. 85 out of 100 proteins were identified. The only protein observed in all three conditions is a small protein, called ep1, involved in eliciting plant-response against phytopathogens. An aspartic protease, previously described as related to mycoparasitism, was only found when T. harzianum was grown with glucose. Gene expression was evaluated and confirmed the gel results. In the media supplemented with FsCW, different hydrolases like chitinases, beta-1,3-glucanases, glucosamylases, alpha-1,3-glucanases, and proteases were identified. Some proteins like a small cystein-rich, alpha-L-arabinofuranosidase and NPP1, with no known function in mycoparasitism were also identified. T. harzianum ALL42 is able to inhibit the growth and parasitize F. solani and showed a complex and diverse arsenal of proteins that are secreted in response to the cell walls, with novel proteins not previously described in mycoparasitism studies.

47. Analysis of carbon catabolite repression (CCR) during cellulase formation by Trichoderma reesei (Hypocrea jecorina) using two-dimensional differential gel electrophoresis (2D-DIGE). Wellington Pedersoli, Lilian Castro, Amanda Antonieto, Vitor M. Faça, Roberto N. Silva. Biochemistry and Immunology, University of São Paulo, Ribeirão Preto, Sao Paulo, Brazil.

The production of cellulases by Trichoderma reesei (Hypocrea jecorina) is fundamental for the production of second generation biofuels from cellulosic wastes. The complex of cellulases of fungi T. reesei is strongly induced by sophorose and cellulose and also antagonized by glucose. Thus, the objective of this study was to compare the differential secretome using 2D-DIGE of T. reesei at induction and repression conditions. The fungus T. reesei (QM9414) were grown in media containing 1% cellulose for 24, 48 and 72 hours and 1% glucose for 24 and 48 hours in a orbital shaker at 180 rpm at 28°C. After filtration of each medium the supernatant was used for protein determination. Samples from each time in the different conditions were mixed in proportional amounts and submitted to isoelectric focusing in 18 cm strip of pH 4.0 - 7.0 and subsequently subjected to electrophoresis on two-dimensional gel - differential (2D-DIGE) for the separation of proteins. The comparative analysis PDQuest software (BioRad) 130 selected spots differentially expressed, wherein the inhibitor glucose showed 41 distinct protein spots and inducer cellulose 89 spots. However, only the 34 spots and 63 spots from glucose and cellulose respectively could be spotted. Identification of these spots was carried out using the mass spectrometer XEVO TQ-S (Waters). A total of 57 proteins were identified, 30 and 27 proteins from cellulose and glucose conditions respectively. The proteins identified from glucose condition were amidases, proteases, isoamyl alcohol oxidases and protein belonging to Glycosyl Hydrolases 16, 17, 43 and 54. On the other hand, the proteins identified from cellulose condition were Glycosyl Hydrolases 3, 7, 54, 55, 72, two Cip 1 (involved in the degradation of cellulose, but with no function described), two proteases and a protein responsible for the regulation of stress. Two other proteins, one of which was used for the identifications of stress as unknown. Thus, the identification of these proteins will allow a better understanding of the mechanism formation of cellulases by T. reesei and thus contribute to improve the production of second generation ethanol. Supported by: FAESP, CAPES and FAPEA-HC/USP-RP.

48. Transposon-associated evolution of a fungal NRPS. Daniel Berry1, Carolyn Young2, Paul Dijkstra1, Barry Scott1. 1) Massey University, Palmerston North, New Zealand; 2) The Samuel Roberts Noble Foundation, Ardmore, Oklahoma.

Epichloë endophytes systemically colonise the aerial tissues of cool season grasses to form mutually beneficial symbiotic associations, one key bioprotective metabolite is the insect feeding deterrent, peramine, which is synthesized by the two-module non-ribosomal peptide synthetase (NRPS) PerA. This NRPS has an A-T-C-A-M-T-R domain structure where the Adenylation-domains provide specificity for and activation of the two amino acid substrates, the Thiolation-domains bind the reaction intermediates, the Condensation-domain catalyzes formation of the peptide bond, the Methylation-domain methylates the substrate amino acid, and the Reductase-domain cyclizes and releases the finished product from the NRPS. The perA gene is found exclusively within the Epichloë, where it is widespread among the different species, but peramine production is somewhat more discontinuous. We show that transposon-mediated deletion of the R-domain present in some E. typhina and E. festucae peramine deficient isolates is associated with a change in the predicted substrate specificity code of the first A-domain. Phylogenetic analysis of this domain groups the peramine negative isolates together whereas the phylogeny based on the whole gene matches the species phylogeny. The recombination mechanism responsible for the evolution of this novel NRPS is still to be resolved.

49. Velvet family control of penicillin production in Penicillium chrysogenum: PcVeI binding to isopenicillin N synthase suggests a novel regulatory mechanism. Sandra Bloemen2, Katarina Kopce, Birgit Hoff, Sarah Milbredt, Alexandra Katurowski, Ulrich Küch. Christian Doppler Laboratory for Fungal Biotechnology, Ruhr-University Bochum, Universitätsstr. 150, 44780 Bochum, Germany.

The filamentous fungus Penicillium chrysogenum is the main industrial producer of the pharmacologically relevant beta-lactam antibiotic penicillin. All three biosynthesis genes are found in a single cluster and the expression of these genes is known to be controlled by a complex network of global regulators. It is supposed that subunits of the velvet complex, which were recently detected for P. chrysogenum, function as such global regulators, although the exact regulatory mechanism still have to be elucidated. Core components of this complex are PcVeIa and PcVeA, which regulate secondary
metabolite production, hyphal morphology, conidiation, and pellet formation [1]. Here we describe the characterization of PcVeLB, PcVeLC, and PcVoA as novel subunits of this velvet complex. Using yeast two-hybrid analysis and bimolecular fluorescence complementation (BiFC), we demonstrate that all velvet proteins are part of an interaction network. Functional analyses using single and double knockout strains generated by the FLP/FRT recombination system [2] clearly indicate that velvet subunits have opposing roles in the regulation of penicillin biosynthesis and light-dependent conidiation. Most strikingly, a direct interaction of PcVeLB with an enzyme of the penicillin biosynthesis pathway, the isopenicillin N synthase, was identified during yeast two-hybrid analysis with PcVeLB as bait. This surprising interaction was confirmed with BiFC in vivo, thereby localizing the interaction in dot-like structures in the cytoplasm. Our discovery of a direct interaction of the isopenicillin N synthase with a subunit of the velvet complex implies a novel regulatory mechanism how enzymes of penicillin biosynthesis are regulated at the molecular level. The results provided here contribute to our fundamental understanding of the function of velvet subunits as part of a regulatory network mediating signals responsible for morphology and secondary metabolism, and will be instrumental in generating mutants with newly derived properties that are relevant to strain improvement programs.


50. Genome mining reveals the evolutionary origin and biosynthetic potential of basidiomycete polyketide synthases. Gerald Lackner, Mathias Misiek, Jana Braesel, Dirk Hoffmeister. Pharmaceutical Biology, Friedrich-Schiller-University Jena, Germany.

Polyketide biosynthesis is a rich source of pharmaceutically active secondary metabolites present in fungi. Besides lipid-lowering drug lovastatin, many infamous toxins are produced via this pathway. While abundant in Aspergillus, only few polyketides have been isolated from basidiomycetes. High throughput genome sequencing projects, however, now help estimate the genetic capacity of basidiomycetes to biosynthesize polyketide derivatives. By inspection of 35 sequenced basidiomycete genomes we identified and annotated 111 iterative type I and three type III polyketide synthase (PKS) genes. Phylogenetic analyses of KS genes imply that all main families of fungal PKS had already evolved before the Ascomycota and Basidiomycota diverged. A comparison of genomic data and metabolomic records shows that the number of polyketide genes surpasses the number of known polyketides considerably. This work might serve as a guide for upcoming genomic mining projects to discover novel polyketide derivatives from mushrooms.


Ninety percent of fatal mushroom poisonings are caused by alpha-amanitin and related bicyclic peptides found in some species of Amanita, Galerina, Lepiota, and Conocybe. We showed that the amatoxins (mainly amanitin) and related phallotoxins are synthesized on ribosomes in A. bisporigera and the unrelated mushroom G. marginata. The primary gene products are short (34-35 amino acid) proproteins that are initially processed by a dedicated prolyl oligopeptidase. A genome survey sequence of A. bisporigera suggested that it has a repertoire of over 40 cyclic peptides, all produced on a single biosynthetic scaffold. Members of this extended gene family are characterized by conserved upstream and downstream amino acid sequences, including two invariant proline residues, flanking a six to ten-amino acid “hypervariable” region that encodes the amino acids found in the mature toxins (or predicted toxins). The evidence indicates that A. bisporigera has evolved a combinatorial strategy that could in principle biosynthesize billions of small cyclic peptides. In order to study the other steps in amanitin biosynthesis, and to engineer novel cyclic peptides, we have developed a transformation strategy for the amanitin-producing mushroom G. marginata. This first transformation method uses Agrobacterium-mediated transformation followed by hygromycin selection. Taking advantage of this platform, we are introducing artificial toxin genes that are deliberately designed to provide insights into the pathway. The synthetic genes include those that encode the cyclic octapeptide beta-amanitin, the heptapeptides phallolidin and phallacin, examples of the toxin gene family known from A. bisporigera but not G. marginata, and randomly generated artificial sequences. Currently, thousands of transformants have been generated through an efficient pipeline and the transformants are being analyzed for production of the expected products. If successful, the novel peptides will be screened in a number of assays including RNA polymerase (the site of action of alpha-amanitin), membrane ion channels, pathogenic bacteria, and cancer cell lines.

52. Spatial assessment of oxidative and enzymatic reactions in brown rotted wood. Jon R. Menke 1, Jae San Ryu 2,6, Gerald N. Presley 3, Shona M. Duncan 1, Joel A. Jurgens 2, Robert A. Blanchette 4, Timothy R. Filley 1, Kenneth E. Hammel 1, Jonathan S. Schilling 1. 1) Department of Bioproducts and Biosystems Engineering, University of Minnesota, St. Paul, MN; 2) Department of Plant Pathology, University of Minnesota, St. Paul, MN; 3) Department of Earth and Atmospheric Sciences and the Purdue Climate Change Research Center, Purdue University, West Lafayette, IN; 4) Institute for Microbial and Biotechnological Technology, U.S. Forest Products Laboratory, Madison, WI; 5) Institute for Microbial and Biotechnological Technology, U.S. Forest Products Laboratory, Madison, WI; 6) Eco-Friendliness Research Department, Gyeongsangnam-do Agricultural Research and Extension Services, Republic of Korea.

Brown rot fungi are theorized to use coordinated free radical oxidations and enzymatic reactions to consume wood. Though likely inconvertible in vitro, it is proposed these reactions occur concurrently during brown rot of wood. We mapped and then compared fungal growth, wood modifications and cellulose during brown rot, we have initiated a study to temporally assess the depth of penetration of an endoglucanase into wood cells during this process. Previous studies have used the marker proteins insulin (5.7 kDa), myoglobin (17.6 kDa), and ovalbumin (44.4 kDa); and immunocytocchemical electron microscopy to demonstrate the ability of the two smaller proteins to infiltrate the cell walls of rotten wood. The Postia placenta endoglucanase Cel5B (PpCel5B) has a theoretical molecular weight of 34.6 kDa, which is considerably lower than the molecular weight of ovalbumin. Our approach involves using a polyclonal antibody raised against PpCel5B heterologously expressed in Pichia pastoris. This antibody will be used to assess the extent to which a native brown rot endoglucanase is able to penetrate Pinus resinosa cells. Given the common supposition that pore size prevents brown rot fungal endoglucanases from accessing wood secondary walls, even in late decay stages, this work will provide a direct assessment of enzyme ingress.

53. Molecular biological basis for statin resistance in naturally statin-producing organisms. Ana Rems, Rasmus Frandsen. DTU Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark.

Secondary metabolites can be toxic to the organism producing them; therefore gene clusters for biosynthesis of secondary metabolites often include genes responsible for the organism’s self-resistance to the toxic compounds. One such gene cluster is the compactin (ML-236B) cluster in Penicillium solitum. Compactin is an inhibitor of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, and is used as a precursor for production of the cholesterol-lowering drug pravastatin. The compactin gene cluster includes two genes encoding proteins that may confer the self-resistance to compactin and its

Full poster session abstracts

This project has taken a multifaceted approach to manipulate the Pleuromutilin. Pleuromutilin is generally synthesised at a low titre by its native producer Clitopilus passeckerianus and as such, research into its biosynthesis mechanisms. Pleuromutilin is one such compound with activity against bacteria such as Methicillin Resistant Staphylococcus aureus, a major wheat pathogen, and a phenotypic acid biosynthesis. This gene is part of a cluster and nearby genes are co-ordinated. The ketoreductase; ACP were reducing with the appropriate domains (KS, KDR, and shunt products). Using this approach we have been able to identify the products of a meroterpenoid pathway were identified as histidyltryptophanyldiketopiperazine (HTD), dehydrohistidyltryptophanyldiketopiperazine (DHTD), roquefortine C, glandicoline A, glandicoline B and meleagrin. Specific genes could be assigned to each enzymatic reaction step. The nonribosomal peptide synthetase RoqA accepts histidine and tryptophan as substrates leading to the production of the diketopiperazine HTD. DHTD, previously suggested to be a degradation product of roquefortine C, was found to be derived from HTD involving the cytochrome P450 oxidoreductase RoqR. The PKS of roquefortine and related compounds in Penicillium chrysogenum is involved in production of roquefortine and related compounds in Penicillium chrysogenum. A branched biosynthetic pathway is involved in production of roquefortine and related compounds in Penicillium chrysogenum. The secretion [1]. The mlcD gene encodes a putative ‘HMG-CoA reductase-like protein’, and mlcE encodes a putative efflux pump. However, the function of these two putative proteins has not yet been confirmed. We aim to elucidate the biological basis for compactin resistance in the compactin-producing organism. A codon-optimized version of the mlcD gene was inserted into the Saccharomyces cerevisiae genome. The constructed yeast strain was tested for sensitivity to lovastatin, a statin structurally similar to compactin, by growing the strain on media containing lovastatin. The strain showed an increased resistance to lovastatin compared to the wild-type strain. Furthermore, we investigated if MlcD confers the resistance by functional complementation of the endogenous HMG-CoA reductases in S. cerevisiae. There are two isoforms of HMG-CoA reductase in yeast, HMG1 and HMG2, both involved in the sterol biosynthetic pathway, which leads to the synthesis of ergosterol. Following deletion of HMG1 and HMG2 genes in S. cerevisiae, we inserted the mlcD gene into the knockout mutants, and tested the resulting strains for sensitivity to lovastatin. The HMG1 and HMG2 knockout mutants were unable to grow on minimal media and had an increased sensitivity to lovastatin on rich media. However, insertion of the mlcD gene restored the growth of the yeast mutants and increased their resistance toLovastatin. These results show that MlcD complements the activity of the deleted HMG-CoA reductases, enabling synthesis of ergosterol in yeast. In addition, MlcD confers statin resistance by being insensitive to the inhibiting effects of statins. Reference: [1] Abe Y., Suzuki T., Ono C., Iwamoto K., Hosobuchi M., Yoshikawa H. Mol Genet Genomics 2002, 267, 5:636-46.

54. Molecular genetic characterization of secondary metabolism pathways in Asperillus species. Clay Wang1, Yiming Chang2, Nancy Keller3, Kenneth Bruno4, Scott Baker4, Chun Jun Guo5, James Sanchez5, Benjamin Knox5, Alexandre Soukup5, Jin Woo Bok5, Mammeet Ahuja5, Ruth Entwistle5, Liz Oakley4, Shu-lin Chang6, Hsu-Hua Yeh7, Mike Praseuth8, Berl Oakley9. 1) Pharma Sci & Chemistry, Univ Southern California, Los Angeles, CA; 2) Department of Molecular Biosciences, University of Kansas; 3) Department of Medical Microbiology and Immunology and Department of Bacteriology, University of Wisconsin Madison; 4) Pacific Northwest National Laboratory.

Advances in next generation DNA sequencing have provided a large number of fungal genome sequences in public databases. Within these genomes are large numbers of cryptic secondary metabolism pathways. Data will be presented where we use a comparative genomics approaches to identify the products of these cryptic pathways. Next we use a gene knock out approach to create mutants followed by isolation and characterization of intermediates and shunt products. Using this approach we have been able to identify the products of a meroterpenoid pathway in A. terreus.

55. A branched biosynthetic pathway is involved in production of roquefortine and related compounds in Penicillium chrysogenum. Hazrat Ali1,2, Marco Ries1, Jeroen Nijland3, Peter Lankhorst1, Thomas Hankemeier1,5, Roel Bovenberg4,6,8, Rob Vreven5,7, Arnold Driessen2,9. 1) Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, Zernike Institute for Advanced Materials, University of Groningen, The Netherlands; 2) Kluiver Centre for Genomics of Industrial Fermentations, Julianaalan 67, 2628BC Delft, The Netherlands; 3) 3Dvision of Analytical Biosciences, Leiden/Amsterdam Center for Drug Research, Leiden University, The Netherlands; 4) DSM Biotechnology Center, Alexander Fleminglaan 1, 2613 AX Delft, The Netherlands; 5) Netherlands Metabolomics Centre, Leiden University, Leiden, The Netherlands; 6) Synthetic Biology and Cell Engineering, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, The Netherlands.

Profiling and structural elucidation of secondary metabolite production by the filamentous fungus Penicillium chrysogenum and derived deletion strains were used to identify the various metabolites and enzymatic steps belonging to the roquefortine/meleagrin pathway. Major abundant metabolites of this pathway were identified as histidyltryptophanyldiketopiperazine (HTD), dehydrohistidyltryptophanyldiketopiperazine (DHTD), roquefortine D, roquefortine C, glandicoline A, glandicoline B and meleagrin. Specific genes could be assigned to each enzymatic reaction step. The nonribosomal peptide synthetase RoqA accepts histidine and tryptophan as substrates leading to the production of the diketopiperazine HTD. DHTD, previously suggested to be a degradation product of roquefortine C, was found to be derived from HTD involving the cytochrome P450 oxidoreductase RoqR. The dimethylallyltryptophan synthetase RoqD prenylates both HTD and DHTD yielding the products roquefortine D and roquefortine C, respectively. This leads to a branch in the otherwise linear pathway. Roquefortine C is subsequently converted into meleagrin with glandicoline A and B as intermediates, involving two monoxygenases (RoqM and RoqQ) and a methyltransferase (RoqN). It is concluded that roquefortine C and meleagrin are derived from a branched biosynthetic pathway rather than a linear pathway as suggested in literature.

56. A biosynthetic gene cluster for the antifungal metabolite phenomonic acid in the plant pathogenic fungus, Leptosphaeria maculans. Candace Elliott1, Damien Callahan2, Daniel Schwenk3, Markus Nett4, Dirk Hoffmeister5, Barbara Howlett6. 1) School of Botany, University of Melbourne, Melbourne, Australia; 2) Metabolomics Australia, School of Botany, The University of Melbourne, Victoria 3010, Australia; 3) Friedrich-Schiller-Universität, Department Pharmaceutical Biology at the Hans-Knoll-Institute, Beutenbergstrasse 11a, 07745 Jena, Germany; 4) Leibniz Institute for Natural Product Research and Infection Biology e.V., Hans-Knoll-Institute, Beutenbergstrasse 11a, 07745 Jena, Germany.

Phenomonic acid, a long chain aliphatic carboxylic acid, is a major metabolite produced by Leptosphaeria maculans, a fungal pathogen of Brassica napus (canola). Early biosynthetic studies suggested that the methyl group derived from S-adenosylmethionine (SAM), whereas the incorporation pattern of [13C]acetate suggested a polyketidic origin of the linear portion of phenomonic acid (Devy et al., 1984). We have used domain modelling to predict a candidate polyketide synthase (PKS) for phenomonic acid biosynthesis. Of the 15 predicted polyketide synthases (PKS) in the L. maculans genome, seven were reducing with the appropriate domains (K5 - keto-synthase; AT - acyltransferase; DH - dehydrogenase; MT - methyltransferase; ER - enoylreductase; KR - keto-reductase; ACP - acyl carrier protein) for the biosynthesis of phenomonic acid. The most highly expressed of these seven genes, PKS2, was silenced to 10% of that of wild type levels and the resultant mutant produced 25 times less phenomonic acid than the wild type did, indicating that PKS2 is involved in phenomonic acid biosynthesis. This gene is part of a cluster and nearby genes are co-regulated. A two-fold reduction in the expression of the adjacent transcriptional regulator C67T, led to at least a 20-fold reduction in expression of PKS2, as well as of other genes in the cluster (P450, YoaA, RTA1 and MFS), but not of the adjacent Chok or a hypothetical gene (Hyp). This down-regulated mutant also showed a marked reduction in phenomonic acid production. Phenomonic acid is toxic towards another canola pathogen Leptosphaeria biglobosa ‘canadensis’, but L. maculans and to a lesser extent the wheat pathogen, Stagonospora nodorum are more tolerant. Phenomonic acid may play a role in allowing L. maculans to outcompete other fungi in its environmental niche.

57. Exploring and Manipulating Pleuromutilin Production. Patrick M Hayes1, Russell J Cox2, Andy M Bailey3, Gary D Foster4. 1) School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK; 2) School of Chemistry, University of Bristol, Bristol BS8 1TS, UK.

Antibiotic resistance has arisen in a significant number of human pathogens, antibiotic discovery and development is, however, currently not keeping pace. This has lead to the reinvestigation of some naturally produced antibiotic compounds which act in a manner that avoids common resistance mechanisms. Pleuromutilin is one such compound with activity against bacteria such as Methicillin Resistant Staphylococcus aureus (MRSA). Pleuromutilin is generally synthesised at a low titre by its native producer Citopilus passeckerianus and as such research into its biosynthesis may enable yield increases. This project has taken a multifaceted approach to manipulate the Pleuromutilin biosynthetic gene cluster in a variety of fungal organisms. Within C.

Filamentous fungi Monascus species have been used to ferment rice producing red mold rice (RMR). They produce several bioactive compounds during fermentation, however, they should have a potential to produce other bioactive compounds considering that most of fungi has many silent secondary metabolism (SM) gene clusters. Therefore, we thought that Monascus species could be improved for functional food production by activation of such silent SM gene clusters. We overexpressed the laeA gene that is known to encode a global positive regulator of secondary metabolism under the alcA promoter in Monascus pilosus. An OE::laeA transformant produced more secondary metabolites including ones not detected under an uninduced condition. RMR fermented with the Monascus pilosus OE::laeA contained 7 times more monacolin K, a cholesterol lowering agent, than non-transformants increasing from 2.45 to 15.59 mg/kg. In addition, the production of pigments was remarkably increased and antioxidant activity was increased as well. This study suggests that Monascus species that are important industrial fermentative fungi in Asia could be improved for the production of functional foods by overexpression of the laeA gene.


Chaetomium globosum has been reported to produce many natural products, secondary metabolites, with complex structures biosynthetic pathways of which are very interesting to be elucidated such as aureonitol, chaetoglobosins, chaetocins and chaetoaviridins. Recently, we developed molecular genetics systems for understanding the secondary metabolism in this fungus: High frequency gene targeting by Cg.ligD disruption and the pyrC marker recycling. Then, we identified biosynthetic gene clusters for various natural products that had been isolated from C. globosum, and analyzed their biosynthetic mechanisms/pathways. We also obtained new natural products by changing an epigenetic regulation. During these studies, we found that some of mutations in biosynthetic genes allowed us to obtain interesting phenotypes: drastic changes in secondary metabolism profiles and defects in production of sexual spores. Here, we present that aureonitol and chaetoglobosins would play a critical role of controlling the productivity of secondary metabolites and producing of sexual spores, respectively. A mutated gene of artH responsible for biosynthesizing aureonitol exhibits activating chaetocinaviridin biosynthesis and inactivating chaetocin A biosynthesis. A qRT-PCR analysis shows that transcriptional expressions of biosynthetic genes for chaetocinaviridin are activated, whereas those for chaetocins are inactivated by the mutated artH gene. Supplementation with aureonitol to DarTH strain is observed to inactivate chaetocinaviridin biosynthesis and transcriptional expression of their biosynthetic gene cluster. These results strongly suggest that aureonitol could be involved in transcriptional regulation of secondary metabolism in C. globosum. On the other hand, we also find chaetoglobosins is essential for production of sexual spores: Mutations in its biosynthetic genes clearly impair meiotic process. It is anticipated that chaetoglobosins are involved in meiotic process, because the mutants don’t affect formation of fruiting bodies (perithecia). Previously, chaetoglobosins were shown to inhibit actin polymerization in vitro. Therefore, chaetoglobosins would play a role of regulating actin or actin-related protein in C. globosum, which has been reported to be associated with regulation of meiotic process as well as morphogenesis in fungi.

60. Identification of T. asperellum CAZyme genes. Lasse Bech1, Morten Nedergaard Grell1, Peter Kamp Busk1, Hai Zhao2, Lene Lange1. 1) Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University Copenhagen, Denmark; 2) Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, PR China.

To understand the complexity of enzymatic hydrolysis of plant cell walls and to discover new enzymes, one approach is to analyze cell wall degrading enzymes (CWDEs) in the transcriptional profile of plant degrading fungi like species of the genus Trichoderma. The Trichoderma spp. are ubiquitous colonizers of cellulosic materials and are often be found where degrading plant material is available as well as in the rhizosphere of plants. Trichoderma spp. are successful colonizers of their habitats, which is shown both by their efficient utilization of the biomass as well as their secretion capacity for antibiotic metabolites and enzymes. This study shows the transcriptional profile of Trichoderma asperellum isolated from duckweed. Duckweed is an aquatic plant that has been shown to clean eutrophic water reservoirs for the production of biomass, which can be used as feed, fertilizer and fuel through the biorefinery platform. The fungus was optimized for the production of CWDEs, where more than 200 glucoside hydrolases from 47 different families were identified. Several group members exhibited novel traits such as larger residue differences in active site and substrate binding site, hence making them interesting subject for expression and further characterization. This data was compared with the hydrolysis of duckweed by enzyme blend from T. asperellum. The enzyme blend showed a promising degree of hydrolysis of duckweed indicating that T. asperellum is a candidate for on-site enzyme production for the enzymatic hydrolysis of certain duckweed species. The transcripome data can further be used to map the expression of CWDEs under different conditions thereby coming closer to understanding the relationship between CWDEs and the plant cell wall.

61. Identification of a lactose permease of Trichoderma reesei that is required for cellulase gene expression. Christa Ivanova1, Jenny Bååth2, Bernhard Seiboth3, Christian Kubicek1. 1) Institute of Chemical Engineering, University of Technology of Vienna, 1060 Vienna, Austria; 2) Lund University, SE-221 00 Lund, Sweden; 3) Austrian Institute of Industrial Biotechnology (ACIB) GmBH c/o Institute of Chemical Engineering, University of Technology of Vienna, 1060 Vienna, Austria.

The disaccharide lactose has been shown to be a potent inducer of cellulases in T. reesei, and it is virtually the only solubilized disaccharide that can be used for commercial enzyme production. To understand the complex regulatory mechanisms underlying cellulase induction by lactose, we performed comparative transcriptomic analysis using oligonucleotide arrays. Among the 410 genes that were upregulated over four-fold on lactose, were all cellulases, cellulase-enhancing proteins, major hemicellulases and also 63 MFS- (major facilitator superfamily) -permeases. The MFS permeases are characterized by 12-transmembrane helices and a well conserved motif between TMS (transmembrane segment) 2 and 9. In order to investigate the function of these transporters, we generated deletion strains in T. reesei. For this, the 14 most-upregulated transporter encoding genes were chosen. One of these disruptant strains showed strongly impaired growth on lactose and was therefore chosen for further analysis. The strain showed impaired growth on lactose, whereas growth on glucose, glycerol and cellulobiose remained unaltered, suggesting that the transporter is required for lactose uptake. The strain was devoid of cellulase gene expression during cultivation on lactose, whereas it formed cellulases upon incubation with sophorose suggesting that it is
involved in cellulase induction by this disaccharide. The deletion of the other 13 transporter encoding genes led to no reduction of growth on any carbohydrate (including glucose and cellobiose), probably due to redundancy of their function. Our data show that lactose leads not only to the formation of a complete cellulase and hemicellulase system in T. reesei, but also to the transcription of a plethora of transporters likely associated with the uptake of the hydrolysis products.

62. The diversity of the Mannosyrythritol lipids depends on the peroxisomal targeting of the Mannosyrythritol acyl transferases Mac1 and Mac2 in Ustilago maydis. Johannes Freitag1, Julia Ast1, Uwe Linne1, Elisa Leisge1, Michael Bölker1, Björn Sandrock1. 1) Biology - Genetics, Philips-University Marburg, Marburg, Germany; 2) Chemistry, Philips-University Marburg, Marburg, Germany.

Under nitrogen starvation the smut fungus Ustilago maydis produces a bunch of secondary metabolites. Among these are the glycolipids Ustilagic acid (UA) and Mannosyrythritol lipid (MEL), which consist of a sugar moiety esterified with fatty acid side chains of variable length (from C2 - C18). The biosynthesis of UA is encoded by the UA gene cluster (11 genes). MEL production depends on the MEL gene cluster composed of the genes mac1, mmf1, mac2, emt1 and mac2. Deletion of mac1, mac2 or emt1 in U. maydis resulted in the complete loss of MELS. Medium-length fatty acids (C4-C14) are derived from longer fatty acids (C16-C18) by partial peroxisomal b-oxidation. After bioinformatic analysis we have identified bona fide peroxisomal targeting sequences 1 (PTS1) at the C-termini of the two Mannosyrythritol lipid acyltransferases Mac1 and Mac2 but not in any other protein involved in the biosynthesis of the MELS or the UAs. Here we show that Mac1 and Mac2 localize in peroxisomes, and that this localization depends on the PTS1 motifs. The analysis of glycolipid production by thin layer chromatography and mass spectrometry from wild type strain MB215 revealed a mixture of MELS with different length of the fatty acid side chains ranging from C12, C14 and C16. Strains expressing both cytoplasmic variants Mac1DPTS and Mac2DPTS showed a reduction of diversity of MELS. In these mutants MELS with C16 and C2 side chains are significantly overrepresented. This indicates that MEL production is coupled to peroxisomal b-oxidation resulting in a more variable distribution in the length of fatty acid side chains. Currently, we investigate the MEL production in strains lacking peroxisomes and the importance of MEL diversity for the life of U. maydis.

63. Metabolic adaptation of the oomycete Phytophthora infestans during colonization of plants and tubers. Carol E. Davis, Howard S. Judelson. Plant Pathology and Microbiology, University of California, Riverside, CA 92521.

Phytophthora infestans is the causative agent of late blight and was responsible for the Irish famine in the 1840’s. Today it still continues to be a global problem and in the USA it has been reported that the economic loss on potato crops alone exceeds $6 billion per year. A successful phytopathogenic relationship depends on the ability of the organism to adapt its metabolism during infection on various nutritional substrates (e.g., plant versus tuber) and at different times throughout infection when nutrients may be limiting. Investigation of this metabolic adaptation is key to understanding how P. infestans succeeds as a pathogen. To do this, tomato plants and potato tubers were infected with zoospores using a “dipping” method. RNA was extracted at 3 dpi and 6 dpi and subsequently used in library preparation. Following this, the libraries were quality checked by analysis on a Bioanalyzer using a high sensitivity DNA chip. Using illumina technology (50 bp, paired-end reads) RNA Sequencing was performed. For each sample an average of 262 million reads was obtained. As a reference for the in planta data, RNAseq was also performed on defined and complex media. Mining of the data shows that the expression profiles of some pathways change, such as glycolysis and gluconeogenesis. Learning how metabolic adaptation occurs will prove useful in the development of novel control strategies for this plant pathogen.

64. Multi-copper oxidase genes of Heterobasidion irregulare. Ming-Chen Hsieh, Bastian Doernte, Ursula Kües. Molecular Wood Biotechnology and Technical Mycology, University of Goettingen, Goettingen, Germany.

The species complex Heterobasidion consists of well-known wood decomposers that infect mainly conifers. The fungi are white rots that decay lignin and cellulose. Laccases are enzymes that potentially attack the lignin. In the sequenced genome of the North American species H. irregulare 18 multi-copper oxidase genes (mcos) are found (1). Phylogenetic sequence analysis divides the encoded proteins into five subclusters of mcos. In total, 14 proteins clustered in two different subfamilies of classical laccases whereas two others are found amongst ferroxidases/laccases (enzymes with often dual activities), one under fungal Fet3-type ferroxidases and one with fungal ascorbate oxidases. The potential three-dimensional structures of all mcos were predicted by homology modelling for further grouping. Six of the potential laccase genes were first chosen for subcloning and expression in the heterologous basidiomycete Coprinopsis cinerea. (1) Olson et al. (2012). Insight into trade-off between wood decay and parasitism from the genome of a fungal forest pathogen. New Phytologist 194: 1001-1013.

65. The two novel class II hydrophobins of Trichoderma stimulate enzymatic hydrolysis of polyethylene terephthalate (PET). Liliana E. Tenorio-Rammer1, Doris Ribitsch1, Annemarie Marold1, Katrin Greimel1, Enrique Herrera Acero2, Georg M. Guebitz1,2, Christian Kubicek1,2,3, Irina S. Druzhinina1,2,3,1) ACIB - Austrian Centre of Industrial Biotechnology, Petersgasse 14, 8010 Graz, Austria; 2) c/o Institute for Environmental Biotechnology, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz Strasse 20, 3430 Tulln, Austria; 3) Microbiology Group, Institute of Chemical Engineering, Vienna University of technology, Vienna, Austria.

Polyethylene terephthalate (PET), a thermoplastic polyester with excellent industrial properties, can be functionalised and/or recycled via hydrolysis by microbial cutinases. Here we tested whether hydrophobins (HFBs), small secreted fungal proteins containing eight positionally conserved cysteine residues, would be able to enhance the rate of enzymatic hydrolysis of PET. To this end, we selected the genus of the mycoparasitic filamentous fungus Trichoderma as it has been previously shown to have the most proliferated arsenal of HFBs among all fungi. Consequently we used the phylogenetic approach to identify the two novel class II HFBs (HFB4 and HFB7) from Trichoderma as the first candidates for the test. HFB4 and HFB7, produced in E. coli as N-terminal glutathione-S-transferase fusion proteins, exhibited subtle structural differences reflected in the hydrophathy plots which were correlated with unequal hydrophobicity and hydrophilicity respectively determined by water contact angle measurements. However they exhibited a dosage-dependent stimulation of PET hydrolysis by cutinase from Humicola insolens with HFB4 displaying an adsorption isotherm, whereas HFB7 was active only at very low concentrations and behaved inhibitory beyond them. We conclude that class II HFBs can stimulate the activity of cutinases on PET, but individual HFBs can display different properties in this process thus warranting a broader screening of HFBs for such industrial applications.
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68. High-yield of cyclosporine peptidomimetics from Scopulariopsis brevicaulis by random mutagenesis. **Linda Paun**, 1, EKibir Hihil, 2 Annemarie Kramer, 2 Antje Labe, 2 Johannes Imhoff, 2 Frank Kempken. 1 Botanical Institute, Christian-Albrechts-University, Kiel, Germany; 2 Kieler Wirkstoff-Zentrum KiWiZ at GEOMAR, Kiel, Germany.

The ascomycete Scopulariopsis brevicaulis, which was isolated from the marine sponge Tethya aurantium, produces two cyclosporine peptidomimetics, scoparides A and B [1]. Both peptides exhibit activity against several tumor cell lines. Within the EU-project MARINE FUNGI (EU FP7, 265926) one of our aims is to enhance the production of these secondary metabolites. We are in the process to establish two ways of random mutagenesis by both UV radiation and transposon-mediated. To this end we created UV-mutants and a miniaturised screening method was developed. UV-radiation was performed at 312 nm and the survival rate was set to 1%. With this method a mutant library was established. To screen these mutants for higher secondary metabolites production, we developed a screening method which includes decreased cultivation volume, fast extraction and an optimised LC-MS analysis format. Using the UV mutagenesis, we identified several mutants with a higher scoparide production in comparison to the wild type. One of these mutants, which produces three times more biomass and more than double the amount of scoparide A, has been used for another round of mutation. Next generation sequencing is being employed to identify the molecular genetic basis of the observed mutations. In parallel we employ transposable elements to introduce mutants [2]. The impact of transposons on gene expression as well as their ability to cause major mutations within the genome makes them an interesting tool for random mutagenesis [3, 4, 5]. We employ the Voder transposon in its homologous host and found that it mostly integrates within or very close to genes thus it appears to be a useful tool for transposon-mediated gene mutagenesis in *A. niger* [6]. At current we try to enhance its usability by modifying the Voder element. [1] Yu, Z.; Lang, G.; Kajahn, I.; Schmaljohann, R.; Imhoff, J. J. Nat. Prod. 2008, 71, 1052-1054 [2] Braumann I, van den Berg M, & Kempken F (2007) Fungal Genet Biol 44(12):1399-1414. [3] Daboussi MJ & Capy P (2003) Annu Rev Microbiol 57:275-299. [4] Kempken F (2003) Applied Mycology and Biotechnology, Vol. 3 Fungal Genomics, eds Arora DK & Khachatourians GG (Elsevier Science Annual Review Series), pp 83-99. [5] Kempken F & Kück U (1998) BioEssays 20:652-659. [6] Hihil E, Braumann I, van den Berg M, Kempken F (2011) Appl Environ Microbiol, 77: 2332-2336.

69. Generation of pathogenic diplonts from heterogeneous conidial populations of Aspergillus flavus. **Farhana Runa**, 1 Ignazio Carbone, 1 Deepak Bhatnagar, 2 Gary Payne. 1 Plant Pathology, North Carolina State University, Raleigh, NC; 2 Southern Regional Research Center, USDA, New Orleans, LA. Aspergillus flavus, a major producer of aflatoxin, has emerged as an opportunistic pathogen for a wide range of hosts. Understanding genetic variation within strains of *A. flavus* is important for controlling disease and reducing aflatoxin contamination. Because conidia of *A. flavus* are multinucleated but haploid, we wanted to know if nuclear condition or ploidy of conidia could be potential sources of genetic variation. The objective of our study is to detect nuclear heterogeneity and ploidy in conidial populations of *A. flavus* and determine their impact on fungal ecology. In order to examine heterokaryosis, protoplasts of two different auxotrophic strains in which nuclei were labeled with yellow (EYFP) and cyan (ECFP) fluorescent markers were fused. Fusants between the two strains were obtained through polyethylene mediated cell fusion and selection on minimal medium, which favored the growth of the fusants over that of either parental strain. Fusants selected for further study showed heterogeneous conidial populations with nuclei predominantly expressing either EYFP or ECFP, or a very few expressing both EYFP+ECFP. Conidia containing nuclei expressing only EYFP+ECFP were separated by Fluorescence-Activated Cell Sorting (FACS) and found to contain both yellow and cyan fluorescent proteins in the same nuclei. Further characterization of conidia having only one nucleus, but expressing both EYFP+ECFP, showed them to be diplonts. Pathogenicity assays using Galleria mellonella showed that


Lichen-forming fungi produce highly diverse and unique secondary compounds such as depsides, depsidones, dibenzofuranus and depsones. The biosynthesis of secondary metabolites is governed by polyketide synthase (PKS). However, the molecular mechanisms underlying the biosynthesis of these metabolites are poorly understood. Here we present analysis of the structure of the PKS gene clusters responsible for secondary metabolite production in the recently sequenced genome Cladonia metacorallifera. We found 37 type I polyketide synthase genes which were composed of 19 reducing PKSs, one partial reducing PKS and 17 non-reducing PKSs. Lichen-forming fungal PKS domains shared common structure with filamentous fungal PKSs. Phylogenetic analysis shows that some lichen-forming fungal PKSs constructed an unique clade in other filamentous fungal PKS clades.

67. Inhibition of benzoate 4-monooxygenase (CYP3A15) from Cochliobolus lunatus by cinnaonic acid derivatives. **Branka Korosec**, 1 Barbara Podobnik, 2 Sabina Berne, 2 Neja Zupanec, 2 Metka Novak, 2 Nada Krasivec, 2 Samo Turk, 2 Matej Sova, 2 Ljerka Lah, 2 Jure Stojan, 2 Stanislav Gobe, 2 Radovan Kome, 1-4. 1 National Institute of Chemistry, Ljubljana, Slovenia; 2 Lek Pharmaceuticals d.d., Verovska 57, SI-1000 Ljubljana, Slovenia; 3) Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, SI-1000 Ljubljana, Slovenia; 4) Chair of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Askerceva cesta 7, SI-1000 Ljubljana, Slovenia.

Fungal infections cause huge economic losses in agriculture. Some of the major phytopathogens also cause serious, and very often lethal, infections in human and animals. Plants may be a good source of antifungals since they have to defend themselves by producing numerous secondary metabolites, such as sterols, terpenes, polyacansols and phenolic compounds. Successful development of antifungal compounds, based on natural defense molecules, could prove useful in combating infectious and toxin-producing fungi in both agriculture and medicine. In recent years several promising antifungal targets have been under exploration. One of such is also fungal CYP35, member of the family of highly conserved CYP proteins, involved in detoxification of benzoate, a key intermediate in metabolism of aromatic compounds in fungi. High specificity and absence of homologue in higher eukaryotes assign CYP3A15 from the filamentous fungus *Cochliobolus lunatus* as interesting drug target. In our latest research we explored chemical properties of isoeugenol for ligand-based similarity searching, and the homology model of CYP3A15 of *Cochliobolus lunatus*, for structure-based virtual screening of a composite chemical library. Two cinnaonic acid derivatives were amongst the highest scoring compounds. In the past few years, several other reports about antifungal activity of cinnaonic acid derivatives have been published. In order to investigate the potential inhibitory activity on benzoate 4-monooxygenase (CYP3A15) we analyzed antifungal activity of 9 commercially available, and 10 representative cinnaonic acid derivatives from our library. Furthermore, to obtain more information about structure-activity relationship 26 additional cinnaonic acid esters and amides were synthesized and included in our assays. Among 45 cinnaonic acid derivatives tested, 7 compounds have shown antifungal activity against *C. lunatus, A. niger* and *P. ostreatus* in vivo inhibition tests. Compounds with antifungal activity were further evaluated for inhibition of CYP3A15 activity with spectral binding titration assay and HPLC. The best two inhibitors of CYP3A15 activity showed 70% inhibition at 600 mM concentration and were selected for further optimization of new lead structures.
70. Inhibition of appressorium formation of Magnaporthe oryzae by Roxithromycin and its possible molecular target. Akira Ishi, Mayu Kumasaka, Megumi Narukawa, Takashi Kamakura. Applied Biological Science, Tokyo Univ. of Science, Noda, Chiba, Japan.

Roxithromycin (RXM), a 14-membered macroclide which was originally active against prokaryote, has beneficial side effects such as anti-inflammatory activities were reported and actually applied to human. However, the mechanisms underlying these side effects are still unclear. In this study, we found that RXM inhibited appressorium formation of rice blast fungus Magnaporthe oryzae (M. oryzae). These results suggest that there are alternative targets in broad eukaryotic organisms and it is interesting to identify the molecular target of the secondary effect on human using M. oryzae. Magnaporthe oryzae is the causal agent of rice-blast disease. M. oryzae enters its host plant using a specialized infection structure known as an appressorium. The developmental stage of appressorium is sensitive to various chemical inhibitors, because large numbers of genes are involved in cellular differentiation. Since appressorium formation by M. oryzae can be observed on artificial surfaces, it can be a useful tool to search new activity of various chemicals. We performed phage display to search novel molecular target(s) of the antibiotic. We found that one candidate gene 32-11 may play important roles in appressorium formation. Expression of 32-11 gene, in a 32-11 mutant, was lower than wild type during developing infection structure, and the mutant was less affected by RXM. Although germinate and formation of appressorium were normal. Over expression of 32-11 gene caused no effect to RXM sensitivity, germination nor appressorium formation compared with the wild type. Over expression of 32-11 caused no effect to RXM activity, germination or appressorium formation compare to the wild type. To investigate whether lower expression of 32-11 causes the less sensitivity to RXM, we introduced 32-11 over expression vector into 32-11 reduced mutant. These mutants restored their wild type phenotype. These results possibly suggest that the complex of 32-11 product and RXM affects another molecule which plays an important role in appressorium formation at M. oryzae.

71. Identification of novel genes involved in induction of appressorium development triggered by plant-derived signals in Colletotrichum orbiculare. Sayo Kodama1, Ayumu Sakaguchi2, Yasuyuki Kubo3. 1) Laboratory of Plant Pathology, Graduate School of Life and Environmental Science, Kyoto Prefectural University, Kyoto, Japan; 2) National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8602, Japan.

Many plant pathogenic fungi initiate infection of host leaves by the germination of conidia and differentiation of appressoria at the tip of germ tubes. These morphological changes are accompanied by various external signals such as physical or chemical signals from the plant surface. In our previous study, cucumber anthracnose fungus Colletotrichum orbiculare CoKEL2, a Schizosaccharomyces pombe tea1 homologue, encoding a kelch repeat protein was identified. The cokel2 mutants formed abnormal appressoria on glass slides, and those appressoria were defective in penetration hyphae development into cellulose membranes, an artificial model substrate for fungal infection. In contrast, the cokel2 mutants formed normal appressoria on the host cucumber plant and retained penetration ability. Moreover, when conidia were incubated in the presence of exudates from cucumber cotyledon, normal appressorium formation on the artificial substrate by the cokel2 mutants was restored. These results suggest that CoKEL2 is essential for normal morphogenesis of appressorium and that there is a bypass pathway that transduces plant-derived signals for appressorium formation independent of CoKEL2. These plant-derived signaling pathways for appressorium formation have not been characterized in fungal pathogens including C. orbiculare. To determine specific components of the plant-derived signaling pathway that leads to appressorium formation, we screened six cokel2 double mutants that formed abnormal appressoria not only on artificial substrates but also on the host plant surface. Furthermore, reintroduction of CoKEL2 into those cokel2 double mutants restored normal appressorium formation on artificial substrates, suggesting that cokel2 double mutants have defects in CoKEL2-independent and plant-derived specific signaling pathway for appressorium formation. We identified and characterized candidate mutated genes by whole genome sequencing of the six cokel2 double mutants. To define the involvement of those candidate mutated genes in appressorium formation, we observed the phenotypes of candidate geneD single mutants, cokel2D candidate geneD double mutants, and complementation strains. As expected, candidate geneD mutants in cokel2D back ground showed same phenotypes as those of screened cokel2D double mutants.

72. Unique protein domains regulate Aspergillus fumigatus RasA localization and signaling during invasive growth. Rachel V. Lovingood1, Praveen R. Juvvadi2, William J. Steinback3, Jarrod R. Fortwendel1. 1) Microbiology and Immunology, University of South Alabama, Mobile AL, USA; 2) Pediatric Infectious Diseases, Duke University, Durham NC, USA.

Invasive pulmonary aspergillosis (IPA) is propagated by inhalation of A. fumigatus spores that germinate and invade the lung tissue in search of nutrients. We have shown that the A. fumigatus RasA GTPase protein is necessary for hyphal morphogenesis, cell wall integrity, and virulence during IPA. Our previous studies focused on conserved protein domains regulating RasA localization and signaling. These studies revealed the requirement for plasma membrane (PM)-localized Ras for proper signaling and regulation of A. fumigatus growth and virulence. Therefore, mechanisms controlling Ras localization are of interest in designing novel antifungal Ras inhibitors. Although Ras pathways may represent valid antifungal targets, the importance of fungal-specific Ras protein domains to Ras function in fungal pathogenesis remains unexplored. To address this important knowledge gap, we identified fungal-specific Ras protein domains by comparing fungal Ras sequences to their human counterpart, H-ras. We hypothesized that such domains could serve as targetable areas to selectively inhibit the fungal Ras protein. This analysis revealed two areas of significant divergence with H-ras: i) the Invariant Arginine Domain (IRD), a novel domain conserved in the RasA homologs of all available fungal genome but not present in H-ras and ii) an extended hypervariable region (HVR). Truncation analysis of the HVR identified a serine-rich region that is necessary for localization to the PM and for RasA signaling during hyphal morphogenesis. Interestingly, mutational analysis of the IRD produced a properly localized yet non-functional RasA protein. However, activation of the IRD RasA mutant was not altered suggesting a role for the IRD during interactions of RasA with downstream effectors. Further characterization of the IRD and HVR, and the protein interactions to which they contribute, will reveal fungal-specific aspects of Ras function and may define a new paradigm for Ras signal transduction in fungal organisms.

73. Light regulates growth, stress resistance and metabolism in the fungal pathogen Aspergillus fumigatus. Kevin K. Fuller, Carol S. Ringleberg, Jennifer J. Lories, Jay C. Dunlap. Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH.

Light serves as an important environmental cue that influences developmental and metabolic pathways in a variety of fungi. Interestingly, orthologs of a conserved blue light receptor, WC-1, promote virulence in two divergently related pathogenic species, Cryptococcus neoformans and Fusarium oxysporum, suggesting that photosensory systems may be conservatively linked to fungal pathogenesis. Aspergillus fumigatus is the predominant mold pathogen of immunocompromised patients, but if and how the organism responds to light has not been described. In this report, we demonstrate that the fungus can indeed sense and distinctly respond to both blue and red portions of the visible spectrum. Included in the A. fumigatus photosensory is a reduction in conidial germination kinetics, increased hyphal pigmentation, enhanced resistance to acute ultra-violet and oxidative stresses, and an increased
susceptibility to cell wall perturbation. Through gene deletion analysis we have found that the WC-1 ortholog, LreA, is a bone fide blue light receptor in *A. fumigatus* that is required for the photopigmentation response. However, the *DireA* mutant retains several blue light mediated responses, including the germination and stress resistance phenotypes, suggesting other blue light receptors are operative in this fungus. We also show that the putative red light sensing phytochrome, FpHa, is involved with some, but not all, blue light specific phenotypes, indicating a complex interaction between red and blue light photosystems in *A. fumigatus*. Finally, whole genome microarray analysis has revealed that *A. fumigatus* displays broad patterns of gene induction and repression upon exposure to light. Affected genes are largely metabolic and include those involved in lipid and sterol synthesis, respiration, carbohydrate catabolism, amino acid metabolism and metal ion homeostasis. Taken together, these data demonstrate the importance of the photic environment on the physiology of *A. fumigatus* and provide a foundation for future studies into an unexplored area of this important pathogen.

74. Analysis of critical domains in calcineurin A required for septal targeting and function in *Aspergillus fumigatus*. Praveen R. Juvvadi1, Jarrod R. Fortwendel1, Christopher Gehre1, Frederic Lamoth1, William J. Steinbach1. 1) Department of Pediatrics, Duke University Medical Center, Durham, NC; 2) Department of Microbiology and Immunology, University of South Alabama, Mobile, AL.

Calcineurin, a calmodulin (CaM)-dependent protein phosphatase, is known to play key roles in virulence, growth and stress responses of pathogenic fungi. Critical understanding of calcineurin regulation and identifying the residues indispensable for calcineurin activity in *vivo* will pave the way for devising new drug targets for combating invasive aspergillosis. Previous studies from our laboratory showed that the calcineurin complex (CnaA and CnaB) in *Aspergillus fumigatus* selectively localizes at the hyphal tip and septum to direct proper hyphal growth and regular septum formation. However, the domains responsible for targeting and function of CnaA at the hyphal septum remain unknown. Here we performed extensive truncational and mutational analyses of the functional domains of CnaA to investigate the relevance of these domains for localization and function of CnaA at the septum. Importantly we found that (1) CaM, the key protein known to activate calcineurin is not required for septal localization of CnaA but is required for its function at the hyphal septum, (2) the PxxIT substrate binding motif in CnaA is required for its localization at the hyphal septum, indicating it localizes at the septum by interacting with other as yet unknown protein(s); (iii) binding of CnaB subunit is not necessary for septal localization of CnaA but the regulatory subunit is required for its activation at the septum, and (iv) triple mutations in the catalytic active site do not affect septal localization of CnaA but completely block hyphal growth revealing that both septal localization and activity of CnaA are required for proper hyphal growth.

75. Calcium imaging and measurement during growth and response to stresses in *Aspergillus fumigatus*. Alberto Muñoz1, Margherita Bertuzzi1, Jan Bettgenhaeuser2, Elaine Bignell1, Nick Read1. 1) Fungal Cell Biology Group, University of Edinburgh, Edinburgh, United Kingdom; 2) Microbiology Section, Imperial College London, London, United Kingdom.

Calcium signalling and homeostasis are essential for the growth, differentiation and virulence of filamentous fungi. During infection, *A. fumigatus* must balance concomitant demands to: (1) withstand toxic levels of exogenous calcium (3-5 mM) in the host environment which can be >100,000x that of the fungal cytosolic free calcium ([Ca²⁺]c) concentration; (2) appropriately integrate homeostatic and stress-responsive adaptations; and (3) undergo normal calcium signalling. There is evidence for calcium signalling regulating numerous processes including spore germination and hyphal tip growth. The low resting level of [Ca²⁺]c (50-100 nM) is maintained by Ca²⁺-pumps and -antiporters, and cytoplasmic Ca²⁺-buffering. However, [Ca²⁺]c becomes an intracellular signal when its concentration is transiently increased. We have developed two methods for measuring and imaging [Ca²⁺]c: (1) 96-well plate luminometry using the genetically encoded, bioluminescent aequorin; and (2) fluorescence microscopy using the genetically encoded calcium-sensitive, fluorescent protein G-CaMP5. Aequorin is ideally suited for quantitative measurements of [Ca²⁺]c, calcium signatures in cell populations whereas fluorescence imaging of the G-CaMP5 is good for single cell and subcellular measurements of [Ca²⁺]c. Using the aequorin methodology we have found that transient increases in [Ca²⁺]c, with specific, reproducible calcium signatures in *A. fumigatus* arise from exposure to stresses such as high external calcium. In our analysis, [Ca²⁺]c spikes in actively growing hyphal tips have been imaged using G-CaMP5. Exposure of conidial germings to high external calcium induces dramatic and very dynamic changes in [Ca²⁺]c, with the generation of localized [Ca²⁺]c transients and waves. Furthermore, there is considerable heterogeneity in the [Ca²⁺]c responses of different germings within the cell population. Calcium imaging and measurement using genetically encoded probes, particularly when combined with pharmacological and genetic analyses, will provide major new insights into calcium signalling in filamentous fungi.

76. WITHDRAWN

77. The copper transporter ctpA in *Aspergillus fumigatus* is critical for conidial melanization and virulence in an invertebrate infection model. Srijana Upadhyay, Xiaorong Lin. Biology, Texas A&M University, College Station, TX.

*Aspergillus fumigatus* is an opportunistic pathogen that causes life-threatening invasive diseases in immunocompromised hosts. This fungus produces abundant, easily aerosolized, and heavily melanized conidia that are the infectious particles. The melanin, or the bluish green pigment coated on the conidial surface, is associated with fungal virulence and resistance to environmental stresses. This melanin is synthesized to the DHN melanin gene. Lack of melanin is caused by growing the wild type in the snxA mutation or deletion of the snxA ortholog, LreA, is a bone fide blue light receptor in *Aspergillus nidulans*. This fungus produces conidia more immune suppressors of ctpA1 ortholog, LreA, is a bone fide blue light receptor in *Aspergillus nidulans*. We conducted a screen for extrageneric suppressors of nimX2 that resulted in the identification of the cold-sensitive, G1-arresting snxA1 mutation. Our data show that snxA1 suppresses defects in regulators of the G2/M transition, including nimX22, nimE6, and nimT23, but does not suppress the G1/S-arresting nimE102 mutation or any of the 45 phase mutations. Furthermore, the snxA1 mutation or deletion of snxA alter localization patterns of NIME102 at the restrictive temperatures for snxA1 and nimX2, supporting a role for SNXA in cell cycle control. snxA encodes the *A. nidulans* ortholog of
Additionally, the cell wall becomes increasingly cross-
function for the protein in transporting arsenic across the plasma membrane, removing arsenic from cells. Arsenic resistance in A. nidulans cells, I have GFP
environments to remove excess copper from cells. Arsenic is also an important environmental contaminant. To begin to understa
and cells expressing t
addition, disruption of the A. nidulans copper transporter gene results in increased sensitivity to copper in the growth medi

79. The Aspergillus nidulans MAPK module AnSte11-Ste50-Ste7-Fus3 controls development and secondary metabolism. Oezguer Bayram1*, Oezlem
Sarikaya Bayram2, Yasar Luqman Ahmed3, Jun-ichi Maruyama4*, Oliver Valerius1, Silvio Rizzoli1, Ralf Ficner1, Stefan Imigert1, Gerhard Braus1. 1) Institute of Microbiology & Genetics, Department of Molecular Microbiology and Genetics, Georg-August-Universitat, Grisebachstr. 8, D 37077 Goettingen, Germany; 2) Department of Molecular Structural Biology, Institute for Microbiology and Genetics, Georg-August-Universitat, Goettingen; 3) European Neuroscience Institute, Deutsche Forschungsgemeinschaft Center for Molecular Physiology of the Brain/Excellence Cluster 171, 37077 Göttingen; 4) Department of Biotechnology, The University of Tokyo, Tokyo, Japan.

The sexual Fus3 MAP kinase module of yeast is highly conserved in eukaryotes and transmits external signals from the plasma membrane to the nucleus. We show here that the module of the filamentous fungus Aspergillus nidulans (An) consists of the AnFus3 MAP kinase, the upstream kinases AnSte7 and AnSte11, and the AnSte50 adaptor. The fungal MAPK module controls the coordination of fungal development and secondary metabolite production. It lacks the membrane docking yeast Ste5 scaffold homolog but similar to yeast the entire MAPK module interacts with each other at the plasma membrane. AnFus3 is the only subunit with the potential to enter the nucleus from the nuclear envelope. AnFus3 interacts with the conserved nuclear transcription factor AnSte12 to initiate sexual development and phosphorylates VeA which is a major regulatory protein required for sexual development and coordinated secondary metabolite production. Our data suggest that not only Fus3 but even the entire MAPK module complex of four physically interacting proteins can migrate from plasma membrane to nuclear envelope.

Institute of Technology, Karlsruhe, Germany.

A continuous flow of secretion vesicles from the hyphal cell body to the growing hyphal tip provides the delivery of proteins and lipids to the tip and is essential for cell wall and cell membrane extension at the tip. Apical sterol-rich plasma membrane domains (SRDs), which can be viewed using the sterol-binding fluorescent dye filipin, are gaining attention for their important roles in polarized growth of filamentous fungi. Although the importance of SRDs is becoming clear, their exact roles and formation mechanisms remain rather unclear. Transport of sterol to hyphal tips is thought to be important for the SRDs organization. Oxysterol binding proteins, which are conserved from yeast to human and involved in vesicular trafficking, signalling, lipid metabolism and non-vesicular sterol transport. Saccharomyces cerevisiae has seven oxysterol binding protein homologues (OSH1-7). Their subcellular distributions are regulated respectively. The OSH proteins are thought to function as a sterol transporter between closely located membranes independently of vesicle transport. In the filamentous fungus Aspergillus nidulans, we found five OSH genes. To investigate their functions for the polarized growth and SRDs organization, their localization are analyzed by GFP tagging. The gene-deletion strains are constructed and analyzed. Their expression levels are analyzed via qRT-PCR.

81. Mechanisms of cellular resistance to copper and arsenic in Aspergillus nidulans. Steven H. Denison, Natural Sciences, Eckerd College, St Petersburg, FL.

Copper is an essential element for cells that is toxic in high concentrations. Understanding cellular mechanisms for survival in high concentrations of copper is important for at least two reasons. Firstly, copper is an important environmental contaminant. In addition, two human genetic disorders, Wilson and Menkes diseases, result from impaired copper transport. I am using the filamentous fungus, Aspergillus nidulans, as a model organism for understanding cellular mechanisms of resistance to high concentrations of copper. I identified a gene in A. nidulans homologous to the copper transporting ATPase-encoding genes mutated in Wilson and Menkes diseases. To determine the location of this copper transporter in A. nidulans cells, I used fusion PCR to construct a GFP-tagged version of the gene, which was then transformed into A. nidulans in a gene replacement. In terms of its location in the cell, the A. nidulans GFP-tagged protein behaves in the same way as the Menkes disease protein: it is located to an intracellular compartment (possibly the Golgi, as in human cells) in low copper medium but appears in the plasma membrane upon addition of excess copper to the medium.

In addition, disruption of the A. nidulans copper transporter gene results in increased sensitivity to copper in the growth medium relative to wild type cells and cells expressing the GFP-tagged protein. Taken together, these data suggest that the transporter functions in the plasma membrane in high copper environments to remove excess copper from cells. Arsenic is also an important environmental contaminant. To begin to understand the mechanism of arsenic resistance in A. nidulans cells, I have GFP-tagged and disrupted a putative arsenic transporter gene from A. nidulans. Disruptants are more sensitive to arsenic than wild type cells and cells expressing the GFP-tagged protein. The GFP fusion protein localizes to the plasma membrane, consistent with a function for the protein in transporting arsenic across the plasma membrane, removing arsenic from cells.


Cell polarity is a defining feature of filamentous fungal growth. However, the complete molecular pathway that regulates this morphogenetic characteristic has not yet been elucidated. In Aspergillus nidulans, a germ tube emerges from a discrete location along the conidium following a brief period of isotropic swelling. Plasma membrane and cell wall components are continuously added to the apex of the germ tube via microtubule and actin-mediated trafficking of vesicles to this region. As growth progresses, germ tube cells undergo cytokinesis and are compartmentalized by septa. Additionally, the cell wall becomes increasingly cross-linked throughout subapical regions forming a hyphal, which continues to grow in the same polarized manner. ANID_05595.1 is located on chromosome 5, contig 96, and encodes a 946 amino acid hypothetical involucrin repeat protein. To investigate the function of ANID_05595.1 in A. nidulans, deletion mutants were generated using pyrG from Aspergillus fumigatus as a selectable marker. This mutation resulted in restricted colony growth, increased hyphal diameter, and dichotomous hyphal branching patterns. These phenotypes suggest that ANID_05595.1 function is important to the maintenance of polarized cell growth in A. nidulans and other ascomycetes. The hypothetical ANID_05595.1 protein shares characteristics with Saccharomyces cerevisiae Pea2, a polarisome component required for bipolar budding and mating. Along with structural similarities, the phenotypes observed in S. cerevisiae DPea2 are similar to A. nidulans D5595. This suggests that ANID_05595.1 may perform a similar mechanistic function to Pea2 in A. nidulans.
83. *Aspergillus nidulans* septin interactions and post-translational modifications. Yainitza Hernandez-Rodriguez¹, Shunsuke Masuo², Darryl Johnson³, Ron Orlando⁴, Michelle Momany⁴. 1) Plant Biology, University of Georgia, Athens, GA, US; 2) Laboratory of Advanced Research AS15, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai, Tsukuba, Ibaraki, JP; 3) Department of Chemistry, University of Georgia, Athens, GA, US; 4) Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, US.

Septins are cytoskeletal elements found in fungi, animals, and some algae, but absent in higher plants. These evolutionarily conserved GTP binding proteins form heterologenic complexes that seem to be key for the diverse cellular functions and processes that septins carry out. Here we used *Aspergillus nidulans*, a model filamentous fungus with well defined vegetative growth stages to investigate septin-septin interactions. *A. nidulans* has five septins: AspA/CDc11, AspB/CDc3, AspC/CDc12 and AspD/CDc10 are orthologs of the “core-filament forming-septins” in *S. cerevisiae*; while AspE is only found in filamentous fungi. Using S-tag affinity purification assays and mass spectrometry we found that AspA, AspB, AspC and AspD strongly interact in early unicellular and multicellular vegetative growth. In contrast, AspE appeared to have little or no interactions with core septins in unicellular stages before septation. However, after septation AspE interacted with other septins, for which we postulate an accessory role. AspE localized to the cortex of actively growing areas and to septa, and localizations are dependent on other septin partners. Interestingly, septin core localizations can also depend on accessory septin AspE, particularly post-septation. In addition, LC-MS/MS showed acetylation of lysine residues in AspA before septation and AspC after septation. Western blot analysis using an anti-acetylated lysine antibody showed that AspC is highly acetylated in all stages examined, while other septins showed acetylation post-septation. Though LC-MS analysis failed to detect phosphorylation of septins, this modification has been widely reported in fungal septins. Using phosphatase treatments and Western Blotting, we found phosphorylation of AspD, but no other septins. This is interesting because AspD belongs to a special group of septins that lack a C-terminal coil-coil found in other septins. However, septin localization is not affected by the absence of AspD/CDc10, but by the absence of filamentous fungi specific septin AspE. Our data suggests that septin interactions and modifications change during development and growth in *A. nidulans*, and that some modifications are septin specific.

84. A highly conserved sequence motif is required for PkcA localization to septation sites and protein function in *Aspergillus nidulans*. Loretta Jackson-Hayes¹, Terry Hill¹, Darlene Loprete², Claire DelBove³, Omolola Dawodu⁴, Jordan Henley¹, Ashley Poullard¹, Justin Shapiro¹. 1) Rhodes College, Memphis, TN 38112; 2) Rust College, Holly Springs, MS 38635; 3) Tougaloo College, Tougaloo, MS 39174.

Many proteins with diverse functions contribute to cell wall synthesis in involved growth and septation. Some of these proteins play similar roles at tips and septa, while others are exclusively involved in one process or the other. In *Aspergillus nidulans*, wild type protein kinase C (PkcA) localizes to growing hyphal tips and septation sites, and a role for PkcA in cell wall synthesis is supported by the inability of PkcA mutant strains to exhibit resistance to cell wall perturbing agents. PkcA localization to septation sites is dynamic. Upon initiation of septum formation PkcA is organized as a ring at the periphery of the septation site. The ring constructs in synchrony with the actin/myosin contractile ring and dissipates when septa are fully matured. To determine which domains are important for septate site formation we used a yeast strain with a well characterized protein core (Kanaya et al. 2010). The localization of PkcA to septation site domains is dynamic. 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resistance mutations. Xiaoxiao Sean He, Shengnan Jili Li, Susan Kaminsky. Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. Systemic fungal infections are estimated to contribute to ~10% of hospital deaths. Systemic fungal infections are most dangerous for the young, the old, and the already sick, since their immune systems are less vigorous. Most antifungal drugs in current clinical use target ergosterol (polymers) or the ergosterol biosynthetic pathway (azoles and allylamines). Drugs against beta-glucan synthesis (echinocandins) are effective against aspergillosis and candidiasis. The use of compounds that target fungal enzymes inevitably leads to the development and natural selection of drug resistant fungal strains. Not only are the anti-fungal drugs in current clinical use losing efficacy in some situations, but in addition the high level of conservation between animal and fungal physiology leaves relatively few relevant targets to explore. However, it is likely that for any drug-enzyme combination there will be relatively few mutations that could increase drug resistance while still maintaining enzyme function. We are using Aspergillus nidulans as an experimental model system to assess the number and identity of mutations that lead to drug resistance. As proof of concept, we grew wild type A. nidulans on replicate plates containing a sub-lethal concentration of Calcifluor. These developed fast-growing sectors beginning at ~ 5 d (70 rounds of mitosis). Preliminary results show that many of these sectors harboured heritable, single-gene mutations. To date, mutated genes that confer robust, heritable resistance to Calcifluor that were identified by next generation sequencing have roles in cell wall synthesis, cell wall integrity regulation, or drug detoxification. We suggest this strategy will be useful for predicting genetically-mediated anti fungal resistance adaptation and help us to be ahead in the drug-resistance arms race.

88. Aspergillus nidulans cell walls lacking galactofuranose are more susceptible to glucan degrading enzymes. Biplab Paul1, Tanya Dahms2, Susan Kaminsky2. 1) Dept Chemistry and Biochemistry, Univ Regina, Regina, SK, Canada; 2) Dept Biology, Univ Saskatchewan, Saskatoon, SK, Canada.

The cell wall of Aspergillus is a dynamic organ, consisting of a semi-permeable network of mannoprotein, and alpha- and beta-glucans. These components are remodeled as fungal cell grows and responds to its environments. By weight, fungal walls are estimated to be 35-45% alpha-(1,3)-glucan, 20-35% beta-(1,3)-glucan, 20-25% galactomannan, 7-15% chitin (beta-1,4-glucan), and 4% beta-(1,6)-glucan. Evidence from literature sources suggest that the Aspergillus wall core is chitin and galactomannan linked to beta-1,6- and beta-1,6-glucan. Galactofuranose (Gal-f) appears to play a central role in Aspergillus cell wall maturation. Previously, we showed that Gal-f biosynthesis is important for wild type chemical, physical, structural properties of the A. nidulans cell wall. We propose that the lack of Gal-f disrupts the proper packing of cell wall components, giving rise to more disordered surface subunits and so to greater deformability. Here, we show results from an investigation of the susceptibility of Aspergillus Gal-f biosynthesis deletion strains to glucan degrading enzyme using atomic force microscopy. Topographic images of glucanase- and laminarinase-treated wildtype strains suggest that glucan is at least one component of the cell surface subunits. Strains that lacked Gal-f were more susceptible to beta-1,3-glucanase.

89. The GATA-type transcription factor NsdD is a key regulator of conidiation and secondary metabolism in Aspergillus. Min-Kyung Lee1, Nak-Jung Kwon1, Im-Soon Lee2, Jae-Hyuk Yu1. 1) Bacteriology, University of Wisconsin Madison, MADISON, WI, USA; 2) Department of Biological Sciences, Konkuk University, Seoul, Republic of Korea.

Asexual development (conidiation for higher fungi) is the most common reproductive mode of many fungi; yet, its regulatory mechanisms remain to be understood. In this study, we carried out a multi-copy based genetic screen in the absence of the represor of conidiation sfgA, which is designed to identify a set of negative regulator(s) of conidiation. Among over 100,000 colonies, 45 transformants showing altered conidiation were isolated, of which 10 defined the nsdD gene (AN5152), a key activator of sexual fruiting. The others have defined AN7507, AN2009, AN1652, AN5833 and AN9141. A series of verification, genetic and mycotoxin analyses revealed that only NsdD is a true negative regulator of brlA (an essential activator of conidiation) and conidiation, and that NsdD acts downstream of flug and fibA-B-E; but upstream of brlA. The removal of NsdD was sufficient to cause hyper-active conidiation even in liquid submerged culture, as well as early and prolonged activation of brlA suggesting that NsdD is indeed a key repressor of brlA and conidiation. Moreover, the deletion of nsdD results in hyper-active conidiation and altered production of mycotoxins in the opportunistic human pathogen Aspergillus fumigatus and the aflatoxin-producing human/plant pathogen Aspergillus flavus. Importantly, we have discovered that NsdD encodes two differentially expressed mRNAs and polypeptides (b and a). Finally, the subsequent transient promoter analysis using the brlA promoter::luciferase fusion constructs have revealed that NsdD negatively regulates the brlAb promoter activity. In summary, NsdD is a key negative regulator of conidiation acting direct upstream of brlA in A. nidulans, and couples conidiation and mycotoxin biosynthesis in Aspergillus.

90. THE velvet regulators in Aspergilli. Heesoo Park, JJaehyuk Yu. Bacteriology, University of Wisconsin Madison, Madison, WI. The velvet regulators are the key players coordinating fungal growth, differentiation and secondary metabolism in response to various internal and external cues. All velvet family proteins contain the conserved velvet homology motif (~190 a.a.), and define a novel class of fungal specific transcription factors with the DNA binding ability. Some velvet regulators form time and/or cell type specific complexes with other velvet regulators or non-velvet proteins. These complexes play differential roles in regulating growth, development, sporogenesis and toxigenesis. Among the velvet complexes, the VelB-VoSA hetero-complex acts as a functional unit conferring the completion of sporogenesis (focal trehalose biogenesis and spores wall completion), and the long-term viability of spore, and the attenuation of conidial germination in the model filamentous fungus Aspergillus nidulans. Both velB and voSA are activated by ABA in developing cells, and the VelB-VoSA complex plays a dual role in activating genes associated with spore maturation and in exerting negative feedback regulation of developmental genes. Interestingly, the VelB-VoSA complex plays similar yet somewhat distinct roles in spore maturation, dormancy and germination in Aspergillus fumigatus and Aspergillus flavus. A comprehensive model depicting the roles of the velvet regulators in aspergilli is presented.

91. Coordinated regulation of asexual development, cell death and autolysis by the C2H2 zinc finger transcription factor BrlA in Aspergillus nidulans. Iriván Pérez1, Jae-Hyuk Yu2, Tamás Emri2. 1) Department of Microbial Biotechnology and Cell Biology, University of Debrecen, Debrecen, Hungary; 2) Departments of Bacteriology and Genetics, University of Wisconsin, Madison, WI, USA.

Carbon starvation elicited in submerged cultures of Aspergillus nidulans triggers all various physiological responses affecting cell wall composition, stress tolerance, protein synthesis and primary and secondary metabolisms. Particularly, function of vacuoles and endoplasmic reticulum is drastically affected leading to the re-utilization of cellular biopolymers through macroautophagy and the removal of damaged cells by apoptosis. Autolytic cell wall degradation is also an integrant part of this highly complicated and delicate regulatory process. Importantly, although the development of conidiophores is initiated in carbon-starving submerged cultures, these structures are underdeveloped and only simple conidia are observable. There is an increasing body of evidence supporting the idea that the transcription factor BrlA, a well-studied central regulator of conidiation in aspergilli, is one of the most important master controllers orchestrating development and autolysis in submerged culture of aspergilli. Major processes subjected to BrlA-dependent regulation under these conditions include the production of autolytic enzymes, rodlet proteins and melasins. In fungal biology, the concerted and well-balanced regulation of conidiogenesis, cell death and autolysis is of primary importance because any overproduction of cell wall hydrolases may affect cell vitality and colony propagation rather disadvantageously. The age-dependent production of autolytic hydrolases coincides with the synthesis of antimicrobial
metabolites and proteins, and all these carbon-starvation-associated products will affect markedly the microbiome in the ecological niche the autolyzing fungus occupies. A deeper understanding of the BrA-mediated spatial and temporal regulatory mechanisms for conidiogenesis, cell death and autolysis may lead to the development of new industrial strains for heterologous protein production and/or novel biocontrol technologies.


Many organelles are transported long distances along microtubules in eukaryotic organisms by dynein and kinesin motors. To identify novel alleles and genes required for microtubule-based transport, we performed a genetic screen in the filamentous fungus, Aspergillus nidulans. We fluorescently-labeled three different organelle populations known to be cargo of dynein and kinesin in Aspergillus: nuclei, endosomes, and peroxisomes. We then used a fluorescence microscopy-based screen to identify mutants with defects in the distribution or motility of these organelles. Using whole-genome sequencing, we found a number of single nucleotide polymorphisms (SNPs) that resulted in misdistribution of peroxisomes, endosomes, or nuclei. Some of these SNPs were novel alleles of cytoplasmic dynein/ nudA, Arp1/ nudK (dynactin), Lis1/ nudT, and kinesin-1/ kiaA. Here, we characterize the in vivo transport defects in these novel mutants and analyze the single molecule in vitro motility properties of purified mutant motor proteins. We also describe our methods for using whole genome sequencing as a tool in mutagenesis studies in A. nidulans.

93. Two methyltransferase protein complexes control fungal development and secondary metabolite production. Öezlem Sarıkaya Bayram1, Öezguer Bayram1, Jong-Hwa Kim1, Keon-Sang Chae2, Dong-Min Han1, Kap-Hoon Han1, Gerhard Braus1. 1) Institute of Microbiology & Genetics, Dept. of Molecular Microbiology and Genetics, Georg-August University, Grisebachstr. 8, D 37077 Goettingen, Germany; 2) Department of Pharmaceutical Engineering, Woosuk University, Wanju, 565-701, Korea; 3) Division of Biological Sciences, Chonbuk National University, Jeonju, 561-756, Korea; 4) Division of Life Sciences, Wonkwang University, Iksan, 570-749, Korea.

Coordination of development and secondary metabolism of the filamentous fungus Aspergillus nidulans requires the trimeric velvet complex consisting of VelB-VeA and the putative methyltransferase LaeA. We discovered a second trimeric protein complex for the same control mechanism consisting of an unusual zinc finger domain protein and even two subunits containing canonical methyltransferase domains. In contrast to velvet, which is assembled in the nucleus, the trimeric protein complex is formed at the plasma membrane. Functional green fluorescent protein fusions revealed that both methyltransferases are released from the membrane-bound zinc finger domain and migrate to the nucleus. The dimeric nuclear methyltransferase complex physically interacts with chromatin factors as heterochromatin protein and has an impact on the expression of sexual or nonsexual developmental genes as well as secondary metabolite gene clusters. Consistently, deletions of the corresponding genes result in defects in light response. Our results support that a trimeric membrane complex initiates a signalling pathway which is mediated by two methyltransferases which transduce the signal to nuclear chromatin and affect gene expression. The interplay between the novel methyltransferase complex and the velvet complex remains to be elucidated.

94. Control of Multicellular Development by the Physically Interacting Deneddylases DEN1/DenA and COP9 Signalosome. Josua Schinke1, Martin Christmann1, Tilo Schmaler2, Colin Gordon3, Xiaohua Huang1, Öezguer Bayram1, Silke Stumpf1, Wolfgang Dubiel1, Gerhard Braus1. 1) Microbiology and Genetics, Georg-August-University, Göttingen, Niedersachsen, Germany; 2) Department of General, Visceral, Vascular and Thoracic Surgery, Division of Molecular Biology, Charité - Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany; 3) MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK.

Deneddylases remove the ubiquitin-like protein Nedd8 from modified proteins. An increased deneddylation activity has been associated to various human cancers. In contrast, we show here that a mutant strain of the model fungus Aspergillus nidulans which is deficient in two deneddylases is viable but can only grow as a filament and has lost most of the potential for multicellular development. The DEN1/DenA and the COP9 signalosome (CSN) deneddylases physically interact in A. nidulans as well as in human cells, and CSN targets DEN1/DenA for protein degradation. Fungal development responds to light and requires both deneddylases for an appropriate light reaction. In contrast to CSN which is necessary for sexual development, DEN1/DenA is required for asexual development. The CSN-DEN1/DenA interaction which affects DEN1/DenA protein levels presumably balances cellular deneddylation activity. A deneddylation disequilibrium impairs multicellular development and suggests that control of deneddylation activity is important for multicellular development.

95. Visualization of apical membrane domains in Aspergillus nidulans by Photoactivatable Localization Microscopy (PALM). Norio Takeshita1, Yuji Ishitsuka1, Yiming Li2, Ulrich Nienhaus3, Reinhard Fischer2. 1) Dept. of Microbiology, Karlsruhe Institute of Technology, Karlsruhe, Germany; 2) Institute for Applied Physics, Karlsruhe Institute of Technology.

Apical sterol-rich plasma membrane domains (SRDs), which can be viewed using the sterol-binding fluorescent dye filipin, are gaining attention for their important roles in polarized growth of filamentous fungi. The size of SRDs is around a few nm, whereas the size of lipid rafts ranges in general between 10-200 nm. In recent years, super-resolution microscope techniques have been improving and breaking the diffraction limit of conventional light microscopy whose resolution limit is 250 nm. In this method, a lateral image resolution as high as 20 nm will be a powerful tool to investigate membrane microdomains. To investigate deeply the relation of lipid membrane domains and protein localization, the distribution of microdomains in SRDs were analyzed by super-resolution microscope technique, Photoactivatated Localization Microscopy (PALM). Membrane domains were visualized by each marker protein tagged with photoconvertible fluorescent protein mEosFP for PALM. Size, number, distribution and dynamics of membrane domains, and dynamics of single molecules were investigated. Time-lapse analysis revealed the dynamic behavior of exocytosis.

In the filamentous fungus Aspergillus nidulans, the transition from hyphal growth to asexual development is associated with dramatic changes in patterns of cellular morphogenesis and division. These changes enable the formation of airborne conidiophores that culminate in chains of spores generated by repeated budding of phialides. Our objective is to characterize the regulatory modules that mediate these changes and to determine how they are integrated with the well-characterized network of transcription factors that regulate conidiation in A. nidulans. Because protein phosphorylation is likely to be a key component of these regulatory modules, we have exploited the availability of A. nidulans post-genomic resources to investigate the roles of protein kinases and phosphatases in developmental morphogenesis. We have used the protein kinase and phosphatase deletion mutant libraries made available by the Fungal Genetics Stock Center to systematically screen for defects in conidiophore morphology and division patterns. Our initial results implicate ANID_11101.1 (yeast His1/Gin4) in phialide morphogenesis, and also reveal the importance of ANID_07104.1 (yeast Yak1) in the maintenance of cell integrity during asexual development. Additional deletion mutants with reproducible defects have been identified and will be described in detail. We will also summarize initial results from double mutant analyses that attempt to place specific protein kinase deletions within the regulatory network that controls conidiation.

97. The Putative Guanine Nucleotide Exchange Factor RicA Mediates Upstream Signaling for Growth and Development in Aspergillus. Nak-Jung Kwon¹, Hee Soo Park², Seunho Jung³, Sun Chang Kim⁴, Jae-Hyuk Yu¹,² 1) Dept Bacteriology, University of Wisconsin, Madison, WI, USA; 2) Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, WI, USA.; 3) Department of Bioscience and Biotechnology, and Center for Biotechnology Research in UBITA, Konkuk University, Seoul, Republic of Korea; 4) Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Dae-Jon, Republic of Korea.

Heterotrimeric G proteins (G proteins) govern growth, development, and secondary metabolism in various fungi. Here, we characterized ricA, which encodes a putative GDP/GTP exchange factor for G proteins in the model fungus Aspergillus nidulans and the opportunistic human pathogen Aspergillus fumigatus. In both species, ricA mRNA accumulates during vegetative growth and early developmental phases, but it is not present in spores. The deletion of ricA results in severely impaired colony growth and the total (for A. nidulans) or near (for A. fumigatus) absence of asexual sporulation (conidiation). The overexpression (OE) of the A. fumigatus ricA gene (AfricA) restores growth and conidiation in theΔAnricA mutant to some extent, indicating partial conservation of RicA function in Aspergillus. A series of double mutant analyses revealed that the removal of RgsA (an RGS protein of the GanB Ga subunit), but not sfgA, fibA, rgsB, or rgsC, restored vegetative growth and conidiation in AnricA. Furthermore, we found that RicA can physically interact with GanB in yeast and in vitro. Moreover, the presence of two OE of pkaA suppresses the profound defects caused by ΔAnricA, indicating that RicA-mediated growth and developmental signaling is primarily through GanB and PkaA in A. nidulans. Despite the lack of conidiation, brlA and vosA mRNAs accumulated to normal levels in the ricA mutant. In addition, mutants overexpressing fluG or brlA (OEfluG or OEBrlA) failed to restore development in theΔAnricA mutant. These findings suggest that the commencement of asexual development requires unknown RicA-mediated signaling input in A. nidulans.


In Aspergillus nidulans spindle pole bodies (SPBs) and septum-associated microtubule-organizing centres (sMTOCs) polymerize cytoplasmic microtubules. Previously, we identified a novel MTOC-associated protein, ApsB (Schizosaccharomyces pombe mot1), whose absence affected MT formation from sMTOCs more than from SPBs, suggesting that the two protein complexes are organized differently (Suelmann et al., 1998; Veith et al., 2005). In addition, we discovered that ApsB localizes to a subclass of peroxisomes apparently without a peroxisomal targeting motif. However, we found that ApsB interacts with the Woronin body protein HexA, which has a PTS1 motif at the C-terminus (Zekert et al., 2010). Our hypothesis is that ApsB is imported to peroxisomes by a piggyback import mechanism along with HexA. To further investigate the role of peroxisomes in microtubule organization, We created a deletion mutant of pexC. PexC is an essential protein for peroxisomal biogenesis (Heiland & Erdmann, 2005). The pexC mutant partially phenocopied the apsB mutant, which shows reduced sporulation and nuclear migration defects in comparison to wild type. The number of astral and cytoplasmic microtubules and the activities of sMTOCs and SPBs was reduced in the pexC mutant in comparison to wild type. sMTOC activity was more affected than the SPB activity, which again resembles the phenotype of the aps8 mutant. In conclusion, peroxisomes play a role in microtubule organization through ApsB.

99. Autophagy promotes survival in aging submerged cultures of the filamentous fungus Aspergillus niger. Maria A. Burggraaf¹,², Benjamin M. Nitsche¹,², Gerda Lamers², Vera Meyer²,³, Arthur F.J. Ram¹,² 1) Institute of Biology Leiden, Molecular Microbiology and Biotechnology, Leiden, The Netherlands; 2) Kluiver Centre for Genomics of Industrial Fermentation, Delft, The Netherlands; 3) Institute of Biotechnology, Applied and Molecular Microbiology, Berlin University of Technology, Berlin, Germany.

The filamentous fungus Aspergillus niger is an important and versatile cell factory commonly exploited for the industrial-scale production of a wide range of enzymes and organic acids. Although numerous studies have been conducted aiming at improving our knowledge of degradative cellular activities that determine product yields in A. niger including secretion of proteases and the unfolded protein response, there is a catabolic pathway that has yet not been studied in this industrially exploited fungus, namely Autophagy. Autophagy is a well conserved catabolic process constitutively active in eukaryotes that is involved in cellular homeostasis by targeting of cytoplasmic content and organelles to vacuoles. Autophagy is strongly induced by limitation of nutrients including carbon, nitrogen and oxygen and is clearly associated with cell death. We previously demonstrated that the accumulation of empty hyphal compartments and secondary regrowth in carbon starved submerged cultures of A. niger were accompanied by a joint transcriptional induction of autophagy genes. In this study we examined the role of autophagy by deleting the atg1, atg8 and atg17 orthologues in A. niger and phenotypically analyzing the deletion strains in surface and submerged cultures. Our results indicate that atg1 and atg8 are essential for efficient autophagy whereas deletion of atg17 has little to no effect on autophagy. Depending on the stressor, autophagy deficiency renders A. niger both more resistant and more sensitive to oxidative stress. Fluorescence microscopy showed that mitochondrial turnover upon carbon depletion in submerged cultures is severely blocked in autophagy impaired mutants. Furthermore, automated image analysis demonstrated that autophagy promotes survival in maintained carbon starved cultures of A. niger. Taken together, our results suggest that besides its function in nutrient recycling, autophagy plays important roles in physiological adaptation by organelle turnover and protection against cell death upon carbon depletion in submerged cultures.
100. Inactivation of flbA results in increased secretome complexity and reduced secretion heterogeneity in colonies of Aspergillus niger. Pauline Krijgheld1, Benjamin M. Nitsche2, Harm Post1, Ana M. Levin1, Wally H. Müller3, Albert J.R. Heck4, Arthur F.J. Ram1, A.F. Maarten Altenaar2, Han A.B. Wösten1; 1) Microbiology and Kluvyer Centre for Genomics of Industrial Fermentation, Utrecht University, Utrecht, The Netherlands; 2) Department of Molecular Microbiology and Biotechnology, Institute of Biology Leiden and Kluvyer Centre for Genomics of Industrial Fermentation, Leiden University, Leiden, The Netherlands; 3) Biomolecular Mass Spectrometry and Proteomics, Netherlands Proteomics Center, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands; 4) Biomolecular Imaging, Utrecht University, Utrecht, The Netherlands.

Aspergilli are among the most common fungi. They colonize substrates by secreting enzymes that degrade organic polymers into small products that can be taken up by the fungus to serve as nutrient. Hyphae at the periphery of the colony are exposed to unexplored organic material, whereas the substrate is (partly) utilized in the colony center. Aspergillus niger is known for its capacity to secrete high amounts of proteins. Interestingly, the fungus secretes proteins in the central part and at the periphery of the colony but not in the sub-peripheral zone. The sporulating zone of the colony overlaps with the non-secreting zone, indicating that sporulation inhibits protein secretion. Indeed, strain ΔflbA that is affected early in the sporulation program secreted proteins throughout the colony. In contrast, the DbrA strain that still initiates but does not completes sporulation did not show an altered spatial secretion. The secretome of 5 concentric zones of 7-day-old xylose-grown ΔflbA mutant colonies of A. niger was assessed by quantitative proteomics using stable isotope dimethyl labeling. In total 171 proteins were identified in the medium of the DflbA colonies, of which 33 proteins did not have a signal sequence for secretion. Out of the 138 secreted proteins, 101 had previously not been identified in the secretome of the 5 concentric zones of xylose-grown wild-type colonies. Moreover, 18 proteins had never been reported to be part of the secretome of A. niger. Taken together, inactivation of flbA, but not brA results in spatial changes in secretion and in a more complex secretome. The latter may be explained by the fact that strain DflbA has a thinner cell wall compared to the wild type, enabling efficient release of proteins. These results can implemented in the industry to improve A. niger as a cell factory.

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101. Functional characterization of A. niger class III and class V chitin synthases and their role in cell wall integrity. Jean-Paul Ouedraogo1, Arthur Ram1, Vera Meyer1; 1) Molecular Microbiology and Biotechnology, Institute of Biology, Leiden, Leiden, Netherlands; 2) Molecular and Applied Microbiology, Institute of Biotechnology, Berlin University of Technology, Berlin, Germany.

Class III and V chitin synthases play an important role in morphogenesis and cell wall integrity in many filamentous fungi. However, their function in the filamentous fungus, A. niger, has not yet been elucidated. To address this, deletion mutants of class III and V chitin synthase-encoding genes of A. niger, chsB and csmB, and their role in cell wall integrity have been studied. Deficiency in condiation and abnormal swollen conidiophores have been observed in chsB and csmB deletion mutants. Using cell wall inhibitor reagents, it was shown that the mutants are hypersensitive towards cell wall stress. However, there are differences between them as regards susceptibility to the antifungal protein AFB. These results suggest that ChsB and CsmB play an important role during sexual development and in ensuring cell wall integrity of A. niger. Interestingly, the data indicate that only chitin synthase csmB is important to counteract AFB inhibitory effects.

102. Exploiting transcriptomic signatures of Aspergillus niger to uncover key gene regulators for high protein traffic through its secretory pathway. Min Jin Kwon1,2, Thomas Jørgensen3, Benjamin M Nitsche4,5, Mark Arentshorst4, Joohae Park5, Arthur F.J. Ram1,2, Vera Meyer1; 1) Molecular Microbiology, Institute of Biology Leiden, Leiden, Netherlands; 2) Kluvyer Centre for Genomics of Industrial Fermentation, P.O. Box 5057, 2600 GA Delft, The Netherlands; 3) Institute of Biotechnology, Department Applied and Molecular Microbiology, Berlin University of Technology, Gustav-Meyer-Allee 25, 13355 Berlin, Germany.

The filamentous fungus Aspergillus niger is well known for its exceptional high capacity to secrete proteins. However, system-wide insights into its secretory capacities are sparse and rational strain improvement approaches are thus limited. To gain a global view on the transcriptional basis of the secretory pathway of A. niger, we have investigated its transcriptomic fingerprint when specifically forced to overexpress the hydrolytic enzyme glucoamylase (GLA). An A. niger wild-type strain and an GLA over-expressing strain where cultivated under maltose-limited chemostat conditions. Elevated glaA mRNA and extracellular GLA levels in the over-expressing strain were accompanied by reinforced transcription of 772 genes and down-regulation of 815 genes when compared to the wild-type strain. Using GO term enrichment analysis, four higher order categories were identified in the up-regulated gene set: i) translocation, ii) protein glycosylation, iii) vesicle transport and iv) ion homeostasis. Among these, about 130 genes have predicted functions for the protein passage through the endoplasmatic reticulum including well-known target genes of the HaA transcription factor, e.g. bipA, chaA, prpA, tplA and pda. To identify those genes, which are generally important for high-level secretion in A. niger, we compared the GLA transcriptome with six other secretion stress transcriptomes of A. niger, including a constitutive active HaA transcriptome, several UPR stress transcriptomes and a carbon-source induced secretion transcriptome. Overall, 40 genes were commonly up-/down-regulated under these three conditions (36 genes up-regulated, 4 down-regulated), thus defining the core set of genes important for ensuring high protein traffic through the secretory pathway.

103. Identification of two Golgi-localized putative UDP-galactofuranose transporters with overlapping function in Aspergillus niger. Joohae Park1, Boris Tefsen2, Ellen Lagendijk1, Irma van Die1, Arthur Ram1,2; 1) Molecular Microbiology, Institute of Biology Leiden, Leiden, Netherlands; 2) Department of Molecular Cell Biology and Immunology, VU University Medical Center, van den Boechorststraat 7, 1081 BT Amsterdam, The Netherlands; 3) Kluvyer Centre for Genomics of Industrial Fermentation, P.O box 5057, 2600 GA Delft, The Netherlands.

Galactofuranose-containing glycoconjugates are present in numerous microbes, many of which are pathogenic for humans. Metabolic aspects of the monosaccharide have proven difficult to elucidate, because galactofuranose metabolites and glycoconjugates are relatively unstable during analyses. Recent advances with genetic approaches have facilitated a better understanding of galactofuranose biosynthesis. Galactofuranose (Galf) the five-ring isomer of galactopyranose (Galp), is an essential component of the cell wall and required for a structural integrity [1-2]. Recently, it has been postulated that UDP-Galp is converted to Galf by a UDP-galactopyranose mutase (UgmA) and subsequently transported into the Golgi by a putative UDP-Galf-transporter for the further biosynthesis of cell wall polymers such as galactomannan, galactoaminogalactan and cell wall glycopolypeptides (galactomannoproteins) [3-4]. Based on homology searches, we have identified two putative UDP-Galf-transporters in A. niger. We have studied the function of the transporters by making deletions mutants (either single or double mutants) and by studying their localization by making GFP fusions. We conclude that the two putative UDP-Galf-transporters (named UgtA and UgtB) have an overlapping function in UDP-Galf-transport and that both proteins are localized in Golgi equivalents. References: [1] Damveld, R.A. et al., 2008. Genetics 178 (2), 873-81; [2] Schmalhorst, P.S. et al. 2008. Euk. Cell 7 (8), 1268-77; [3] Engel, J. et al., 2009. J. Biol. Chem. 284; [4] Bernard, M., Latge, J. P., 2001. Med. Myc. 39, 9-17.
104. Maltose permease-encoding mRNA is cleaved under induction condition of amylolytic gene expression in Aspergillus oryzae. Mizuki Tanaka, Takahiro Shintani, Katsuya Gomi. Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan.

Eukaryotic mRNA is degraded by two degradation pathways: the 5’ to 3’ degradation pathway by Xrn1 and the 3’ to 5’ degradation pathway by exosome-Ski complex. To investigate the mRNA degradation mechanism in filamentous fungi, we generated the disruptions of orthologous genes encoding mRNA degradation machinery in Aspergillus oryzae. Interestingly, the disruptants of ski2 and ski3, which encode the components of Ski complex, showed the remarkable growth defect on minimal medium containing maltose or starch as a sole carbon source, whereas they normally grew on the medium with glucose or fructose as a sole carbon source. Northern blot analysis showed that the 3’-truncated fragment of mRNA encoding maltose permease (malP) was accumulated in Ski complex deficient mutants. Circularized RT-PCR analysis revealed that the malP mRNA was cleaved at a large stem-loop structure situated within the coding region. These results suggested that the 3’-truncated fragment of malP mRNA is cleaved by endonuclease and the resultant 3’-truncated malP mRNA is degraded rapidly by 3’ to 5’ degradation pathway. In higher eukaryotes, it has been reported that the mRNAs encoding secreted and membrane proteins were cleaved by endoplasmic-reticulum (ER) endonuclease Ire1 during ER stress. Since A. oryzae produces copious amounts of amylolytic enzymes in the presence of maltose, we presumed that malP mRNA is cleaved by Ire1 with the induction of amylolytic gene expression. Therefore, we generated the double deficient mutant for Ski complex and AmyR, the regulator of amylolytic gene expression. The resultant double mutant showed normal growth on maltose medium, and 3’-truncated fragment of malP mRNA was not detected by Northern blot analysis. This result clearly indicated that malP mRNA is cleaved under induction condition of amylolytic gene expression in A. oryzae.

105. Functional characterisation of Rac GTPase in Botrytis cinerea reveals impact on polarity, cell cycle and pathogenicity. Anna Minz-Dub1, Leonie Kokkelink1, Paul Tuzdynski1, Amir Sharon2. 1) Department of Plant Sciences, Britannia 536, Tel-Aviv University, Tel-Aviv 69978; 2) Universität zu Köln, Biozentrum, Institut für Botanik, Zülpicher Str. 47 b, 50674 Koeln, Germany; 3) Institut für Biologie und Biotechnologie der Pflanzen, Westfälische Wilhelms-Universität Muenster, Schloßplatz 8, D-48143 Muenster, Germany.

Small GTPases of the Ras superfamily are interconnected with many different signalling pathways. In this study we describe functional characterisation of a Ras-type GTPase BcRac from the necrotrophic plant pathogen Botrytis cinerea. Role of this protein in cell cycle, development and pathogenicity is described. Expression of a constitutively active (CA) version of the BcRac protein, or deletion of the gene had a severe impact on fungal growth and differentiation. The mutant strains have polarity defects, they do not produce conidia, disease symptoms on plants are delayed, and they produce and accumulate increased amounts of ROS in culture. In addition, nuclear content and actin localization were altered in the CA-BcRac strain as compared to wild type. An effect of Rac-specific inhibitor NSC23766 on spore germination of wild type strain indicated that BcRac might be necessary for spore germination during G2/M phase. Based on our observations, BcRac is an important regulator of development in B. cinerea, and alteration of its activity disrupts the morphogenetic program and influences fungal infection.

106. Light matters: The transcription factor LF11 regulates virulence and light responses in the necrotrophic plant pathogen Botrytis cinerea. Julia Schumacher1, Adeline Simon2, Kim Cohrs1, Muriel Viaud1, Paul Tuzdynski1. 1) IBBP, WWU Muenster, Schloßplatz 8, 48143 Muenster, Germany; 2) INRA, BIOGER, Avenue Lucien Brétignières, 78850 Grignon, France.

The lifecycle of Botrytis cinerea/ Botryotinia fuckeliana includes the formation of white mycelia generating pigmented conidiophores with macroconidia for propagation, pigmented sclerotia for over-wintering and sexual reproduction, microconidia for spermatization of the sclerotia, and the formation of aspergoid bodies as spermatized sclerotia. Full-spectrum light induces the differentiation of conidia and apothecia, while sclerotia are exclusively formed during incubation in constant darkness. The relevance of light for virulence of the fungus is not that clear, however, infections are observed under natural illumination conditions as well as in constant darkness. By a T-DNA insertional mutagenesis approach, we identified a novel virulence-related gene encoding a GATA-type transcription factor (TF) with homologues in A. nidulans (NsdD) and N. crassa (SUB-1). As transcription is induced by light (2.5-fold), it was called BcLF11 for ‘Light-regulated TF 1’. By deletion and over-expression of BcLF11, we confirmed the predicted role of the TF in virulence, and discovered furthermore its extraordinary functions in regulating light-dependent differentiation processes (growth defect of BcLF11 in light, loss of sclerotia formation in darkness), the equilibrium between production and scavenging of reactive oxygen species (ROS), and secondary metabolism. Hence, microarray analyses (WT, BcLF11; dark vs. exposure to light for 1h) revealed that the expression levels of 206 out of 313 light-dependent genes are modulated by BcLF11, including the genes of the putative carotenoid gene cluster and six out of eleven genes encoding TF. In addition, the mutation of bcl11 affects the expression of 1,516 genes irrespective of the light conditions, including the over-expression of known and so far uncharacterized secondary metabolism gene clusters. The over-expression of the gene encoding the alternative oxidase (AOX) and the under-expression of genes involved in oxidative stress responses are in accordance with the observed phenotypes of the deletion mutant, i.e. the hypersensitivity to exogenously applied oxidative stress even in the absence of light and the restoration of growth rates in continuous light by offering antioxidants, indicating that BcLF11 is required to cope with oxidative stress that is caused by the exposure to light.


Botrytis cinerea is a heterothallic ascomycete with two mating types, MAT1-1 and MAT1-2, each containing two genes. Besides the archetypal genes encoding the MAT1-1-1 (alpha-domain) protein and the MAT1-2-1 (HMGB-box) protein, each idiomorph contains one additional gene, designated MAT1-1-5 and MAT1-2-4, respectively. Homologs of these genes are only found in closely related taxa, and their function is as yet unknown. Knockout mutants were generated in all four genes in the B. cinerea MAT locus, either in the MAT1-1 strain SAS56 or in the MAT1-2 strain SAS405. Mutants were crossed with a strain of the opposite mating type, either the wild type or a knockout mutant, in all possible combinations. Knockout mutants in the MAT1-1-1 gene and the MAT1-2-1 gene fail to show any sign of primordial outgrowth and are entirely sterile. This confirms the essential role of the alpha-domain protein and the HMGB-box protein in the mating process. By contrast, mutants in the MAT1-1-5 gene and the MAT1-2-4 gene do produce stipes, but these fail to develop further into an apothecial disk. The MAT1-1-5 and MAT1-2-4 mutants show identical phenotypes, suggesting that these two genes jointly control the transition from stipe to disk development. RNA-seq data were obtained from a cross between two wild type strains and from a cross involving a MAT1-1-5 knockout mutant, from tissue at the stage of transition from stipe to disk. Differential gene expression analysis was performed to identify genes that are possibly involved in development of the apothecial disk.
108. The role of hydrophobins in sexual development of Botrytis cinerea. Razak Bin Terhem1, Matthias Hahn1, Jan van Kan1. 1) Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands; 2) Department of Biology, University of Kaiserslautern, Kaiserslautern, Germany.

Hydrophobins are small secreted proteins that play a role in a broad range of developmental processes in filamentous fungi, e.g. in the formation of aerial structures. Hydrophobins allow fungi to escape their asexual environment and confer hydrophobicity to fungal surfaces. In Botrytis cinerea (teleomorph Botryotinia fuckeliana), one class I and two class II hydrophobin genes have been identified, as well as a number of hydrophobin-like genes. Previous studies showed that hydrophobins are not required for concurring surface hydrophobicity to conidia and aerial hyphae. We investigated the role of hydrophobins in sclerotium and apothecium development. RNA-seq analysis of gene expression during different stages of apothecium development revealed high expression of the Bhp1 (class I hydrophobin) gene and of the Bhh1 (hydrophobin-like) gene in certain stages, whereas Bhp2 and Bhp3 (class II hydrophobin) genes were always expressed at very low levels. We characterized different hydrophobin mutants: four single gene knockouts, three double knockouts as well as a triple knockout. Sclerotia of D8hp1/D8hp3 (double knock out) and D8hp1/D8hp2/D8hp3 (triple knock out) mutants showed easily wettable phenotypes. These results indicate that hydrophobins Bhp1 and Bhp3 are important for normal development of sclerota of B. cinerea. For analyzing apothecial development, a reciprocal crossing scheme was set up. Morphological aberrations were observed in crosses with some hydrophobin mutants. When the D8hp1/D8hp2 (double knock out) and D8hp1/D8hp2/D8hp3 (triple knockout) mutants bearing a MAT1-1 mating type were used as maternal parents (sclerotia), and fertilized with microconidia of a wild type MAT1-2 strain, the resulting apothecia were swollen, dark brown in color and had a blotted surface. Instead of growing upwards, the apothecia in some cases fell down. This aberrant apothecial development was not observed in the reciprocal cross, when the same mutants were used as paternal parent (microconidia). These results indicate that the presence of hydrophobins Bhp1 and Bhp2 in maternal tissue is important for normal development of apothecia of B. cinerea.

109. The pescadillo homolog, controlled by Tor, coordinates proliferation and growth in response to Candida albicans yeast. Tahmeena Chowdhury1, Niketa Jan1, Folkert J. Van Werven2, Robert J. Bastidas1, Joseph Heitman1, Julia R. Köhler1. 1) Division of Infectious Diseases, Boston Children’s Hospital/Harvard Medical School, Boston, MA; 2) Institute for Integrative Cancer Research, MIT, Cambridge, MA; 3) Dept. of Genetics and Molecular Biology, Duke University, NC.

Candida albicans has evolved as a colonizer and opportunistic pathogen of mammals. Among fungi infecting humans, it is unique in the frequency with which it switches between growth as budding yeast and growth as pseudohyphal and hyphal filaments. In vitro and presumably in vivo, filamentous constitutively produce yeast from their sub-apical compartments. This developmental step is required for dispersal of planktonic yeast from biofilms. The C. albicans pescadillo homolog PES1 is required for this lateral facet of yeast growth. In eukaryotes, pescadillo homologs are involved in cell cycle progression and ribosome biogenesis, processes that respond to nutrient availability. This work investigated the potential role of C. albicans PES1 in the Tor signaling pathway, which is a major nutrient signaling cascade. Results show that Tor signaling controls PES1 expression and localization. C. albicans yeast but not hyphae require PES1 for proliferation, and for proliferation arrest upon Tor1 inhibition with rapamycin. PES1 inactivation via a temperature-sensitive allele leads to defective exit of starved cells from the cell cycle. Pes1 inactivation also leads to rapid loss of phosphorylation of ribosomal protein S6, a marker of translational activity, as does Tor1 inhibition and genetic perturbation of Tor1 activation. These data support a role for Pes1 downstream of Tor1 in coordinating cell cycle progression with protein synthesis. As all cells must coordinate proliferation and growth, investigating why the requirement for Pes1 in this role is yeast-specific will inform our understanding of morphogenesis and Tor signaling in C. albicans.

110. Uncovering the mechanisms of thermal adaptation in Candida albicans. Michelle Leach1,2, Susan Budge2, Louise Walker2, Carol Munro2, Alistair Brown2, Leah Cowen2. 1) Department of Molecular Genetics, University of Toronto, Medical Sciences Building, 1 Kings College Circle, Toronto, Ontario, Canada, M5S 1A8; 2) Aberdeen Fungal Group, School of Medical Sciences, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen, AB25 2DD, UK.

The heat shock response is governed by one of the most highly conserved networks in eukaryotic cells. Upon sensing a sudden temperature upshift, the heat shock transcription factor (Hsf1) is rapidly phosphorylated and activated, leading to the induction of numerous genes that mediate thermal adaptation, including heat shock genes that encode molecular chaperones. We have shown that the major fungal pathogen of humans, Candida albicans, has retained a bona fide heat shock response even though it is obligatorily associated with warm-blooded animals [Molec. Micro. 74, 844]. Furthermore, this thermal adaptation is essential for the virulence of C. albicans [Fungal Gen. Biol. 48, 297]. To identify signalling pathways that contribute to long-term thermal adaptation resistance in C. albicans we performed unbiased genetic screens for protein kinase mutants that display temperature sensitivity. This screen reproducibly highlighted several key signalling pathways associated with cell wall remodelling: the Hog1, Mkc1 and Cek1 pathways. None of these pathways are essential for Hsf1 phosphorylation and activation; each pathway contributing to heat shock adaptation independently of Hsf1. We demonstrate that these pathways are differentially activated during heat shock, and that there is crosstalk between these pathways, with high temperatures contributing to increased resistance to cell wall stress in the long term, and oxidative stress in the short term. Critically, this crosstalk between thermotolerance and other types of stress adaptation is mediated by the molecular chaperone Hsp90, whose down-regulation reduces the resistance of C. albicans to proteotoxic stresses. Hsp90 depletion also affects cell wall biogenesis by impairing activation of these signalling pathways. Furthermore, we show that Hsp90 interacts with and down-regulates Hsf1 thereby modulating short-term thermal adaptation. Therefore, Hsp90 lies at the heart of heat shock adaptation, modulating the short-term Hsf1-mediated activation of the classic heat shock response, coordinating this response with long term thermal adaptation via Mkc1- Hog1- and Cek1-mediated cell wall remodelling.

111. Characterisation of contact-dependant tip re-orientation in Candida albicans hyphae. Darren Thomson, Silvia Wehmeier, Alex Brand. Aberdeen Fungal Group, Aberdeen University, Aberdeen, United Kingdom.

Candida albicans is a pleomorphic fungus that lives as a commensal yeast in the human body but can become pathogenic in susceptible patient groups. Virulence is strongly linked with the production of penetrative hyphae that can adhere to and invade a wide range of substrates, including blood vessels, organ tissue, keratinised finger-nails and even soft medical plastics. Using live-cell imaging and nanofabricated surfaces, we are characterising the spatio-temporal dynamics of contact-induced hyphal tip behaviour (thigmotropism). To test whether tip re-orientation responses positively correlate with levels of hyphal adhesion, we generated substrates with increasing adhesive force. Hyphal tip re-orientation was absent in poorly-immobilised hyphae and a threshold adhesive force was required sub-apically to generate the hyphal tip pressure required for re-orientation. Interestingly, sub-threshold adhesion resulted in sub-apical hyphal bending. Localization of fluorescent protein markers for the Spitzenkörper and the Polarisome (Mlc1-YFP and Spa2-YFP, respectively) showed that C. albicans hyphal tips grow in an asymmetric, ‘nose-down’ manner on a surface. Additionally, hyphal tips can detect surface stiffness and show a distinct preference for nose-down growth on the softer of two substrates. Localisation of fluorescent cell-cycle reporter proteins over time revealed that hyphal tip contact slowed the cell-cycle, suggesting that tip-contact perturbs cell-cycle mechanics. Finally, we examined the role of cytoskeleton regulators in thigmotropism and determined the force that can be generated by the hyphal tip. Our results suggest that C. albicans hyphae
can exert sufficient force to penetrate human epithelial tissue without the need for secreted enzyme activity. This is consistent with the observed hyphal penetration of medical-grade silicone, which has a similar Young’s modulus to human cartilage.

112. Cdc14 association with basal bodies in the oomycete Phytophthora infestans indicates potential new role for this protein phosphatase. Audrey M.V. Ah-Fong, Howard S. Judelson. Plant Pathology & Microbiology, University of California, Riverside, CA.

The dual-specificity phosphatase Cdc14 is best known as a regulator of cell cycle events such as mitosis and cytokinesis in yeast and animal cells. However, the diversity of processes affected by Cdc14 in different eukaryotes raises the question of whether its cell cycle functions are truly conserved between species. Analyzing Cdc14 in Phytophthora infestans should provide further insight into the role of Cdc14 since this organism does not exhibit a classical cell cycle. Prior study in this organism already revealed novel features of its Cdc14. For example, instead of being post-translationally regulated like its fungal and metazoan relatives, PiCdc14 appears to be mainly under transcriptional control. It is absent in vegetative hyphae where mitosis occurs and expressed only during the spore stages of the life cycle which are mitotically quiescent, in contrast to other systems where it is expressed constitutively. Since transformants overexpressing PiCdc14 exhibit normal nuclear behavior, the protein likely does not play a critical role in mitotic progression although PiCdc14 is known to complement a yeast Cdc14 mutation that normally arrests mitosis. Further investigation into the role of PiCdc14 uncovered a novel role. Subcellular localization studies based on fusions with fluorescent tags showed that PiCdc14 first appeared in nuclei during early sporulation. During the development of biflagellated zoospores from sporangia, PiCdc14 transits to basal bodies, which are the sites from which flagella develop. A connection between Cdc14 and flagella is also supported by their phylogenetic distribution, suggesting an ancestral role of Cdc14 in basal bodies and/or flagellated cells. To help unravel the link between PiCdc14 and the flagella apparatus, searches for its interacting partners using both yeast two hybrid and affinity purification are underway. Together with colocalization studies involving known basal body/centrosome markers such as cenitin and gamma-tubulin, the location and hence the likely roles of PiCdc14 will be revealed.

113. Colletotrichum orbiculare Bub2-Bfa1 complex, a spindle position checkpoint (SPOC) component in Saccharomyces cerevisiae, is involved in proper progression of cell cycle. Fumi Fukada, Ayumu Sakaguchi, Yasuyuki Kudo. 1) Laboratory of Plant Pathology, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan; 2) National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8602, Japan.

Colletotrichum orbiculare is an ascomycete fungus that causes anthracnose of cucumber. In Saccharomyces cerevisiae, the orientation of the mitotic spindle with respect to the polarity axis is crucial for the accuracy of asymmetric cell division. A surveillance mechanism named spindle position checkpoint (SPOC) prevents exit from mitosis when the mitotic spindle fails to align along the mother-daughter polarity axis. BUB2 is a component of SPOC and constitutes the main switch for the mitotic exit network (MEN) signaling. We identified and named this homolog as CoBUB2 in C. orbiculare and generated gene knock-out mutants. First, we observed morphogenesis and pathogenesis of the cobub2 mutants. The cobub2 mutants formed abnormal appressoria and penetration hyphae on model substrates, and the cobub2 mutants also showed attenuated pathogenesis to cucumber leaves. Second, we observed mitosis based on mitotic spindle behavior and nuclear DAPI staining during appressorium development. In the wild type, mitosis occurred in appressorium developing conidia after 4h incubation, whereas interestingly, in the cobub2 mutants, mitosis occurred in pre-germinated conidia after 2h incubation. After development of appressorium, in some germlings the daughter nucleus was delivered from conidia to appressoria, and the others perform second round of mitosis in appressorium developing conidia after 4h incubation. Third, we evaluated the timing of S phase and M phase during appressorium development in wild type and the cobub2 mutants by cell cycle specific inhibitors. In the cobub2 mutants, it was shown that the transition period from G1 phase to S phase accelerated about 2h than that of the wild type. Last, in S. cerevisiae, Bub2 forms GTPase activating protein (GAP) complex with Bfa1, and Bub2-Bfa1 GAP complex constitutes SPOC. Then we named homolog of BFA1 as CoBFA1 in C. orbiculare and generated cobfa1 mutants. From observation of nuclear division, the cobfa1 mutants showed similar behavior of nuclear division to the cobub2 mutants. Therefore, it is assumed that CoBub2 forms GAP complex with CoBfa1, however, CoBub2-CoBfa1 GAP complex has different function from that in S. cerevisiae maintaining G1 phase duration or setting up the proper time of S phase.

114. Metazoan-like mitotic events in the basidiomycetous budding yeast Cryptococcus neoformans - a human fungal pathogen. L. Kozubowski1,2, V. Yadav5, G. Chatterjee1, M. Yamaguchi1, I. Bose2, J. Heitman3, K. Sanyal1. 1) Department of Medicine, Division of Infectious Diseases, Duke University Medical Center, Durham, NC, USA; 2) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA; 3) Molecular Mycology Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India; 4) Department of Biology, Western Carolina University, Cullowhee, NC, USA; 5) Medical Mycology Research Center, Chiba University, Chiba, Japan.

Mitosis in ascomycetous budding yeasts is characterized by several features that are distinct from those of metazoans. In Saccharomyces cerevisiae, centromeres are always clustered in a single spot, the kinetochores are fully assembled for the majority of the cell cycle, and the nuclear envelope (NE) does not break down (closed mitosis). Currently it is not clear how these mechanisms evolved or whether these features are a universal characteristic hallmark of the budding mode of cellular division. Here we report an analysis of key mitotic events in the basidiomycetous human fungal pathogen Cryptococcus neoformans. The dynamics of microtubules, the kinetochore, NE and the nucleolus were analyzed by time-lapse microscopy using fluorescently tagged proteins. In striking contrast to ascomycetous budding yeast, centromeres in C. neoformans were not clustered in non-dividing cells. Prior to mitosis, centromeres underwent gradual clustering, eventually forming a single spot, which then migrated into the daughter cell where the chromosomal division occurred. One set of chromosomes migrated back to the mother cell and subsequent de-clustering of centromeres occurred in both cells. Analysis of individual components of the kinetochore indicated that kinetochores assemble in a step-wise manner in C. neoformans. While the inner kinetochore (Cse4, Mif2) was present throughout the entire cell cycle, the middle kinetochore (Mt1) assembled prior to mitosis when centromeres underwent clustering, and this was then followed by assembly of the outer kinetochore (Dad1, Dad2). Formation of the outer kinetochore during mitosis, as observed in metazoans that undergo an open mitosis, prompted us to examine the fate of the NE at various cell cycle stages. Several lines of evidence suggested that C. neoformans undergoes a semi-open mitosis. The nuclear pore marker GFP-Nup107, and a nucleolar marker GFP-No1 dispersed into the cytoplasm during metaphase, a nuclear membrane marker Ndc1 exhibited a localization pattern that also suggests a partial opening of the NE during mitosis. A semi-open mitosis was further confirmed by transmission electron microscopy. In summary, our data demonstrate that key mitotic events in C. neoformans are similar to that of metazoan cells. This study sheds new light on the evolution of mitosis during fungal speciation.

115. Distinctive Mitotic Localization of a Novel Suppressor of nimA1 Provides New Insight into NIMA Function. Jennifer R. Larson, Stephen A. Osmani. Department of Molecular Genetics, The Ohio State University, Columbus, OH.

The NIMA kinase is an essential regulator of mitotic events in Aspergillus nidulans. Not only is NIMA essential for initiating mitosis its overexpression can prematurely induce mitotic events including DNA condensation and nuclear pore complex (NPC) disassembly in A. nidulans and human cells. One of the key roles for NIMA at the onset of mitosis is its regulation of NPCs. A previous study aimed at identifying suppressors of the temperature-sensitive nimA1
allele isolated two NPC proteins, which were named SONA and SONB for Suppressors of Nima1. Although NIMA is essential for mitotic entry there is also evidence that NIMA and conserved related kinases have functions later in mitosis and in the DNA damage response. To further characterize the roles of NIMA we designed a genetic screen to isolate additional suppressors of nima1 that also cause conditional temperature-dependent DNA damage sensitivity. Our expectation was the identification of additional genes involved in NIMA regulation and in the DNA damage response. Here we describe one such gene, which we have named sonC. SonC contains a unique Zn(II)Cys6 binuclear DNA binding domain, which is highly conserved among the Ascomycota. Deletion of sonC results in swollen, ungerminated spores, suggesting it is essential for a core growth process. As expected for a DNA binding protein, SonC localizes to nuclei during interphase. Interestingly, dual fluorescence imaging of SonC with histone H1 during mitosis revealed that a portion of SonC localizes with histone H1 along a distinct projection of chromatin that juts away from the main, condensed chromatin mass, which we hypothesize may be the NOR. Supporting this hypothesis, the region of DNA that likely forms the projection is cradled by the nucleolus prior to mitosis as seen by colocalization studies of SonC with the nucleolar protein Bop1. As mitosis proceeds, the H1 histones are evicted from the middle region of this projection but not at its distal end. This indicates that the chromatin in this region of the genome is altered during mitotic progression and we are testing the idea that SonC might be important for NOR condensation and/or nucleolar disassembly during its mitotic segregation. Because SonC was identified as a suppressor of NIMA we propose that NIMA may have a function in regulating nucleolar disassembly during mitosis.


The rice blast fungus Magnaporthe oryzae elaborates specialized infection structures called appressoria to gain entry into rice plant tissue. The initiation of appressorium morphogenesis has previously been shown to require a single round of mitosis in the germ tube, shortly after spore germination. On daughter nucleus migrates to the incipient appressorium at the germ tube tip and the other daughter nucleus moves back to the conidial cell from which the germ tube originates. We reasoned that an S-phase checkpoint mediates the apical-isotropic switch leading to swelling of the germ tube tip. Perturbation of DNA synthesis by hydroxyurea (HU) blocks the initiation of appressorium formation, but only when applied within 3-4h of spore germination, prior to S-phase. Here, we report investigations regarding the interplay between cell cycle control and operation of the Pmk1 Mitogen-activated protein kinase cascade, which is essential for appressorium morphogenesis in M. oryzae. Furthermore we report changes in the global pattern of gene expression of HU-treated conidia which has been carried out in order to determine the identity of morphogenetic genes that are controlled by the S-phase checkpoint. Progress on understanding the genetic control of early appressorium development will be presented.

117. THE ROLE AND TRAFFIC OF CHITIN SYNTHASES IN Neurospora crassa. R. Fajardo1, R. Roberson2, B. Jöhnk1, Ô. Byram3, G.H. Braus4, M. Riquelme4. 1) Department of Microbiology, CICESE, Ensenada, Mexico; 2) School of Life Sciences, Arizona State University, Arizona, USA; 3) Molecular Microbiology and Genetics, Georg-August University, Göttingen, Germany.

Chitin is one of the most important carbohydrates in the cell wall in filamentous fungi. Chitin synthases (CHS) are involved in the addition of N-acetylglucosamine monomers to form chitin microfilibrils. The filamentous fungus Neurospora crassa has one representative for each of the seven CHS classes described. Previous studies have shown that in N. crassa, CHS-1, CHS-3 and CHS-6, are concentrated at the core of the Spizizenkörper and in forming septa and seem to be transported in different populations of chitosomes. In this study we have endogenously tagged chs-2, chs-4, chs-5 and chs-7 with GFP to study their distribution in living hyphae of N. crassa. CHS-5 and CHS-7 both have a myosin motor-like domain at their amino termini, suggesting that they interact with the actin cytoskeleton. CHS-2 and CHS-7, appeared solely involved in septum formation. As the septum ring developed, CHS-2-GFP moved centripetally until it localized exclusively around the septal pore. CHS-4 and CHS-5 were localized both at nascent septa and in the core of the Spk. We observed a partial colocalization of CHS-1-mCherry and CHS-5-GFP in the Spk. Total internal reflection fluorescence microscopy (TIRFM) analysis revealed putative chitosomes containing CHS-5-GFP moving along wavy tracks, presumably actin cables. Collectively our results suggest that there are different populations of chitosomes, each containing a class of CHS. Mutants with single gene deletions of chs-1, chs-3, chs-5, chs-6, or chs-7 grew slightly slower than the control strain (FGSC#9718 and FGSC#988); only chs-60 displayed a marked reduction in growth. Both chs-3D and chs-7D strains produced less aerial hyphae and conidia. The double mutant chs-5D; chs-7D showed less growth, aerial hyphae production and conidiation than the single mutant chs-5D, but not than the chs-7D single mutant. A synergistic effect was observed in double mutant chs-1D; chs-3D, in which growth, aerial hyphae production and conidiation were significantly reduced. During the sexual cycle, after homoygous crosses, chs-3D and chs-7D strains did not produce perithecia and chs-5D produced less perithecia. We are analyzing chitin and glucan synthase activities in these single and double mutants. Additionally, we are conducting pulldown assays, and mass spectrometry to identify putative proteins that are interacting with CHS.

118. DFG5 and DCW1 cross-link Cell Wall Proteins into the Cell Wall Matrix. Abhiram Maddi, Jie Ao, Stephen J. Free. Dept Biological Sci, SUNY Univ, Buffalo, Buffalo, NY.

The cell wall is an essential organelle for the growth and survival of a fungus. The cell wall structure consists of matrix of cross-linked chitin, glucans, and cell wall glycoproteins. In Neurospora crassa, we have shown that the DFG5 and DCW1 proteins function in cross-linking the cell wall proteins into the cell wall matrix. We have also shown that the Candida albicans DFG5 and DCW1 proteins are required for the cross-linking of cell wall proteins into the cell wall. The DFG5 and DCW1 proteins are predicted to have a-1,6-mannanase activity. Our results suggest that they function in transglycosylation reactions between a-1,6- and a-1,3-mannans, which are found in galactomannan and the outer chain mannans present as modifications on cell wall proteins, and cell wall glucans. These galactomannans and outer chain mannans are modifications to the N-linked oligosaccharides attached to cell wall glycoproteins. As a result of these transglycosylation reactions, the cell wall proteins are effectively cross-linked into the cell wall. The DFG5 and DCW1 enzymes are excellent targets for the development of anti-fungal agents that could disrupt cell wall biosynthesis.


The cell wall is a dynamic extracellular compartment protecting the cell, providing rigidity, and playing an essential role in the uptake of molecules and signalling. In pathogenic organisms, the cell wall is at the forefront of disease, providing contact between the pathogen and host. Using a multidisciplinary approach, we seek to understand the role of the cell wall in oomycete disease, both as a communication centre with the host organism and as a compartment that is continually reshaped and strengthened throughout the lifecycle, to penetrate and colonise the host. Understanding these mechanisms in more detail will pave the way for better control of oomycete diseases. We are combining novel chemical genomics approaches with state-of-the-art biochemistry and biophysics to study the cell wall and to develop new anti-oomycete drugs. P. infestans produces a variety of spores and infection structures that are essential for disease development throughout its lifecycle. In particular thick-walled sporangia release wall-less motile zoospores that rapidly synapse a cell wall upon contact with host plant cells. These cysts further differentiates to produce appressoria which build up
turgor pressure and act as a focal point for cell wall degrading enzymes to penetrate the host cell. A highly strengthened cell wall is thus essential for the onset of infection. Here we present the results of our detailed biochemical analyses, using GC-MS and methylation analysis to determine the neutral sugar composition and glycosidic linkages of the cell wall structural carbohydrates present at these key points in the lifecycle. Having previously established an essential role for a cellulosic cell wall in appressorium production and infection of potato by P. infestans (Grenville-Briggs et al 2008), we are now working to elucidate the precise functions of the individual cellulose synthase (CesA) genes. Silencing each CesA using RNAi reveals overlapping functions with subtle differences in phenotype. These results will be presented. Since the genome of P. infestans also contains a putative chitin synthase, but hyphal cell walls are devoid of measurable chitin we are also investigating the role of this gene in the P. infestans cell wall and in pathogenicity and here we present the latest findings of this work.

120. Analysis of the cell wall integrity (CWI) pathway in Ashbya gossypii. Klaus B Lengeler, Lisa Wasserström, Andrea Walther, Jürgen Wendland. Carlsberg Laboratory, Yeast Biology, DK-1799 Copenhagen V, Denmark.

Fungal cells are constantly exposed to rapidly changing environmental conditions, in particular considering their osmotic potential. The cell wall takes on an important function in protecting the fungal cell from external stresses and controlling intracellular osmolarity, but it is also required to maintain regular cell shape. At the same time, cells must still be able to remodel the rigid structure of the cell wall to guarantee cell expansion during cell differentiation processes. While several signaling pathways contribute to the maintenance of the cell wall, it is the cell wall integrity (CWI) pathway that is most important in regulating the remodeling of the cell wall structure during vegetative growth, morphogenesis or in response to external stresses. To characterize the CWI pathway in the filamentous ascomycete Ashbya gossypii we generated deletion mutants of several genes encoding for the most important components of the CWI pathway including potential cell surface sensors (e.g. AgWSCI), the following downstream protein kinases including a MAPK signaling module (AgPKC1, AgBK1, AgMK1 and AgMPK1), and transcription factors known to be involved in CWI signaling (e.g. AgRML1). An initial characterization of the corresponding mutants is presented. While a mutant in Agpkc1 shows a strong general growth defect, mutants in several other components of the CWI pathway, in particular in the MAPK module, show a noticeable colony lysis phenotype. Finally, we show that the colony lysis phenotype may be useful to easily isolate recombinant proteins from A. gossypii.


Morphogenesis in filamentous fungi depends principally on the establishment and maintenance of polarized growth. This is accomplished by the orderly migration and discharge of exocytic vesicles carrying cell wall components. We have been searching for evidence that endocytosis, an opposite process, could also play a role in morphogenesis. Previously, we found that coronin deletion (Neurospora crassa mutant, Dcrn-1) causes a decrease in endocytosis (measured by the rate of uptake of FM4-64) together with marked alterations in normal hyphal growth and morphogenesis accompanied by irregularities in cell wall structure. The absence of coronin destabilizes the cytoskeleton and leads to interspersed periods of polarized and isotropic growth of the hyphae. We used CRIB fused to GFP as an exocytic reporter of activated Cdc-42 and Rac-1. By confocal microscopy, we found that CRIB-GFP was present in wild-type hyphae as a thin hemispherical cap under the apical dome, i.e. when growing in a polarized fashion and with regular hypohid morphology. In the Dcrn-1 mutant, the location of CRIB-GFP shifted between the periods of polarized and isotropic growth, it migrated to the subapical region and appeared as localized patches. Significantly, cell growth occurred in the places where the CRIB-GFP reporter accumulated, thus the erratic location of the reporter in the Dcrn-1 mutant correlated with the morphological irregularity of the hyphae. We found that the Dcrn-1 mutant had a higher proportion of chitin than the WT strain (14.1% and 9.1% respectively). We also compared the relative cell wall area (TEM images) and we found a different ratio wall/cytoplasm between the Dcrn-1 mutant and the WT strain. In conclusion, we have found that the mutant affected in endocytosis has an altered pattern of exocytosis as evidenced by its distorted morphology and displaced exocytic markers. A direct cause-effect relationship between endocytosis and exocytosis remains to be established.

122. Comprehensive genome-based analysis of cell wall biosynthesis in the filamentous phytopathogen Ashbya gossypii. R. Capaul1, M. Finlayson1, S. Voegeli1, A. I. Martinez2, Q. Y. Yin3, C. de Koster3, F. M. Klis4, P. Philippse4, P. W. J. de Groot1. 1) Biozentrum, Molecular Microbiology, University of Basel, Klingelbergstr. 50-70, CH 4056 Basel, Switzerland; 2) Regional Center for Biomedical Research, Albacete Science & Technology Park, University of Castilla-La Mancha, Spain; 3) Swarmendam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands.

The filamentous ascomycete Ashbya gossypii and the yeast Saccharomyces cerevisiae are phylogenetically closely related. It is well established that the fungus A. gossypii contains very little chitin and orthologs of genes required for cell wall remodeling and degradation of septa during hyphal growth can restart in the differentiated state. We found that (HO) and results in growth arrest, adhesion, aerial hyphae growth and conidia development [1]. Each transition is started by an up-regulation of the putative chitin synthase CesA genes. Silencing each CesA using RNAi reveals overlapping functions with subtle differences in phenotype. These results will be presented. Since the genome of P. infestans also contains a putative chitin synthase, but hyphal cell walls are devoid of measurable chitin we are also investigating the role of this gene in the P. infestans cell wall and in pathogenicity and here we present the latest findings of this work.


In N. crassa, conidiation is started when an aerated liquid culture is filtered and the resulting mycelial mat is exposed to air. Three morphogenetic transitions take place: hyphae adhesion, aerial hyphae growth and conidia development [1]. Each transition is started by an unstable hyperosmotic state (HO) and results in growth arrest, autophagy, antioxidant response and an insulation process from dioxygen [2,3]. These responses stabilize the system and growth can restart in the differentiated state. We found that ras-1 mutation has increased ROS formation during conidiation resulting in increased aerial mycelium growth and increased submerged conidiation. Different ras-1 point mutations were generated that affected growth and conidiation. Only three proteins have a predicted RAS association domain: NRC-1, the STE50p orthologue (STE50) and adenylate cyclase (AC). The Dncr-1 was more resistant whereas the Dste50 more sensitive to added H2O2. The AC mutant strain cr-1 affects vegetative growth and aerial hyphae formation. Oxidative stress and RAS-1 determined partially CAMP levels during the first two HOs of the conidiation process. Higher CAMP levels than WT were observed in ras-1. In both strains, cAMP decreased within minutes at the start of the first two HOs and thereafter, as rapidly, levels recover to initial values. N. crassa has a high

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(PDEs) and a low affinity (PDEL) phosphodiesterases. The Dpde5 strain grows slowly and does not conidiate; no evident phenotype was reported for Dpde6. We found that PDE6 was mainly responsible for the cAMP decrease during the first HO and that hyphal adhesion was retarded in Dpde6. Both PDEs, and PDE, were responsible for the cAMP decrease during the second HO. H2O2 and low Ca+ activated PDE, and inhibited PDEs. This opposite regulation can explain the cAMP decrease during the HOs of the N. crassa conidiation process. [1] Toledo I et al. (1986) Aerial growth in Neurospora crassa: characterization of an experimental model system. Exp Mycol. 10:114-125. [2] Hansberg W; Aguirre J (1990) Hyperoxidant states cause microbial cell differentiation by cell isolation from dioxygen. J Theoret Biol 142:201-221. [3] Hansberg W et al. (2008) Cell differentiation as a response to oxidative stress. In: Stress in Yeasts & filamentous fungi. (Ed. Avery et al.) Elsevier ISBN 978-0-12-374184-4.

124. Ste12 is a negative regulator of conidiation and cell wall lytic enzymes production in response to nitrogen deprivation and light in Trichoderma atroviride. Maria Fernanda Nieto-Jacobs1, Alfredo Herrera-Estrella2, Alison Stewart1, Artemio Mendoza-Mendoza1. 1) Bioprotection Research Centre, Lincoln University, Lincoln, Canterbury, New Zealand; 2) Laboratorio Nacional de Genómica para la Biodiversidad, Centro de Investigación y de Estudios Avanzados del IPN Sede Irapuato, Irapuato 36821, Guanajuato, Mexico.

Ste12 is a transcription factor found exclusively in the fungal kingdom. In Saccharomyces cerevisiae, Ste12 regulates mating and invasive/pseudohyphal growth, while in saprophytic and parasitic filamentous fungi Ste12-like proteins control mating, plant penetration and invasive growth. Ste12 and Ste12-like proteins are downstream components of the MAPK PMK1 pathway which are capable of regulating several genes encoding fungal virulence factors involved in both plant and animal infection. Among the virulence factors are diverse range of lytic enzymes and cell surface components. Several members Trichoderma genus are mycoparasites of plant fungal pathogens; so they are widely used as biocontrol agents. In addition, Trichoderma spp. penetrate plant roots and establish beneficial relationships with their host. One crucial element in biocontrol activity and root colonization of Trichoderma is the synthesis of lytic enzymes. Several lytic enzymes in Trichoderma are regulated by nitrogen metabolite repression. Here we observed that the ste12-like transcription factor gene is highly up-regulated when Trichoderma is grown on nitrogen depleted medium. To find the role of ste12 in Trichoderma, a ste12-like orthologue gene was deleted in T. atroviride and the effects on fungal development and response to different biotic and abiotic stimuli evaluated. Our results demonstrate that growth and conidiation of a T. atroviride Ste12-like mutant was only slightly altered in complete media. We evaluated the ability of the Dste12-like mutant to use a variety of nitrogen sources using Biolog microtiter plates. We noticed that when essential amino acids are used as the sole nitrogen source, the deletion mutant grew faster than the wild type, however this situation did not occur when the same amino acids were used as the sole carbon source. In addition, induction of conidiation in response to light or mechanical injury was stronger in the Dste12-like mutants than in the wild type but only when a secondary nitrogen source was used in the medium. Finally we observed that some lytic enzymes are differently produced between the wild type and Dste12-like mutants under nitrogen deprivation conditions. We propose that the T. atroviride Ste12-like orthologue regulates lytic enzymes and conidiation by a mechanism that involves nitrogen catabolite repression.


Genome instability and mutations provoked by transposon movement are counteracted by novel defense mechanisms in organisms as diverse as fungi, plants, and mammals. In the human fungal pathogen Cryptococcus neoformans we have previously characterized an RNAi silencing pathway that defends the genome against mobile elements and artificially introduced repeats of homologous DNA. Repetitive transgenes and transposons are silenced by an RNAi-dependent pathway during sexual development (sex-induced silencing, SIS) and during vegetative mitotic growth (MIS). RNAi silencing pathways are conserved in the Cryptococcus pathogenic species complex and are mediated by core RNAi components, including an RNA-dependent RNA polymerase (Rdp1), Argonaute (Ago1) and Dicer (Dcr1 and Dcr2). Surprisingly, all of the canonical known RNAi components are missing from all C. gattii VG1 strains, the molecular type responsible for the North American Pacific Northwest outbreak. To identify novel components of the RNAi pathway, we surveyed the genome of the C. gattii R265 isolate for missing genes. One of the most interesting is ZNF3. In previous studies we found that Znf3, a protein with three zinc finger domains, is required for opposite- and same-sex mating in C. neoformans var. neoformans. Surprisingly, in C. neoformans var. grubii ZNF3 is not required for sexual development. However, it is required for mitotic- and sex-induced silencing via RNAi. SIS is less efficient in znf3D unilateral matings and is abolished in znf3D x znf3D bilateral matings, similar to the phenotypes of rdp1D mutants. Znf3 is also required for transgene-induced mitotic silencing; znf3D mutations abrogate silencing of repetitive transgenes during vegetative growth. Znf3 tagged with mCherry is localized in the cytoplasm in bright, distinct foci. Co-localization of Znf3 with the P-body marker Dcp1-GFP further supports the hypothesis that Znf3 is a novel element of the RNAi pathway and operates to defend the genome during sexual development and vegetative growth.

126. The Crz1/Spl1 transcription factor of Cryptococcus neoformans is activated by calcineurin and regulates cell wall integrity. Sophie Levy1, Desmarini Desmarini1, Methee Chayakulkeeree1, Tania Sorrell1, Julienne Djordjevic. 1) Centre for Infectious Diseases and Microbiology, Sydney Medical School and Westmead Millennium Institute, University of Sydney, Westmead 2145 NSW, Australia; 2) Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Cryptococcus neoformans survives host temperature and regulates cell wall integrity via a calcium-dependent phosphatase, calcineurin. However, downstream effectors of C. neoformans calcineurin are largely unknown. In S. cerevisiae and other fungal species, a calcineurin-dependent transcription factor Crz1 translocates to nuclei upon activation and triggers expression of target genes. We now show that the C. neoformans Crz1 ortholog (Crz1/Spl1), previously identified as a protein kinase C target during starvation, is a bona fide target of calcineurin under non-starvation conditions, during cell wall stress and growth at high temperature. Both the calcineurin-defective mutant, DcnA1, and a CRZ1/SPL1 mutant (Dcrz1) were susceptible to cell wall perturbing agents. Furthermore, expression of the chitin synthase encoding gene, CHS6, was reduced in both mutants. We tracked the subcellular localization of Crz1-GFP in WT C. neoformans and DcnA1 in response to different stimuli, in the presence and absence of the calcineurin inhibitor, FK506. Exposure to elevated temperature (30-37°C vs 25°C) and extracellular calcium caused calcineurin-dependent nuclear accumulation of Crz1-GFP. Unexpectedly, 1M salt and heat shock triggered calcineurin-independent Crz1-GFP sequestration within cytosolic and nuclear puncta. To our knowledge, punctate cytosolic distribution, as opposed to nuclear targeting, is a unique feature of C. neoformans Crz1. We conclude that Crz1 is selectively activated by calcium/calcineurin-dependent and independent signals depending on the environmental conditions.

127. A Fungal Adhesin Guides Community Behaviors by Autoinduction and Paracrine Signaling. Linqi Wang, Xunyun Tian, Rachana Gyawali, Xiaorong Lin. Biology, Texas A&M University, College Station, TX.

Microbes live mostly in a social community rather than in a planktonic state. Such communities have complex spatiotemporal patterns that require intercellular communication to coordinate gene expression. Here, we demonstrate that Cryptococcus neoformans, a model eukaryotic pathogen, responds to an extracellular signal in constructing its colony morphology. The signal that directs this community behavior is not a molecule of low molecular weight.
like pheromones or quorum sensing molecules, but a secreted protein. We successfully identified this protein as the conserved adhesin Cfl1 in the extracellular matrix. The released Cfl1 acts as an auto-induction signal to stimulate neighboring cells to phenocopy Cfl1-expressing cells. We propose that such adhesin/matrix-initiated communication system exists in divergent microbes and our work represents the first adhesin/matrix-mediated signaling mechanism in simple eukaryotes.


As is common in the ascomycetes, the Neurospora crassa life cycle has both asexual and sexual developmental phases. Sexual development in N. crassa is characterized by the formation of a protoperithecium, fertilization, and the maturation of the protoperithecium to form a perithecium. In a screening experiment, we identified over 600 isolates from the N. crassa single gene deletion library that are unable to complete sexual development. Many of these are affected in the process of anastomosis, and we have previously reported on these mutants. We now report on the other female development defective mutants identified in our screening experiments. Co-segregation and complementation experiments were carried out on these mutants and we identified 80 genes that are required for female development (in addition to the 24 genes needed for anastomosis). We find that these genes fall into 5 general classes: 1) signal transduction pathway genes (25 genes), 2) transcription factor genes (7 genes), 3) chromatin remodeling genes (17 genes), 4) genes required for autophagy (11 genes), and 5) miscellaneous genes (20 genes). The PacC pathway genes are among the identified signal transduction pathway genes needed for female development. The activation of the PacC signal transduction pathway is a key signaling event in sexual development. Our experiments also suggest that autophagy and anastomosis are important for the movement of nutrients from the hyphal tissues supporting the developing perithecium.

129. Aspergillus flavus MAP kinase AflmpkB positively regulates developmental process but not aflatoxin production. Sang-Cheol Jun1, Dong-Soon Oh1, Jong-Hwa Kim1, Kwang-Yeop Jahn1, Kap-Hoon Han1. 1) Dept. of Pharmaceutical Engineering, Woosuk Univ, Wanju, Korea; 2) Div. of Biological Sciences, Chonbuk National University, Jeonju, Korea.

Developmental process of eukaryotes is controlled by the multiple regulatory systems including signal transduction pathways and transcription factors. One of the central signaling mechanisms includes mitogen-activated protein kinase (MAPK) pathway that transfer extracellular signals into nucleus, generating cellular responses. Previously, we have showed that Aspergillus nidulans MpkB, the yeast Fus3 MAP kinase ortholog, regulates sexual development and secondary metabolism. Here, we identified and characterized the ortholog of the A. nidulans mpkB gene in Aspergillus flavus, AflmpkB, to understand whether the AflmpkB gene has conserved function with A. nidulans mpkB. Deletion of AflmpkB did not affect hyphal growth but showed reduced conidia production. Furthermore, AflmpkB null strain didn't produce any sclerotia while WT and recipient strain produced a lot of sclerotia in normal conditions. However, loss of AflmpkB resulted in normal aflatoxin biosynthesis, suggesting that the major function of AflmpkB is positive regulation of conidiation, sclerotia development but not mycotoxin production. These results indicate that A. nidulans and A. flavus MpkBs have conserved and divergent roles in development and secondary metabolism.

130. Subcellular localization and kinase activity of GK4, a Phytophthora infestans GPCR-PIPK involved in actin cytoskeleton organisation. Chenlei Hu1, Harold Meijer1, Kiki Kots2, Tijs Ketelaar3, Francine Govers1. 1) Laboratoires de Phytopathologie, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; 2) Laboratories of Cell Biology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

For dispersal and host infection plant pathogens largely depend on actin cytoskeleton. Disassembly and disruption of the actin cytoskeleton is required for motility. Moreover, actin-based motility is required for cell-to-cell signaling. In P. infestans, we identified over 600 isolates from the N. crassa single gene deletion experiment, we identified over 600 isolates from the N. crassa single gene deletion library that are unable to complete sexual development. Many of these are affected in the process of anastomosis, and we have previously reported on these mutants. We now report on the other female development defective mutants identified in our screening experiments. Co-segregation and complementation experiments were carried out on these mutants and we identified 80 genes that are required for female development (in addition to the 24 genes needed for anastomosis). We find that these genes fall into 5 general classes: 1) signal transduction pathway genes (25 genes), 2) transcription factor genes (7 genes), 3) chromatin remodeling genes (17 genes), 4) genes required for autophagy (11 genes), and 5) miscellaneous genes (20 genes). The PacC pathway genes are among the identified signal transduction pathway genes needed for female development. The activation of the PacC signal transduction pathway is a key signaling event in sexual development. Our experiments also suggest that autophagy and anastomosis are important for the movement of nutrients from the hyphal tissues supporting the developing perithecium.

131. External calcium ions and deletion of per-1 gene suppressed the abnormal morphology of och-1 and frost mutants in Neurospora crassa. Masayuki Kamei, Yoko Tsukagoshi, Shinpei Banno, Masakazu Takahashi, Akihiro Ichishi, Makoto Fujimura. Faculty of Life Sciences, Toyo University, ORA-GUN, GUNMA, Japan.

Calcium ions play important roles in the growth and development in filamentous fungi. The frost mutant show slow growth and hyperbranching phenotypes that can be corrected by Ca2+ addition in Neurospora crassa. The frost gene is an ortholog of S. cerevisiae cdc1 which encodes positive Mnt-dependent lipid phosphatase. We found that the abnormal morphology of the och-1 mutant was quite similar to that of the frost mutant and its abnormality was also corrected by external Ca2+. The och-1 gene encodes an alpha-1,6-mannosyltransferase that is probably involved in sugar processing for GPI-anchor proteins. In yeast, the mutation of per1 gene, encoding a protein required for GPI remodeling pathway, suppresses the abnormal growth phenotype of cdc1 mutant. To examine the effect of per1 gene, an ortholog of per1 in S. cerevisiae, on the phenotypes of the frost and och-1 mutants, we isolated two double mutants, frost; Dper-1 and och-1; Dper-1. Although per1 gene disruptant showed the normal growth phenotype, per2 gene deletion suppressed the slow growth and hyperbranching phenotypes of frost and also och-1 mutants. Addition of Ca2+ did not affect the growth and morphology of the two double mutants. These results suggest the connection between FROST and OCH-1 may participate in lipid remodeling or calcium signaling in Neurospora crassa.

132. Functional analysis of carbonic anhydrases from the filamentous ascomycete Sordaria macrospora. Ronny Lehneck1, Piotr Neumann2, Achim Dickmanns3, Ralf Ficner1, Stefanie Pöggeler1. 1) Institute of Microbiology and Genetics, Department of Genetics of Eukaryotic Microorganisms, Georg-August-University Göttingen; 2) Institute of Microbiology and Genetics, Department of Molecular Structural Biology, Georg-August-University Göttingen.

Carbonic anhydrases (CA) are widely distributed enzymes, which catalyses the reversible hydration of carbon dioxide to bicarbonate and protons. Based on their amino acid sequence and structure, they can be divided into five distinct groups (a, b, d, x) which share no sequence similarity and have supposable evolved independently. All known fungal CAs belong either to the a-class or to the b-class. Our model organism Sordaria macrospora encodes at least four carbonic anhydrases: three of the b-type, termed cas1, cas2 and cas3 (carbonic anhydrase of Sordaria) and one a-type, termed cas4. Previously, the functions of CAS1, CAS2 and CAS3 have been intensively studied (Elleuche and Pöggeler 2009) and displayed an involvement in fruiting-
body development and ascospore germination. Here, we present a functional characterization of the secreted a-CA CAS4. CAS4 seems to be involved in ammonium metabolism but not in ascospore germination. The Dcas4 mutant displayed a slightly reduced vegetative growth rate and a delayed fruiting-body development. Based on real time PCR analysis cas4 is upregulated during the sexual development. Moreover, we present the phenotype of a quadruple mutant without any CAS genes. The complete CAS deletion strain (Dcas12/3/4) is able to grow under ambient air but the vegetative growth rate is drastically reduced and the mutant is only able to form thin hyphae. The mutant is even under elevated CO2 levels (5 %) not able to form fruiting bodies. Heterologous expression in Saccharomyces cerevisiae demonstrated that CAS1 and CAS2 are active enzymes, but only CAS1 displays considerable in vitro activity. Furthermore, X-ray and gel filtration analyses revealed a tetrameric structure of CAS1 with a conserved histidine and two cysteine residues in the active center.


We have mutagenized a homokaryotic fruiting strain, R326, of Coprinopsis cinerea and isolated a mutant that fails to enlarge the cap tissue on the primordial shaft in fruiting. Genetic analysis of this mutant, cap-growthless, indicated that the mutant phenotype is brought about by a single gene, designated as cag1. The cag1 locus was mapped on chromosome IX by linkage analysis using RAPD markers mapped to each chromosome. The cag1 gene was identified by transformation experiments using BAC DNAs and their subclones derived from chromosome IX, and found to encode a homolog of Saccharomyces cerevisiae Tup1. The Coprinopsis genome includes another Tup1 homologous gene, designated Cc.tupA. Expression levels of these two tup1 paralogs were examined using a real-time quantitative PCR method. Cc.tupA is predominantly expressed in vegetative mycelium. In contrast, in the cap tissue, transcript levels of cag1 are similar to that of Cc.tupA. Since it is known that S. cerevisiae Tup1 forms heterotrimeric interactions, Cag1 with itself and Cc.TupA were examined using yeast two-hybrid system. Cag1 interacts with itself through the N-terminal region and with Cc.TupA. Like Tup1, which interacts with Cyc8, the N-terminal region of Cag1 also interacts with the N-terminal region of Cc.Cyc8, which contains tetratricopeptide repeats. Based on expression and yeast two-hybrid analyses of Cag1 and Cc.TupA, combined with information on S. cerevisiae Tup1, we speculate that, in vegetative mycelium, Cc.TupA represses expression of genes required for cap growth, and Cag1, which might become expressed at the top of primordial shafts to produce the cap tissue and continue to be expressed in the cap tissue, might derepress and activate the expression through interaction with Cc.TupA.

134. Adaptation of the microtubule cytoskeleton to multinuclearity and chromosome number in hyphae of Ashbya gossypii as revealed by electron tomography. R. Gibeaux, C. Lang, A. Z. Politi, S. L. Jaspersen, P. Philipsen, C. Antony. 1) European Molecular Biology Laboratory, Heidelberg, Germany; 2) Biozentrum, Molecular Microbiology, University of Basel, CH 4056 Basel, Switzerland; 3) Stowers Institute for Medical Research, Kansas City, USA.

The filamentous fungus Ashbya gossypii and the yeast Saccharomyces cerevisiae evolved from a common ancestor based on the high level of gene order conservation. Interestingly, A. gossypii lost the ability of cell divisions and exclusively grows as elongating multinucleated hyphae. Using electron tomography we reconstructed the cytoplasmic microtubule (cMT) cytoskeleton in three tip regions with a total of 13 nuclei and also the nuclear microtubules (nMTs) of four mitotic bipolar spindles. Each spindle pole body (SPB) nucleates three cMTs on average, similarly to S. cerevisiae SPBs. 80% of cMTs were growing as concluded from the structure of their plus-ends. Very long cMTs closely align for several microns along the cortex to generate dynein-dependent pulling forces on nuclei. The majority of nuclei carry duplicated side-by-side SPBs, which together emanate an average of six cMTs, in most cases in opposite orientation with respect to the hyphal growth axis. Such cMT arrays explain why many nuclei undergo short-range back and forth movements. Following mitosis, daughter nuclei carry a single SPB. The increased probability that all three cMTs orient in one direction explains the high rate of long-range nuclear bypassing observed in these nuclei. These results demonstrate how cMT arrays, despite a conserved number of microtubules, could successfully adapt to the demands of multinuclearity during evolution from mono-nucleated budding yeast-like cells to multinucleated hyphae. The modelling of A. gossypii mitotic spindles revealed a very similar structure to mitotic spindles of S. cerevisiae in terms of nMT number, length distribution and three-dimensional organisation even though A. gossypii carries 7 and S. cerevisiae 16 chromosomes per haploid genome. Our results suggest that the nMT cytoskeleton remained largely unaltered during the evolution and that two nMTs attach to each kinetochore in A. gossypii and three in S. cerevisiae.

135. High resolution proteomics of spores, germlings and hyphae of the phytopathogenic fungus Ashbya gossypii. L. Molzahn1,2, A. Schmidt2, P. Philipsen1. 1) Biozentrum, Molecular Microbiology, University of Basel, CH4056 Basel, Switzerland; 2) Biozentrum, Proteomics Facility, University of Basel, CH4056 Basel, Switzerland.

Growth of the filamentous ascomycete A. gossypii is regulated by a genome very similar to the Saccharomyces cerevisiae genome even though the growth modes of both organisms differ significantly. During the previous decade progress was made to better understand some of these differences. 1. Cytokinesis in A. gossypii is not coordinated with mitosis and cell separation does not occur due to loss of specific genes which most likely led to the evolution of multinucleated hyphae. 2. Short nuclear cycle times and dynein-dependent pulling forces exerted on nuclei by autonomous cMT arrays with fast growing microtubules maintain a high nuclear density also in fast growing hyphae. 3. Polar growth sites once established support permanent and constantly accelerating polar surface expansion at hyphal tips at rates of up to 40mm2/min compared to 1mm2/min of yeast buds. Very efficient exocytosis and endocytosis could be documented in hyphal tips of A.gossypii. We want to understand on a system level the differences between both organisms and have started a proteomic approach. Total protein extracted from spores and developing A. gossypii hyphae was digested with trypsin, mixed with heavy isotope-labeled reference peptides and subjected to high resolution tandem MS analyses. We could identify 3900 proteins at each developmental stage. Significant quantitative changes of these proteins with respect to clusters of orthologous groups (COG) or gene ontology (GO) terms were identified during A.gossypii development and between log-phase growing S. cerevisiae cells and fast growing A. gossypii hyphae. Important differences concern ribosome biogenesis and translation, mitochondria biogenesis and respiration, glycolysis and gluconeogenesis, chromatin remodeling, chaperones, cell wall biosynthesis and the first reaction in several biosynthetic pathways.

Indoor fungi are present in a considerable part of the European dwellings and cause cosmetic and structural damage. The presence of indoor fungi poses a potential threat to human health as a result of continuous exposure as they are able to form allergens and mycotoxins. Indoor fungal growth does not exist without the presence and availability of water. Not much is known on the response of fungi to humidity dynamics during different stages of their development. Relative humidity (RH) and water activity (aw) are used in many studies for the amount of water available for the fungus. A RH of 80% or higher is thought to be required for fungal growth to occur. On average the RH is below 50% in normal buildings, suggesting a crucial role of humidity dynamics for fungal growth. In order to study the fungal response to humidity dynamics, two indoor fungal species, Cladosporium halotolerans and Penicillium rubens, were dried in controlled humidity vessels to stop growth and are rehydrated under high humidity conditions after a week. Non-linear Spectral Imaging Microscopy (NSIM) is a non-intrusive method to follow the response of fungal cells under varying relative humidity conditions by looking at the metabolic activity of separate cells. The different developmental stages of C. halotolerans and P. rubens before and after periods of a certain level of humidity are determined by using Cryo Scanning Electron Microscopy (CryoSEM). A different response to humidity dynamics was seen between several developmental stages and both fungi used. More in depth research will be done on the specific cellular response of the fungi to humidity dynamics.

137. Essentiality of Ku70/80 in Ustilago maydis is related to its ability to suppress DNA damage signalling at telomeres. Carmen de Sena-Tomás1, Eun Young Yu2, Arturo Calzada3, William K. Holloman4, Neal F. Lue1, Jose Perez-Martin1. 1) IBFG (CSIC-USAL), Zacarías González 3, 37037 Salamanca, Spain; 2) Cornell University Medical College, 1300 York Avenue, 10021 New York; 3) CNB (CSIC), Darwin 3, 28049 Madrid, Spain.

Ku heterodimer is formed of two subunits Ku70 and Ku80 that bind with high affinity to DNA ends in a sequence independent manner. Ku has a role in several cellular processes including DNA repair, telomere maintenance, transcription and apoptosis. Ku heterodimer is essential in human cells as well as in Ustilago maydis, a well-characterized fungal system used in DNA repair studies. We found that depletion of Ku proteins in U. maydis elicits a DNA damage response (DDR) at telomeres resulting in a permanent cell cycle arrest, which depends on the activation of the Atr1-Chk1 signalling cascade. A consequence of this inappropriate activation is the induction of aberrant homologous recombination at telomeres manifested by the formation of extrachromosomal telomere circles, telomere lengthening and the accumulation of unpaired telomere C-strand. Abrogation of the DDR response by deleting either chk1 or atr1 genes alleviates much of these aberrant recombination process suggesting that one of the roles of Ku proteins at telomeres in Ustilago maydis is related to the suppression of unscheduled DNA damage signalling at telomeres, in addition to the protection of telomeres.

138. Magnaporthe oryzae effectors with putative roles in cell-to-cell movement during biotrophic invasion of rice. Miwha Yi1, Xu Wang2, Jung-Yoon Lee2, Barbara Valent1. 1) Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506, USA; 2) Department of Plant and Soil Sciences, University of Delaware, Newark, Delaware 19711, USA.

Previous studies implicated rice plasmodesmata in two different aspects of rice blast disease caused by the hemibiotrophic ascomycetous fungus, Magnaporthe oryzae. First, effectors that are translocated into the cytoplasm of living rice cells move ahead into unwounded host plant cells by a mechanism that depends on effector protein size and rice cell type. This suggested that these effectors move through plasmodesmata to prepare surrounding host cells for fungal infection. Second, biotrophic invasive hyphae (IH) search for locations to move into neighboring rice cells and they undergo extreme constriction when crossing the host cell wall. These findings and additional evidence suggested that IH manipulate host pit fields containing plasmodesmata for cell-to-cell movement. Our goals are to test these hypotheses, and to understand the molecular mechanisms responsible for cell-to-cell movement in blast disease. We have identified six biotrophy-associated secreted (Bas) proteins that accumulate around IH at the point where they have crossed the rice cell wall to invade neighboring rice cells. We designated these effectors as putative fungal movement proteins (fMPs). When imaged as fluorescently labeled fusion proteins, the fMPs show unique localization patterns at the cell wall crossing points. Functional analysis of the fMPs is underway. Precise microscopic characterization with correlative light and electron microscopy (CLEM) and time-course, live-cell imaging is being performed to decipher how the fungus manipulates the rice cell wall junction area for effector trafficking and its own cell-to-cell spread. The fMPs will be localized relative to each other and to plasmodesmata-specific fluorescent markers. We will compare the structure and function of rice plasmodesmata in invaded versus non-invaded rice cells. Our results will identify novel host targets exploited by the fungus and related infection mechanisms at the wall crossing sites to facilitate colonization in planta.

139. Functional characterization of autophagy genes Smatg8 and Smatg4 in the homothallic ascomycete Sordaria macrospora. Stefanie Poeggele1, Oliver Voigt. Genetics of Eukaryotic Microorganisms, Georg-August University, Göttingen, Germany.

Autophagy is a degradation process involved in various developmental aspects of eukaryotes. However, its involvement in developmental processes of multicellular filamentous ascomycetes is largely unknown. Here, we analyzed the impact of the autophagic proteins SmATG8 and SmATG4 on the sexual and vegetative development of the filamentous ascomycete Sordaria macrospora. A yeast complementation assay demonstrated that the S. macrospora SmATG8 and SmATG4 genes can functionally replace the yeast homologs. By generating homokaryotic deletion mutants, we showed that the S. macrospora SmATG8 and SmATG4 orthologs were associated with autophagy-dependent processes. SmATG8 and SmATG4 deletions abolished fruiting-body formation and impaired vegetative growth and ascospore germination, but not hyphal fusion. We demonstrated that SmATG4 was capable of processing the SmATG8 precursor. SmATG8 was localized to autophagosomes and SmATG4 was distributed throughout the cytoplasm of S. macrospora. Furthermore, we could show that SmATG8 and SmATG4 are not only required for nonselective macroautophagy, but for selective macropexophagy as well. Our results suggest that in S. macrospora autophagy seems to be an essential and constitutively active process to sustain high energy levels for filamentous growth and multicellular development even under nonstarvation conditions. (Voigt O, Pöggeler S Autophagy genes Smatg8 and Smatg4 are required for fruiting-body development, vegetative growth and ascospore germination in the filamentous ascomycete Sordaria macrospora. Autophagy. 2012 Oct 12;9(1). [Epub ahead of print]).

140. Laser microdissection and transcriptomics of infection cushions formed by Fusarium graminearum. Mariske Boenisch1, Stefan Scholten2, Sebastian Piehler2, Martin MünterKötter4, Ulrich Güldener2, Wilhelm Schäfer1. 1) Molecular Phytopathology and Genetics, Biocenter Klein Flottbek, University of Hamburg, Germany; 2) Developmental Biology and Biotechnology, Biocenter Klein Flottbek, University of Hamburg, Germany; 3) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum Münich (GmbH), Neuherberg, Germany.

The fungal plant pathogen Fusarium graminearum Schwabe (teleomorph Gibberella zeae (Schwien) Petch) is the causal agent of Fusarium head blight (FHB) of small grain cereals and cob rot of maize worldwide. Trichothecene toxins produced by the fungus e.g. nivalenol (NIV) and deoxynivalenol (DON) contaminate cereal products and are harmful to humans, animals, and plants. We demonstrated recently, that F. graminearum forms toxin producing infection structures during infection of wheat husks, so called infection cushions (Boenisch and Schäfer, 2011). The aims of the presented study were to further clarify the penetration mechanism of infection cushions by histological studies and to identify molecular characteristics of infection cushions by expression analysis. Structural characteristics of infection cushions were visualized by 3D images following laser scanning microscopy. We observed
multiple penetration events underneath infection cushions by scanning electron microscopy. Colonization of the underlying plant tissue was studied by bright field microscopy and transmission electron microscopy of LR-White serial sections. To understand the molecular basis of initial colonization of the leaf surface followed by infection cushion development, a laser capture microdissection (LCM) approach was established to isolate specifically runner hyphae and infection cushions. Several hundred runner hyphae and infection cushions grown on wheat glumes were collected using the PALM system (Zeiss) avoiding contamination with plant tissue. Total mRNA of runner hyphae and infection cushions were isolated and amplified. The cDNA library of each developmental stage was used for next generation sequencing with Illumina HiSeq 2000. Quantitative expression analysis show marked differences in gene expression patterns between runner hyphae and infection cushions. Different functional pathways specific for each infection stage were identified. Thereby new insights in the initial infection process of FHB disease are gained. To our knowledge, we provide the first transcriptome data of runner hyphae and infection cushions from a fungal plant pathogen obtained under in planta conditions. In summary, the power of combined microscopic and molecular approaches to analyze cell type-specific gene expression during fungal-plant-interactions is demonstrated.

141. Biochemical and biophysical analysis of the CarO rhodopsin of Fusarium fujikuroi. Jorge Garcia-Martinez1, Marta Castrillo1, Javier Avalos1, Ulrich Terpitz2. 1) Departamento de Genética, Universidad de Sevilla, Sevilla, Spain; 2) Lehrstuhl für Biotechnologie und Biophysik, Julius-Maximilians-Universität Würzburg, Biozentrum / Am Hubland, Würzburg, Germany.

Light controls many substantial processes in filamentous fungi, such as reproduction and pathogenicity. Fungi naturally possess light sensors, which react to a broad range of wavelengths with absorption maxima in the blue, green or red regions of the spectrum. Rhodopsins are green light-absorbing membrane-integrated photoreceptors consisting of seven transmembrane helices forming an interior pocket for the chromophore, either all-trans or 11-cis retinal, covalently bound to the protein via a protonated Schiff-base. Type I rhodopsins, predicted to bind all-trans retinal, are widespread in ascomycota and basidiomycota. Upon light-activation, type I rhodopsins act as proton pumps or sensory proteins; however, detailed knowledge of their physiological function and biological role in fungi is still missing. The gibberelin-producing fungus Fusarium fujikuroi contains two rhodopsin encoding genes, carO and opsA, whose mutations produce no external phenotypic alterations. The carO gene is linked and co-regulated with genes coding for enzymes for retinal-synthesis, whose expression is strongly induced by light. To gain information on CarO biological role, we have combined biophysical methods to analyse the localisation and function of this rhodopsin in F. fujikuroi mycelia. We established a strain expressing CarO fused to a yellow fluorescent protein (YFP) under control of the carO promoter. This strain was investigated with confocal laser scanning microscopy (CLSM) and super-resolution fluorescence imaging (dSTORM) to reveal the subcellular localisation of CarO. Protein-localisation was compared with data recorded from a S. cerevisiae DYS3 strain overexpressing CarO-YFP. Additionally, the carO-YFP gene fusion was expressed in neuroblastoma cells, where it exhibited an efficient ion-pump-activity, as demonstrated by Patch-clamp techniques. The results suggest a light dependent ion-pumping role in the fungus, nonessential under standard laboratory conditions.

142. Roles of membrane and organelle calcium channels and transporters in controlling pulsatile [Ca2+]i signatures. Hyo-Seon Kim1, Jung-Eun Kim2, Kirk Czymmek1, Robert Cinco1, Randall Duncan1, Hokeyoung Son1, Yin-Won Lee3, Seogchan Kang1. 1) Department of Biological Sciences, University of Delaware, Newark, DE 19711; 2) Department of Plant Pathology & Environmental Microbiology, The Pennsylvania State University, University Park, PA 16802; 3) Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921.

Calcium ions translate diverse environmental stimuli into many different physiological and developmental functions in fungi ascomycota and basidiomycota. Upon light stimulation, calcium entry from a fungal plant pathogen obtained under in planta conditions. In summary, the power of combined microscopic and molecular approaches to analyze cell type-specific gene expression during fungal-plant-interactions is demonstrated.

143. Characterization of positive regulator for asexual and sexual reproduction in the cereal head blight pathogen Gibberella zeae. Jungkwon Lee1, Boknam Jung1, Hokeyoung Son1, Yin-Won Lee2. 1) Department of Applied Biology, Dong-A University, Busan 604-714, Republic of Korea; 2) Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea.

Gibberella zeae is an important plant pathogen that causes cereal head blight and produces mycotoxins that are harmful to animals and humans. Ascosporas and conidia contribute to the primary inoculums and propagation for disease epidemics. In this study, we identified one putative C2H2 zinc finger transcription factor (prd1) that is required for both conidiation and sexual reproduction, as screening transcription factor mutant collection we previously generated. prd1 deletion mutants impaired conidial production and lost both self-fertility and sexual fertility, but retain male fertility. The overexpression of the gene increased the amount of conidial production and resulted in earlier maturation of fruiting body formation than the wild-type strain. The vegetative growth of deletion and overexpression mutants was increased and decreased on nutrient-rich media, respectively, but was not different from the wild-type strain on nutrient-poor media. This study was the first report for transcription factor which positively regulates both conidiation and sexual reproduction, and the characterization of genes regulated by this gene will be further studied.

144. Functional analysis of Elongator complex protein 3 in Gibberella zeae. Y.-J. Lee1, H. Son1, J.-C. Kim2, G. J. Choi2, Y.-W. Lee1. 1) Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea; 2) Eco-friendly New Materials Research Group, Research Center for Biobased Chemistry, Division of Convergence Chemistry, Korea Research Institute of Chemical Technology, Daejeon 305-343, Republic of Korea.

Gibberella zeae (anamorph: Fusarium graminearum) is a causal agent of Fusarium head blight (FHB) which causes huge economic losses in cereal crops such as wheat and barley. In addition to yield reduction, mycotoxin contamination of grain presents a threat to human safety. We examined one of Restriction-Enzyme-Mediated Integration (REMI) mutants Z43R9282 showing defects in virulence and sexual development and identified a gene encoding Elongator complex protein 3 (ELP3). ELP3 is a catalytic subunit of Elongator complex and contains histone acetyltransferase (HAT) domain. The biological function of ELP3 gene was studied by targeted deletion in G. zeae. Deletion of ELP3 resulted in retarded growth and delay of sexual development compared to the wild-type strain. Most of the ascosporas had two cells in the ELP3 deletion mutants, while wild-type ascosporas usually had four cells. The
length of the mutant conidia was approximately 25% longer than the wild type. Deletion mutants of ELP3 were sensitive to stress conditions, such as high-salt stress (NaCl and KCl), suggesting a role in adaptation to environmental condition. Virulence on wheat heads was greatly reduced in the ELP3 deletion mutants. These results demonstrate that ELP3 is required for normal sexual and asexual development and ELP3 could be involved in cell size regulation in G. zeae.


G protein signaling proteins play key roles in the regulation of fungal development, secondary metabolism, and virulence. Regulators of G protein signaling (RGS) proteins make up a highly diverse and multifunctional protein family that plays a critical role in controlling heterotrimeric G protein signaling. The genome of the plant pathogenic fungus Gibberella zeae contains seven RGS genes (FgFibA, FgFibB, FgRgsA, FgRgsB, FgRgsB2, FgRgsC, and FgGprK). We functionally characterized the function of these genes in various cellular processes. Mutant phenotypes were observed for deletion mutants of FgRgsA and FgRgsB in vegetative growth, FgFibB and FgRgsB in conidia morphology, FgFibA in conidia production, FgFibA, FgRgsB, and FgRgsC in sexual development, FgFibA and FgRgsA in spore germination and mycotoxin production, and FgFibA, FgRgsA, and FgRgsB in virulence. Furthermore, FgFibA, FgRgsA, and FgRgsB acted pleiotropically, while FgFibB and FgRgsA deletion mutants exhibited a specific defect in conidia morphology and sexual development, respectively. Site-directed Ga subunits mutagenesis and overexpression of the FgFibA gene revealed that deletion of FgFibA and dominant active GzGPA2 mutant, ggpxozn, had similar phenotypes in cell wall integrity, perithecia formation, mycotoxin production, and virulence, suggesting that FgFibA may regulate asexual or sexual development, mycotoxin biosynthesis, and virulence through GzGPA2-dependent signaling in G. zeae. Especially, GzGPA2 might activate trichothecene production in a FgFibA-dependent manner.

146. A novel gene, GEA1, is required for ascus cell wall development in the ascomycete fungus, Gibberella zeae. H. Son, J. Lee, Y.-W. Lee. 1) Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea; 2) Department of Applied Biology, Dong-A University, Busan 604-714, Republic of Korea.

The ascomycete fungus Gibberella zeae is a devastating plant pathogen for major cereal crops. Ascospores are produced via sexual reproduction and forcibly discharged from mature perithecia, which function as the primary inocula. Perithecia development involves complex cellular processes and is under polygenic control. In this study, a novel gene, GEA1, was found to be required for ascus wall development in G. zeae. GEA1 deletion mutants produced normal-shaped perithecia and ascospores, yet ascospores were observed to precociously germinate inside of perithecia. Moreover, GEA1 deletions resulted in abnormal ascus walls that collapsed prior to ascospore discharge. Based on localization of GEA1 to the endoplasmic reticulum (ER), GEA1 may be involved in protein export from the ER to the ascus wall biogenesis. This is the first report to identify a unique gene required for ascus wall development in G. zeae.


Fusarium graminearum is a notorious fungal plant pathogen that causes head blight disease in small grain cereals and ear rot disease in maize. Infection with F. graminearum leads to yield losses and mycotoxin contamination. Mycotoxin formation and asexual development are thought to share common nodes of genetic regulation. However, the regulatory networks connecting salt/osmotic stress to either is limited or undefined. Salt tolerance is a complex trait that remains poorly understood. Very few genes have been identified that are required for salt tolerance in plants, animals, or fungi. To address this, we screened >5,000 insertional mutants of F. graminearum (PH-1) for gain-of-function or loss-of-function phenotypic classes specific to both asexual development (conidiation) and osmotic stress responses. These screens yielded strains representing all classes and one outlier from each were chosen for additional analyses. Mutant 9E1 exhibits an “osmotic hyper-tolerant” phenotype when cultured on growth media containing either NaCl or glycerol. In contrast, mutant 11B8 displays an “osmotic-overly sensitive” phenotype, where growth is severely limited on concentrations of solute that have a negligible effect on growth by control strains. Both 9E1 and 11B8 grow normally on non-osmotically adjusted media and were subsequently chosen for transcription-profiling experiments. Additionally, microarray expression data of developmental mutants B8S (aconidial) and B8E (hyperconidial) have revealed coordinately expressed, putative candidate regulatory genes. Based on a transcriptomics framework, we applied a bioinformatics approach to identify shared gene regulatory networks involved in osmotic stress responses and conidiation.

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Fusarium oxysporum (Fo) is a pathogenic species complex with a broad host range. Comparative genomics revealed lineage-specific (LS) genomic regions in Fusarium oxysporum f. sp. lycopersici (FoI) that account for more than 25% of the genome. At least two LS chromosomes can be transferred horizontally to non-pathogenic Fo strains, resulting in acquired pathogenicity in the recipient [1]. Here we want to elucidate the chromosome transfer pathway and the mechanisms by which the incompatibility reaction between strains is avoided. It has been suggested that heterokaryon formation is necessary for horizontal chromosome transfer in Colletotrichum gloeosporioides [2] and that heterokaryon incompatibility is suppressed after conidial anastomosis tube (CAT) fusion [3]. To study nuclear dynamics during formation of heterokaryotic cells in Fo, we observed green or red fluorescent protein labeled nuclei of Fo and non-pathogenic Fo strains in a vegetatively incompatible interaction.

While in rich medium co-cultivation of both strains revealed no heterokaryotic cells, co-cultivation under starvation conditions led up to ~30% heterokaryotic colonies (red and green nuclei). We were able to distinguish between different types of heterokaryotic conidia. In some cases after germination only one of the nuclei was able to propagate, which always originated from the pathogenic strain. In other cases both nuclei were able to propagate and these colonies in turn produced uninucleate conidia (yellow nuclei). Another intriguing finding was that the pathogenic strain used fared better under starvation conditions (higher germination and growth rate). We conclude that under starvation condition Fo is the dominant/fitter strain and that heterokaryon formation in Fo is greatly enhanced, possibly by further suppressing non-self recognition machinery in CATs and/or increased hyphal
149. Requirements for horizontal chromosome transfer in the plant pathogenic fungus Fusarium oxysporum, Ido Vlaardingerbroek, Martijn Rep. FNWI, University of Amsterdam, Amsterdam, Netherlands.

Strains within the Fusarium oxysporum species complex are clonal and diverse. A number of them are pathogenic to plants but rarely can they infect more than one host. Host specificity is determined by the presence of a set of secreted effector genes. These genes typically reside on Lineage Specific (LS) chromosomes that can be transferred between strains, even if they are vegetatively incompatible. These extra chromosomes typically carry no housekeeping genes and have many more transposable elements than the non-LS or core chromosomes. If a strain receives one of these chromosomes it can acquire the ability to infect a new host, compatible with the effector genes the chromosome harbours. Our main interests at this moment are (1) determining which chromosomes are amenable for transfer, and (2) which cellular processes are involved in transfer. To determine which chromosomes can be transferred, we created a bank of random insertional mutants carrying an antibiotic resistance marker. These have been tested for chromosome transfer. A few of these showed consistent transfer of the chromosome tagged with the marker. By screening a large number of transformants we should cover the entire genome. In addition to the screen we tagged an LS chromosome that we know can be transferred, as well as the smallest of the core chromosomes, with GFP. In this way we can directly compare transfer capability of these chromosomes. We hypothesize that the LS chromosomes’ unique make-up is required for transfer. We will also test these strains for stability of the tagged chromosome by screening spores for the loss of GFP expression using FACS. In this way we can test whether transferrable (LS) chromosomes differ in stability from core chromosomes under varying conditions. To identify cellular processes involved in chromosome transfer, we are making deletion mutants for genes required for cellular processes we suspect might be involved in chromosome transfer. These will be tested for transfer efficiency compared to the wild-type strains. We are currently investigating hyphal fusion, heterochromatin formation and programmed cell death. By combining the results from these two research lines we should be able to discover which chromosomes can be transferred as well as the chromosomal features and processes involved.

150. Characterization of the endocytotic proteins Yel1-Arf3-Gts1 in Ashbya gossypii and the role of Gts1 in endocytosis, actin localization and filamentous growth, Therése Oskarsson, Klaus Lengeler, Jürgen Wendland. Carlsberg Laboratory, Copenhagen, Denmark.

Endocytic vesicle formation and regulation thereof is performed by a complex protein machinery, coordinating every detail of the endocytic process from initiation and pit formation to vesicle scission and uncoating. We have used the filamentous fungi Ashbya gossypii to study three proteins that are involved in uncoating of vesicles in clathrin-mediated endocytosis. We deleted the corresponding genes encoding the GTP-binding protein Arf3 and its regulators; the Guanine nucleotide Exchange Factor Yel1 and the ArfGAP protein Gts1, using PCR-based gene targeting methods. We then characterized these mutant strains under various conditions. While no deletion-specific phenotypes could be observed in the Darf3 and Dye1l, the Dgts1 strain shows several severe mutant phenotypes. Deletion of GTS1 results in a strong growth defect and renders mycelia with severe endocytotic deficiencies indicated by distinctly reduced endocytotic rates, and large immobile vacuoles. Other phenotypic observations in A. gossypii Dgts1 strains indicate that Gts1 may have additional functions other than regulating the activity of Arf3. We have observed effects of Gts1 on temperature stress resistance, actin localization and polar filamentous growth.

The importance of GTS1 for polarized hyphal growth leads us to studying the GTS1 homolog of the human fungal pathogen Candida albicans in an effort to elucidate its role for the yeast-to-hyphal transition in this dimorphic fungi.


Late Embryogenesis Abundant (LEA) proteins were first characterized in cotton and wheat and are synthesized in abundance during the late maturation stage of seed development. As the seed matures, water content decreases greatly inducing severe desiccation stress. Expression of LEA proteins is linked to the acquisition of desiccation tolerance. Using BLAST to search for LEA like proteins in various filamentous fungal genome databases, we found orthologs in all filamentous species and processes involved.

152. Coordination of polarized secretion by the exocyst complex is critical for filamentous growth and cytokinesis in Ustilago maydis, Michaela Wehr, Kay Oliver Schink, Michael Böker. 1) Philippus University, FB Biologie, AG Boelker Marburg, Hessen, Germany; 2) Department of Biochemistry, Institute for Cancer Research The Norwegian Radium Hospital, Montebello, N-0310 Oslo, Norway.

To establish and sustain their polarity, cells have to transport proteins and membrane lipids to defined locations at the growing tip. This is achieved by directional transport of vesicles that fuse with the plasma membrane. Vesicle fusion and active exocytosis requires the presence of an octameric protein complex, the exocyst. In S. cerevisiae, two proteins of the exocyst complex, Sec3 and Exo70, were shown to serve as landmark proteins for exocytosis. The other components of the exocyst tether secretory vesicles carrying the Rab GTPase Sec4 to the membrane. Fusion of secretory vesicles occurs via interaction of the exocyst with SNARE proteins. To elucidate the function and regulation of the exocyst complex and its associated proteins in Ustilago maydis, we have characterized the Rab GTPase Sec4 and the exocyst proteins Sec3, Exo70 and Sec15 by genetic, cell biological and biochemical approaches. We found that the two landmark proteins, only one is important for polar growth in U. maydis. Interestingly, this gene is not essential, suggesting that in U. maydis exocytosis sites can also be marked by alternative mechanisms. Another essential player for polar growth in U. maydis is the exocyst subunit Sec15, which mediates the interaction of the exocyst with incoming secretory vesicles. Conditional mutants of sec15 are defective in hyphal tip growth and are affected in long-distance transport of secretory vesicles. In contrast to S. cerevisiae where Sec4 vesicles are transported along the actin cytoskeleton, long distance transport of vesicles depends in U. maydis on the microtubule cytoskeleton. Furthermore, we studied mutants of different motor proteins to get insights into the molecular mechanisms of secretory vesicle trafficking.

153. Localization of Neurospora crassa Cell Fusion Proteins, Chi Fu, Stephen J. Free. Biological Sciences, University at Buffalo, Buffalo, NY.
A screen of mutants in Neurospora crassa single gene deletion library identified 24 cell fusion genes. Bioinformatics studies indicate that 14 of these genes are likely to function in signal transduction pathways, 4 genes are transcription factors, 3 genes are likely to be involved in the process of vesicular trafficking, and 3 genes are highly conserved in fungal species with unknown functions. GFP and RFP fusion proteins were constructed for 2 vesicular trafficking proteins AMPH-1 and HAM-10, and 1 conserved hypothetical protein HAM-8 to study their functions during cell fusion process. Fluorescent protein markers for cellular organelles (including nucleus, mitochondria, golgi apparatus, endoplasmic reticulum, vacuole and vesicle), and for cytoskeleton (including actin filament and microtubule) were obtained from Fungal Genetic Stock Center. Strains expressing individual fluorescent protein marker were used to study the cellular localization of AMPH-1, HAM-10 and HAM-8 by using fluorescent confocal microscopy. The fluorescent protein marker strains were also used to study the dynamics of organelle movements during cell fusion by using time-lapse fluorescence microscopy. Fluorescent signals from AMPH-1, HAM-10 and HAM-8 were compared with two signaling molecules MAK-2 and SO to study their potential involvement in signal transduction. Results shown AMPH-1, HAM-8 and HAM-10 all colocalize with vesicle marker. One of the conserved hypothetical proteins, HAM-6, was modified with a FLAG tag to study its functions during cell fusion.


Hyphal fusion of Neurospora crassa germblings is a highly regulated process involving -among others- the conserved MAP kinase MAK-2 and the SO protein of unknown biochemical function. During chemotrophic interactions between two genetically identical germblings, MAK-2 and SO alternately localize at the conidial anastomosis tubes (CATs) every 4 minutes, perfectly out of phase of each other. How this process is initiated, maintained and what other proteins are involved is still unknown. One conserved fungal target of MAK-2 is the yeast Ste12-like transcription factor, named PP-1. Similar to mak-2, pp-1 is also required for hyphal fusion and normal mycelial growth. To identify downstream targets of MAK-2 and PP-1 that may play a role in germling fusion, micro-array and RNAseq analyses were performed on wild type (WT) and Dpp-1 strains. Combining the micro-array and RNAseq data, 32 genes were identified that showed at least 2-fold differential expression in WT as compared to Dpp-1. These include six genes, which are homologs of yeast Ste12 targets. To test the involvement of these genes in this hyphal fusion process, a deletion strain was obtained or constructed and assayed for germling fusion phenotype. Three deletion strains were completely devoid of fusion: Dham-7, Dasm-1 and Dham-11, and one deletion strain, Dham-12 showed reduced fusion frequencies when compared to WT. ham-7 was previously identified as fusion gene while asm-1 was shown to be involved in meiosis. When Dham-7 + Dham-7 or Dham-7 + WT germlings are confronted which each other, chemotropic interactions are not initiated, CATs are not observed and MAK-2 and SO are localized predominantly to the cytoplasm. ham-11 is a newly identified gene involved in germling fusion; Dham-11 + Dham-11 germlings do not show chemotrophic interactions or cell fusion. However, in contrast to Dham-7, Dham-11 germling fuse normally with WT germlings. MAK-2 and SO also show normal oscillation in WT and Dham-11 germlings undergoing chemotrophic growth. The observations suggest that HAM-11 might be involved in the production or proper release of a signal capable of inducing cell recognition and the germling fusion process.

155. N-acetylglucosamine (GlcNac) Triggers a Morphogenetic Program in Systemic Dimorphic Fungi. Sarah A. Gilmore1, Shamoon Naseem2, James B. Konopka1, Anita Sit1. 1) Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, CA; 2) Department of Molecular Genetics and Microbiology, Stony Brook University, Stony Brook, NY.

Cellular differentiation is an essential process for the development and growth of multicellular eukaryotic organisms. Similarly, many unicellular organisms undergo a program of cellular differentiation to produce a new cell type specialized for survival in a distinct environmental niche. Systemic dimorphic fungal pathogens, such as Histoplasma capsulatum (Hc) and Blastomyces dermatitidis (Bd), can switch between a unicellular parasitic yeast form adapted for growth within mammals and an infectious soil-growing filamentous form as part of their natural life cycles. Temperature is thought to be the predominant environmental cue that promotes cellular differentiation of systemic dimorphic fungi; however, work with other fungi indicates that additional environmental cues including CO2, light, and nutrient availability can influence how an organism responds to its environment. Recent work suggests that the ubiquitous monosaccharide N-acetylglucosamine (GlcNac) can play a role in cell signaling in fungi. We identified GlcNac as a potent inducer of the yeast-to-filament transition in Hc and Bd. Micromolar concentrations of exogenous GlcNac were sufficient to induce a robust morphological transition of Hc yeast cells to filamentous cells at room temperature, indicating that dimorphic fungal cells may be sensing GlcNac, or one of its catabolic byproducts, to promote filamentation. Using GlcNac as a tool to induce a robust and more synchronous phase transition of Hc yeast cells to filaments, we examined the temporal regulation of the Hc transcriptome during morphogenesis to reveal candidate genes involved in establishing the filamentous growth program. Two genes we identified during transcriptome analysis included NGT1 and NGT2, which encode GlcNac major facilitator superfamiy transporters. RNAi depletion of NGT1 or NGT2 rendered Hc cells unable to respond to exogenous GlcNac. Furthermore, wild type levels of NGT1 and NGT2 transcripts were important for efficient Hc yeast-to-filament conversion even in the absence of exogenously added GlcNac. These data suggest that Ngt1 and Ngt2 may monitor endogenous GlcNac as part of an autoregulatory system that allows Hc to regulate its filamentous growth.


Understanding fungal growth on construction materials is important to control problems with mould growth in buildings. The indoor environment is generally a harsh environment for a fungus. The climate is relatively dry and only during certain events at specific locations in the building (cooking, showering, etc.) there are peaks in the humidity. The porous nature of construction materials seems to play an important role in the survival of organisms, because it buffers the climate at the surface of materials by storing water: A model has been developed that describes the thermodynamic state and flow of water inside porous materials in connection to the growth of the organism. The model shows that the activity of water in a material is the key parameter controlling growth. However, the model also proves that growth cannot be predicted on the basis of experiments performed on idealized microbiological media (agar) with a well defined water activity. In those media water is always abundantly present irrespective of the activity. In porous materials however the amount of water dramatically reduces with the water activity. It is shown that porous materials with small pores in general contain more water than materials with big pores. A drop in the amount of water due to a decreasing activity has direct consequences for the food supply. Whereas in idealized media the amount of water is very high and therefore the mobility of nutrients, in porous materials the mobility of nutrients will decrease with decreasing water activity. To understand the behavior of a fungus on materials, its growth has to be really studied on these materials.

157. Identification and characterization of two genes required in the control of a cell degeneration in the filamentous fungus Podospora anserina. Herve Lalucque1,2, Fabienne Malnagnac1,2, Pierre Grognet1,2, Philippe Sila1,2. 1) Univ Paris Diderot, Sorbonne Paris Cité, Laboratoire Interdisciplinaire des Energies de demain (LIED), 75205 Paris France; 2) Institut de Génétique et Microbiologie (IGM), UMR 8621 CNRS Univ Paris Sud, 91405 Orsay France.

For several years, we use the coprophilous fungus Podospora anserina to study a cell degeneration called Crippled Growth (CG) triggered by an
epigenetic and cytoplasmic element. In the wild-type strain, this element is produced during stationary phase and eliminated at growth renewal. However, in some particular growth conditions, the element is not eliminated in growing hyphae triggering CG. Previous results showed that CG is controlled by two MAPK modules, the PaNox1 NADPH oxidase and IDC1, a protein with unknown activity. Here, we describe the identification and characterization of two new partners involved in the control of CG, IDC2 and IDC3. Data show that IDC2 and IDC3 likely act downstream of PaNox1 to regulate the ppaMpk1 MAPK. We will present a thorough analysis of the phenotypic of the IDC2 and IDC3 mutants and the phylogenetic studies of the IDC2 and IDC3 proteins.

158. Dynein drives oscillatory nuclear movements in the phytopathogenic fungus Ashbya gossypii and prevents nuclear clustering. S. Grava, M. Keller, S. Voegeli, S. Cegar, L. P. Philipp, Biozentrum, Molecular Microbiology, University of Basel, CH 4056 Basel, Switzerland.

In the yeast Saccharomyces cerevisiae the dynein pathway has a specific cellular function. It acts together with the Kar9 pathway to position the nucleus at the bud neck and to direct the pulling of one daughter nucleus into the bud. Nuclei in the closely related multinucleated filamentous fungus Ashbya gossypii are in continuous motion and nuclear positioning or spindle orientation is not an issue. A. gossypii expresses homologues of all components of the Kar9/Dyn1 pathway, which apparently have adapted novel functions. Previous studies with A. gossypii revealed autonomous nuclear divisions and, emanating from each MTOC, an autonomous cytoplasmic microtubule (cMT) cytoskeleton responsible for pulling of nuclei in both directions of the hyphal growth axis. We now show that dynein is the sole motor for bidirectional movements. Surprisingly, deletion of Kar9 shows no phenotype. Dyn1, the dynactin component Jnm1, the accessory proteins Dyn2 and Ndi1, and the potential dynein cortical anchor Num1 are involved in the dynamic distribution of nuclei. In their absence, nuclei aggregate to different degrees, whereby the mutants with dense nuclear clusters grow extremely long cMTs. Like in budding yeast, we found that dynein is delivered to CMT +ends, and its activity or processivity is probably controlled by dynactin and Nm1. Together with its role in powering nuclear movements, we propose that dynein also plays (directly or indirectly) a role in the control of cMT length. Those combined dynein actions prevent nuclear clustering in A. gossypii and thus reveal a novel cellular role for dynein.

159. Quantification of the thigmotropic response of Neurospora crassa to microfabricated slides with ridges of defined height and topography. Karen Stephenson1, Fordyce Davidson2, Neil Gow1, Geoffrey Gadd1. 1) Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Dundee, United Kingdom; 2) Division of Mathematics, University of Dundee, Dundee, United Kingdom; 3) Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom.

Thigmotropism is the ability of an organism to exhibit an orientation response to a mechanical stimulus. We have quantified the thigmotropic response of Neurospora crassa to microfabricated slides with ridges of defined height and topography. We show that mutants that lack the formin BNI-1 and the Rhop-GEF Cdc42-1, an activator of BNI-1, has an attenuated thigmotropic response. In contrast, null mutants that lacked cell end-marker protein TEA-1 and KIP-A, the kinesin responsible for its localisation, exhibited significantly increased thigmotropism. These results indicate that vesicle delivery to the hyphal tip via the actin cytoskeleton is critical for thigmotropism. Disruption of actin in the region of the hyphal tip which contacts obstacles such as ridges on microfabricated slides may lead to a bias in vesicle delivery to one area of the tip and therefore a change in hyphal growth orientation. This mechanism may differ to that reported in Candida albicans in so far as it does not seem to be dependent on the mechanosensitive calcium channel protein Mid1. The N. crassa Mid1 mutant was not affected in its thigmotropic response. Although it was found that depletion of exogenous calcium did not affect the thigmotropic response, deletion of the spray gene, which encodes an intracellular calcium channel with a role in maintenance of the tip-high calcium gradient, resulted in a decrease in the thigmotropic response of N. crassa. This predicts a role for calcium in the thigmotropic response. Our findings suggest that thigmotropism in C. albicans and N. crassa are similar in being dependent on the regulation of the vectorial supply of secretory vesicles, but different in the extent to which this process is dependent on local calcium-ion gradients.

160. Specificity determinants of GTPase recognition by RhoGEFs in Ustilago maydis. Britta A.M. Tillmann, Kay Oliver Schink, Michael Bölker. 1) Philipps-Universität Marburg FB Biologie, AG Bölker Karl-von-Frisch-Str. 8 35032 Marburg, Germany; 2) Department of Biochemistry, Institute for Cancer Research The Norwegian Radiation Hospital, Montebello, N-0310 Oslo, Norway.

Small GT-Pases of the Ras family act as molecular switches and are involved in the regulation of many important cellular processes. They are activated by specific guanine nucleotide exchange factors (Rho-GEFs). Rho-GTPases interact in their active, GTP-bound state with downstream effectors and trigger various cellular events. The number of Rho-GEFs and downstream effectors exceeds the number of GTPases. This raises the question how signalling specificity is achieved. In recent years it became evident that correct signalling depends on both the specificity of the activators with specific downstream effectors. Here, we analysed the Cdc42-specific U. maydis Rho-GEFs Don1, Its1 and Hot1 and the Rac1-specific Rho-GEF Cdc42 for their role in Cdc42 and Rac1 signalling both in vivo and in vitro. We observed that the recognition mechanisms for Cdc42 differ between Hot1 and the other Cdc42-specific Rho-GEFs. While a single amino acid at position 56 of Cdc42 and Rac1 is critical for specific recognition by Don1, Its1 and Cdc42, Hot1 is insensitive to changes at this position. Instead, Hot1 relies on a different set of amino acids to bind its specific target Cdc42. We could demonstrate that this unusual mechanism to discriminate between different Rho-type GT-Pases is also used by the mammalian orthologue of Hot1, TUBA1. These data allowed us to generate a chimeric Cdc42/Rac1 GT-Pase which can be activated by both Cdc42- and Rac1-specific Rho-GEFs with comparable efficiency. Importantly, such a chimeric GT-Pase was able to complement the morphological phenotypes of Cdc42 and Rac1 deletion mutants in vivo.


Fungal growth indoors can lead to both disfigurement of the dwelling and medical problems such as asthma. It is generally accepted that the primary cause for mould growth is the presence of moisture. Strategies to prevent fungal growth are therefore often based on controlling indoor humidity. Still, mould is often encountered in ventilated buildings that are considered to be relatively dry. Preliminary experiments showed that fungi can survive on porous materials due to short intervals of favorable circumstances; even when - on average - conditions for growth are not met. This suggests that the interactions between porous materials and the fluctuating indoor humidity play an important role in a colony's survival. We studied this interplay between indoor climate, substrate water household and fungal growth. A property of water that is crucial for fungal growth is water activity (a_w). This property determines a fungus’s ability to take up water. The effect of a_w on fungal growth has been determined in the past by extensive growth experiments on agar, and many previous studies of growth on building materials take this parameter into account. Up till now, however, little attention has been paid to the water content (q) of a substrate, which represents the amount of water that is physically present in a system. In most porous materials, even when a_w is relatively high, only little water is present. We suspect therefore that growth on porous substrates is limited by water content (whereas on agar, q is always close to 100% and will therefore be of little concern). We performed growth experiments with P. rubens inoculated on gypsum while separately controlling q and a_w. Video microscopy was used to monitor the germination and subsequent growth of hyphae. The early development of the fungus was
then quantified by determining parameters such as germination time and growth speed from the movies. The experiments show that the germination rate, growth speed and growth density of *P. rubens* on gypsum increase with q while aw is constant, and increase with aw while q is constant. We conclude from this that q and aw have separate effects on growth on porous substrates. An explanation for the effect of q could be that it limits a fungus’s access to both water and nutrients. Follow up research will focus on modeling and explaining these effects.

162. Localization of Ga proteins during germination in the filamentous fungus, *Neurospora crassa*. Illy Esther Cabrera1, Carla Eaton1, Jacqueline Servin1, Katherine Borkovich1. 1) Plant Pathology and Microbiology, University of California, Riverside, Riverside, CA; 2) Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand.

Heterotrimeric G protein signaling is essential for normal hyphal growth in the filamentous fungus *Neurospora crassa*. We have previously demonstrated that the non-receptor guanine nucleotide exchange factor RIC8 acts upstream of the Ga proteins GNA-1 and GNA-3 to regulate hyphal extension. Germination assays revealed essential roles for RIC8 and GNA-3 during this crucial developmental process. Localization of the three Ga proteins during conidial germination was probed through analysis of cells expressing fluorescently tagged proteins. Functional TagRFP fusions of each of the three Ga subunits were constructed through insertion of TagRFP in a conserved loop region of the Ga subunits. The results demonstrated that GNA-1 localizes to the plasma membrane and vacuoles, and also to septa throughout conidial germination. GNA-2 localizes to both the plasma membrane and vacuoles during early germination, but is then found in vacuoles later during hyphal outgrowth. Interestingly, in addition to y plasma membrane and vacuolar localization, GNA-3 was found in distinct patches on the plasma membrane of the original conidium during early germination. This distinct localization of GNA-3 supports the hypothesis that GNA-3 is needed for proper conidial germination, and this specific localization may be required for development. Further investigation is under way to determine the consequence of this localization. Colocalization of RIC8-GFP with GNA-1-TagRFP or GNA-3-TagRFP was not detected in cells expressing two fluorescent proteins. This finding suggests that their interaction may be transient not able to be captured via this method. A more sensitive microscopic approach is being implemented to better test for colocalization.


The transport of proteins through different compartments of the secretory pathway is mediated by vesicles. It is well known that vesicular trafficking is regulated by Rab GTPases, which in their active state interact with the membrane of the vesicles. Subsequently, through protein-protein interactions, they coordinately associate with factors involved in transport and/or tethering to the receptor organelle. In contrast to other eukaryotic model systems, most filamentous fungi contain a Spitzenkörper (Spk), which is a multi-vesicular complex found at the hyphal apex to which cargo-carrying vesicles arrive before being redirected to specific cell sites. The exact regulatory mechanisms utilized by the hyphae to ensure the directionality of the secretory vesicles that reach the Spk are still unknown. Hence, we have analyzed the *N. crassa* Rab-GTPases YPT-1 and SEC-4, key regulators of the secretory pathway rather well characterized in *S. cerevisiae*. YPT-1 regulates ER-Golgi and late endosome-Golgi traffic steps, while SEC-4 regulates post-Golgi vesicle traffic en route to the plasma membrane. Laser scanning confocal microscopy of strains expressing fluorescently tagged versions of the proteins revealed that YPT-1 localizes at the Spk microvesicular core and at cytoplasmic pleomorphic punctate structures, suggesting its participation in different traffic steps. YPT-1 accumulation at the Spk might suggest its function in mediating the traffic of vesicles from early endosomes as a recycling process. The pleomorphic structures could correspond to late Golgi equivalents. The localization of SEC-4 at the Spk, suggests the participation of this Rab in late traffic steps of Golgi-derived vesicles previous to exocytic events. The relative distribution of both Rab compared to the molecular motor MYO-2 (presumably involved in secretory vesicle transport), the long coiled-coil protein USO-1 (tethering factor), the secreted protein INV-1, and proteins involved in cell wall biosynthesis is being analyzed and will provide better clues on the nature of the identified compartments.

164. Functional characterization of CBM18 proteins, an expanded family of chitin binding genes in the *Batrachochytrium dendrobatidis* genome. Peng Liu, Jason Stajich. Plant Pathology & Microbiology, Univ California, Riverside, Riverside, CA.

*Batrachochytrium dendrobatidis* (Bd) is the causative agent of chytridiomycosis, one of the major causes of worldwide decline in amphibian populations. Little is known about the molecular mechanisms of its pathogenicity. Our previous work from the initial analysis of the Bd genome revealed a unique expansion 18 copies of the carbohydrate-binding module family 18 (CBM18), specific to Bd, and evolving under positive directional selection. CBM18 is predicted to be a sub-class of chitin recognition domains. Our hypothesis is that some of these copies of CBM18 can bind chitin, a major component of fungal cell walls, in vitro. In order to investigate CBM18’s intracellular localization, four CBM18 genes, representing tyrosinase-like, deacetylase-like and lectin-like groups, were cloned into a yeast GFP expression vector. Only two genes from lectin-like group fused with GFP, showing cell boundary localization. Furthermore, intracellular signals were observed on both GFP fusion proteins. According to the TargetP database, both proteins are predicted to have the secretion signal peptide. When co-stained with FM4-64, a dye to label vacuole membranes, the FM4-64 and GFP signals were mutually exclusive, indicating that the GFP fusion proteins were not destined for degradation. Expression of the proteins from the pHIL-S1 vector in the *Pichia* system will enable purification and characterization of binding properties of these molecules and affinity for chitin and other substrates. 1. Abramyan and Stajich, mBio 2012; 3(3): e00150-12.

165. The exocyst complex is necessary for secretion of effector proteins during plant infection by *Magnaporthe oryzae*. Yogesh K. Gupta1, Martha Giraldo2, Yasin Dagdas1, Barbara Valenta1, Nicholas J. Talbot1. 1) School of Biosciences, University of Exeter, EX4 4QD, UK; 2) Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA.

*Magnaporthe oryzae* is a devastating plant pathogenic fungus, which causes blast disease in a broad range of cereals and grasses. A specialized infection structure called the appressorium breaches the leaf cuticle and subsequently the fungus colonizes host epidermal cells. Colonization of host tissue is facilitated by small secreted proteins called effectors, that suppress plant immune responses and may also mediate invasive growth. Some of these effectors have been shown to localize at the appressorium pore prior to plant infection, at the tips of primary invasive hyphae and in a specialized plant-derived, membrane-rich structure called the Biotic Interfacial Complex (BIC). However the underlying mechanism controlling polarized secretion is not well defined in *M. oryzae*. The exocyst is an octameric protein complex (composed of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84) that appears to be evolutionary conserved in fungi and to play a crucial role in vesicle tethering to the plasma-membrane. The exocyst plays an important role in polarized exocytosis and interacts with various signaling pathways at the apex of fungal cells. We are currently characterizing components of exocyst complex during infection related development of *M. oryzae*. We have shown that the exocyst localizes to hyphal tips as in other fungi during hyphal growth in culture. Interestingly, exocyst components also localize around the appressorium pore, which suggests the pore is an active site for secretion at the point of plant infection. We have recently shown that organization of the appressorium pore requires a hetero polymeric septin network and we show...
here that localization of the exocyst at the appressorium pore is septin dependent. The exocyst is furthermore involved in secretion of symplastic (host cell-delivered) effectors but not apoplastic effectors. Targeted gene deletion of exocyst components Exo70 and Sec5 causes significant virulence defects because of impaired secretion. We will present new information on the role of the exocyst during invasive growth of *M. oryzae*.

166. Functional analysis of protein ubiquitination in the rice blast fungus *Magnaporthe oryzae*. Yeonjee Oh, Hayde Eng, William Franck, David Muddiman, Ralph Dean. Dept Plant Pathology, NCSU, Raleigh, NC.

Rice blast is the most important disease of rice worldwide, and is caused by the filamentous ascomycete fungus, *Magnaporthe oryzae*. Protein ubiquitination, which is highly selective, regulates many important biological processes including cellular differentiation and pathogenesis in fungi. Gene expression analysis revealed that a number of genes associated with protein ubiquitination were developmentally regulated during spore germination and appressorium formation. We identified an E3 ubiquitin ligase, MGG_13065 is induced during appressorium formation. MGG_13065 is homologos to fungal F-box proteins including Saccharomyces cerevisiae Grl1, a component of the Skp1-Cullin-F-box protein (SCFGr1) E3 ligase complex. Targeted gene deletion of MGG_13065 resulted in pleiotropic effects on *M. oryzae* including abnormal conidia morphology, reduced growth and sporulation, reduced germination and appressorium formation and the inability to cause disease. Our study suggests that MGG_13065 mediated ubiquitination of target proteins plays an important role in nutrient assimilation, morphogenesis and pathogenicity of *M. oryzae*.

167. The role of autophagy in *Cryphonectria hypovirus 1* (CHV1) infection in *Cryphonectria parasitica*. M. Rossi, M. Vallino, S. Abba’, M. Turina. Institute of Plant Virology, National Research Council (ICN), Torino, Italy.

The interaction between *Cryphonectria parasitica*, the causal agent of chestnut blight, and *Cryphonectria hypovirus 1* (CHV1) results in fungal hypovirulence associated with alterations of fungal development, reduced sporulation and pigmentation, accumulation of cytosolic vesicles. The role of these vesicles is to support CHV1 maintenance and replication, but the origin of these compartments is still under debate. Due to the phylogenetic proximity between CHV1 and poliovirus, which induces autophagosome proliferation in infected cells, we decided to explore the involvement of autophagy in vesicle accumulation and virus replication in CHV1-infected mycelium. We are studying the autophagy dynamic in CHV1-infected *Cryphonectria* expressing GFP-CpAtg8. Atg8 is the fungal orthologue of the mammalian LC3, an essential protein for autophagosome formation which is considered a reliable autophagosome marker. In CHV1-free hyphae, GFP-CpAtg8 distribution was mostly cytosolic, but in presence of CHV1 we observe a punctate distribution of fluorescence which is compatible with the binding of GFP-CpAtg8 with autophagosome membranes. The induction of autophagy is also supported by the observed increase of accumulation of GFP-CpAtg8 in presence of CHV1 compared with virus-free mycelium which could be due to an activation of gene transcription and/or to protein stabilization. Overall our results seem to confirm the activation of autophagy by CHV1. We are now testing through various approaches if CHV1 is able to induce autophagosomes proliferation to support its own replication or if this is an effect of fungal defense against hypovirus infection.

168. *Neurospora crassa* protein arginine methyl transferases are involved in growth and development and interact with the NDR kinase COT1. D. Feldman, C. Ziv, M. Efrat, O. Yarden. Dept of Plant Pathology and Microbiology, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel.

The protein arginine methyltransferase (PRMT) family is conserved from yeast to human, and regulates stability, localization and activity of proteins. We have characterized deletion strains corresponding to genes encoding for PRMT1/3/5 (designated pmr-1, pmr-3 and Rsk-1, respectively) in *N. crassa*. Deletion of PRMT-encoding genes conferred reduced growth rates and altered Arg-methylated protein profiles (as determined immunologically). Dprm-1 exhibited reduced hyphal elongation rates (70% of wild type) and increased susceptibility to the ergosterol biosynthesis inhibitor voriconazole. In Dprm-3, distances between branches were significantly longer than the wild type, suggesting this gene is required for proper regulation of hyphal branching. Deletion of *skb-1* resulted in hyper conidiation (2-fold of the wt) and increased tolerance to the chitin synthase inhibitor polyoxin D. Inactivation of two PRMTs responsible for asymmetric dimethylation (Dprm-1;Dprn-3) conferred changes in both asymmetric as well as symmetric protein methylation profiles, suggesting either common substrates or cross-regulation of different PRMTs. Taken together, all *N. crassa* PRMTs are involved in fungal growth, hyphal cell integrity and affect asexual (but not sexual) reproduction. The PRMTs in *N. crassa* apparently share cellular pathways which were previously reported to be regulated by the NDR (Nuclear DBF2-related) kinase COT1, whose dysfunction leads to a pleiotropic change in hyphal morphology. Using co-immunoprecipitation experiments, we have shown that SKB1 and COT1 can physically interact. To date, two isoforms of COT1 (67 and 73KDa) have been identified and studied. We have now identified a third, 70KDa, isoform of COT1, whose abundance was increased in a Dskb-1 background. This isoform, as well as the two others, are Arg-methylated, as determined on the basis of immunological detection and results indicate that the methylation observed involves the activity of more than one PRMT enzyme. The fact that *skb-1* background links these PRMTs to the environmental response associated with COT1 function. Based on the highly conserved structure of the PRMTs and the NDR kinases in eukaryotes, it is likely that these proteins undergo similar interactions in other organisms.

169. Role of tea1 and tea4 homologs in cell morphogenesis in *Ustilago maydis*. Flora Banuett, Woraratanadharinn Tad, Lu Ching-yu, Valinluck Michael. Biological Sciences, California State University, Long Beach, CA.

We are interested in understanding the molecular mechanisms that govern cell morphogenesis in *Ustilago maydis*. This fungus is a member of the Basidiomycota and exhibits a yeast-like and a filamentous form. The latter induces tumor formation in maize (*Zea mays*) and teosinte (*Zea mays* subsp. *parviglumis* and subsp. *mexicana*). We used a genetic screen to isolate mutants with altered cell morphology and defects in nuclear position. One of the mutants led to identification of *tea4*. *Tea4* was first identified in *Schizosaccharomyces pombe*, where it interacts with Tea1 and other proteins that determine the axis of polarized growth. *Tea4* recruits a formin (For3), which nucleates actin cables towards the site of growth, and thus, polarizes secretion (Martin et al., 2005). *Tea1* and *Tea4* have been characterized in *Aspergillus nidulans* and *Magnaporthe oryzae* (Higashitruji et al., 2009; Patkar et al., 2010; Takeshita et al., 2008; Yasin et al., 2012). Here we report the characterization for the first time of the *Tea4* and *Tea1* homologs in the Basidiomycota. The *U. maydis tea1* ORF has coding information for a protein of 1684 amino acid residues that contains a Src homology (SH3) domain, a RAS-associating domain, a phosphatase binding domain, a putative NLS, and a conserved domain of unknown function. All *Tea4* homologs in the Basidiomycota contain a RA domain. This domain is absent in *Tea4* homologs in the Ascomycota, suggesting that *Tea4* performs additional functions in the Basidiomycota. We also identified the UmTea1 homolog, which codes for a putative protein of 1698 amino acid residues. It contains three Kelch repeats. The *Tea1* homologs in the Ascomycota and Basidiomycota contain variable numbers of Kelch repeats. The Kelch repeat is a protein domain involved in protein-protein interactions. The *tea1* gene was first identified in *S. pombe* and is a key determinant of directionality of polarized growth (Mata and Nurse, 1997). To understand the function of *tea1* and *tea4* in several cellular processes in *U. maydis*, we generated null mutations. We demonstrate that *tea4* and *tea1* are necessary for the axis of polarized growth, cell polarity, normal septum positioning, and organization of the microtubule cytoskeleton. We also determined the subcellular localization of *Tea1::GFP* and *Tea4::GFP* in the yeast-like and filamentous forms.
170. Sex determination directs uniparental mitochondrial inheritance in *Phycomyces blakesleeanus*. Viplendra P.S. Shkya, Alexander Idnurm, School of Biological Sciences, University of Missouri-Kansas City, MO.

Uniparental inheritance (UPI) of mitochondria is common among eukaryotes. Various mechanisms have been suggested for UPI, but the underlying molecular basis is yet to be fully explained. We used a series of genetic crosses to establish that the *sexM* and *sexP* genes in the mating type locus control the UPI of mitochondria in the Mucoromycotina fungus *Phycomyces blakesleeanus*. Inheritance is from the (+) sex type, and is associated with degradation of the mitochondrial DNA from the (-) parent in the developing zygospore. Hence, the UPI of mitochondria in *Phycomyces* shows that this process can be directly controlled by genes that determine sex identity, independent of cell size or the complexity of the genetic composition of a sex chromosome.

171. Exploring the role of a highly expressed, secreted tyrosinase in Histoplasma capsulatum mycelia. Christopher F. Villalta1, Dana Gebhart2, Anita Sil3.

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The human pathogen *Histoplasma capsulatum* is a dimorphic ascomycete that resides in the soil at ambient temperature as a mycelium. Infection of immunocompetent individuals with *H. capsulatum* occurs when mycelial fragments and associated conidia are inhaled. These fungal cells undergo a conversion to a budding-yeast form in response to mammalian body temperature. We are interested in genes that specify the biological attributes of either the infectious form (mycelia or conidia) or the parasitic form (yeast). Previous work from our lab compared the gene expression profiles of mycelia, conidia, and yeast cells to determine genes that were preferentially expressed in each developmental form. We determined that the *TYR1* gene, which encodes a putative polyphenol oxidase, or “tyrosinase”, is highly differentially expressed in the mycelial form of *H. capsulatum*. Notably, the *H. capsulatum* genome contains seven tyrosinases, all of which are more highly expressed in mycelia and conidia compared to yeast. These enzymes contain a conserved tyrosinase domain, but their function in pathogenic fungi has not been investigated. Our expression data suggest that tyrosinases play a specific role in the biology of *H. capsulatum* filaments and spores. Strains that either lack *TYR1* or express deregulated *TYR1* display altered growth properties during the mycelial phase. Interestingly, our preliminary results indicate that *Tyr1* is secreted into the media during mycelial-phase growth. We are currently investigating whether *Tyr1* affects mycelial growth by modifying a cell-surface or secreted molecule. Additionally, we are determining if *Tyr1* is important in the production of infectious spores.


Wild-type Neurospora grows with the same branch density (statistical distribution of physical distances between branch points along a growing hypha) at a wide range of incubation temperatures. Previous work highlighted the impact of reactive oxygen species (ROS) control on branch density. Here we report the branching effects of selected ROS control gene knockout mutants; the impact of exogenously added anti-oxidants. In all ROS control mutants tested, growth was shown to branch tighter when grown at higher temperatures and looser when grown at lower temperatures. The branch density displayed by the ROS mutants at low temperature is measurably hypobranchered. In tests on wild type Neurospora, added Ascorbic Acid and Glutathione produced unusual branching patterns. Hypha exposed to Ascorbic Acid or Glutathione display a distribution of branching with two distinct maxima. They show an increase in both very closely spaced branching as well as an increase in more distantly spaced branching. At lower doses however, hypobranching, again, is observed with average branch density being linearly related to the dose of added anti-oxidants. We also report on the interaction between ROS mutants and added anti-oxidants.

173. Septum formation starts with the establishment of a septal actin tangle (SAT) at future septation sites. Diego Delgado-Alvarez1, S. Seiler2, S. Bartnicki-Garcia1, R. Mouriño-Pérez1. 1) CICESE, Ensenada, Mexico; 2) Georg August University, Goettingen, Germany.

The machinery responsible for cytokinesis and septation formation in mycelial fungi is well conserved among eukaryotes. Its major components are actin and myosin, which form a contractile actomyosin ring (CAR). The constriction of the CAR is coupled to the centripetal growth of plasma membrane and deposition of cell wall. In filamentous fungi, such as Neurospora crassa, cytokinesis in vegetative hyphae is incomplete and results in the formation of a centrally perforated septum. We have followed the molecular events that precede formation of septa and constructed a timeline that shows that a tangle of actin filaments is the first element to conspicuously localize at future septation sites. We named this structure the SAT for septal actin tangle. SAT formation seems to be the first event in CAR formation and precedes the recruitment of the anillin Bud-4, and the formin Bni-1, known to be essential for septum formation. During the transition from SAT to CAR, tropomysin is recruited to the actin cables. Constriction of the CAR occurs simultaneously with membrane internalization and synthesis of the septal cell wall.

174. Characterization of the Neurospora crassa STRIPAK complex. Anne Dettmann1, Yvonne Heilig1, Sarah Ludwig1, Julia Illeghen2, Andre Fleischer2, Stefan Seiler2. 1) Institute for Biology II, Molecular Plant Physiology, Freiburg, Germany; 2) Biozentrum, Technische Universität Braunschweig, Germany.

The majority of fungi grow by polar tip extension, branching and intercellular fusion to generate a supra-cellular, syncitial mycelium. This hyphal network formation increases the fitness of the organism and is central to the organization and function of the fungal colony. Multiple mutants deficient in hyphal fusion and/or intercellular signaling were characterized in *Neurospora crassa*, the currently best understood model for interhyphal signaling. Among them are components of the two MAK1 and MAK2 MAP kinase cascades and a cell fusion-specific phosphatase 2A termed the STRIPAK complex. While the MAK2 cascade is central for signaling through oscillatory recruitment of the MAK2 module to opposing tips of communicating cells, the MAK1 cell wall integrity pathway is assumed to play a critical role in the cell wall rearrangement after the physical contact of the two partner cells. The mechanistic function of the STRIPAK complex and the functional relationship of the three modules is not resolved. By a combination of genetic, biochemical and life cell imaging techniques, we present the characterization of the STRIPAK complex of *N. crassa* that consists of HAM2/STRIP, HAM3/striatin, HAM4/SLMAP, MOB3/phocin, PPG1/PP2AC and PP2AA. We further describe that the fungal STRIPAK complex localizes to the nuclear envelope and regulates the nuclear accumulation of the MAP kinase MAK1 in a MAK2-dependent manner.

175. Does the CENP-T-W-S-X tetramer linker centromere to kinetochores? Jonathan Galazka, Mu Feng, Michael Freitag. Biochemistry and Biophysics, Oregon State University, Corvallis, OR.

In vertebrates, the centromeric proteins, CENP-T, -W, -S and -X, form a tetramer (CENP-T-W-S-X) in vitro that binds DNA [1]. Furthermore, the unstructured N-terminus of CENP-T interacts with the Ndc80 complex at kinetochores [2]. This suggests that CENP-T-W-S-X has a central role in linking centromeric DNA to kinetochores. Despite the appeal of this model, there is no evidence that this complex forms in vivo, no information of the DNA sequences it may bind at centromeres and little understanding of how it interacts with canonical nucleosomes. CENP-T, -W, -S, and -X are conserved in fungi, including *Neurospora* [1-3]. *Neurospora* is an attractive model in which to understand the function of the CENP-T-W-S-X complex as its centromeric
164. DNA is nearly completely assembled, allowing ChIP-seq reads to be mapped unambiguously [4]. Here, we report on our investigations of the *Neurospora* CENP-T-W-S-X complex, including its interactions with centromeric DNA and canonical centromeric nucleosomes.


176. Proper actin ring formation and septum constriction requires coordination of SIN and MOR pathways through the germinal centre kinase MST1. Yvonne Heilig, Anne Dettmann, Stephan Seiler. Institute for Biology II, Molecular Plant Physiology, Freiburg, Germany.

The highly conserved nuclear Dbf2p-related (NDR) kinases control polar morphogenesis and cell proliferation. In fungi, NDR kinases function as effectors of the morphogenesis (MOR) and septation initiation (SIN) networks and are activated by germinal centre (GC) kinases. The *Neurospora crassa* SIN kinases SIO1 and DBF2 are essential for septum formation. In contrast, the MOR kinases POD6 and COT1 promote apical tip growth and function as negative regulators of septation. We identified a third GC kinase MST1 that functions as promiscuous enzyme, activating DBF2 and COT1. As typical for SIN components, MST1 localized to spindle pole bodies and constricting septa. Moreover, Dmst-1 displayed synthetic interactions with sin, but not mor mutants, placing MST1 in parallel to the central SIN kinase cascade CDC7-SIO1-DBF2. Consistent with these genetic data, we determined that the two GC kinases MST1 and SIO1 are regulated by CDC7 in an opposite manner. Lifeact- and formin-GFP reporter constructs revealed the formation of aberrant cortical actin rings in Dmst-1, which resulted in mispositioned septa and irregular spirals in the mutant. In summary, our data identify an antagonistic relationship between the SIN and MOR during septum formation that is, at least in parts, coordinated through the GC kinase MST1.

177. Regulation of the BUD3-BUD4 landmark complex by the NDR kinases DBF2 and COT1 during septum formation in *Neurospora crassa*. Yvonne Heilig, Stephan Seiler. Institute for Biology II, Molecular Plant Physiology, Freiburg, Germany.

Cytokinesis is essential for cell proliferation, yet the mechanisms for determining the site of cell division are poorly understood. Our data indicate that the anillin BUD4 marks septum placement by organizing the RHO4-BUD3-BUD4 GTPase module and that this complex is controlled through two NDR kinase signaling cascades, the septation initiation network (SIN) and the morphogenesis network (MOR). Epistasis analysis of sin and mor mutants places the SIN upstream of the MOR. DBF2 functions as competitive inhibitor of COT1 by forming hetero-dimers, thereby replacing the COT1 co-activators MOB2B/A. In turn, COT1 functions as negative regulator of septum formation. We demonstrate that COT1, but not DBF2, binds to and phosphorylates BUD3 and BUD4. Mutational analysis of BUD3 identifies Ser798, located within an amphiphatic helix of BUD3 that is phosphorylated by COT1. Localization of this amphiphatic helix at septa is only possible in its nonphosphorylated form. In summary, our data suggest a model, in which the MOR kinase COT1 phosphorylates BUD3 and BUD4 and that this modification inhibits cortical localization and function of the BUD complex. Interference of the SIN with MOR activity at the septum relieves this inhibition and allows initiation of septation.

178. Development of a Protein-Protein Interaction Platform in *Neurospora Crassa*. Shouqiang Ouyang, Katherine Borkovich. Plantn Pathology and Microbiology, University of California, Riverside, Riverside, CA.

The objective of this study is to generate a protein-protein interaction platform for *Neurospora crassa*. We have constructed Dmus-S1::nat and Dmus-S2::nat strains that also carry the Drid::nat mutation to eliminate RIP. These strains are used as recipients for transformation. Ten genes were solicited as candidates from the N. crassa community, including SAD-1/SAD-2, WC-1/WC-2, FQG/FQH, OS-4/ RG-1and GNB-1/GNG-1. We construct vectors for each protein by amplifying the ORFs from wild type *N. crassa* genomic DNA using gene-specific primers. Protein constructs are expressed with a V5-GFP or S-tag-RFP tag from the pan-2 or iri locus, respectively in *N. crassa*. Protein complexes can be isolated by immunoprecipitation using antibody to the GFP/V5 or RFP/S-tag epitope. Both immunoprecipitation and the overlap localization of fluorescent proteins (GFP and RFP) data will streamline our ability to monitor protein-protein interactions and co-localization in vivo in *N. crassa*.

179. Specific Structural Features of Sterols Affect Cell-Cell Signaling and Fusion in *Neurospora crassa*. Martin Weichert, Ewald Priegnitz, Raphael Brandt, Thorben Nawrath, Stefan Schull, Andre Fleissner. 1) Institut für Genetik, Technische Universität Braunschweig, Spielmannstrasse 7, 38106 Braunschweig, Germany; 2) Institut für Organische Chemie, Technische Universität Braunschweig, Hagenring 30, 38106 Braunschweig, Germany.

Sterols are major components in the plasma membrane of microorganisms. They modulate the physical properties of the lipid bilayer, e.g. fluidity. By interacting with certain lipids and proteins in the plasma membrane, sterols cluster into microdomains which might act as platforms for many biological functions, such as signal transduction. In the early stages of colony formation in *Neurospora crassa*, germinating spores direct their growth towards each other, establish physical contact, and fuse. Cell-to-cell signaling requires the coordinated dynamic recruitment of the MAP kinase MAK-2 and the cytoplasmic protein PRM1 to the tips of interacting cells. Subsequent plasma membrane fusion is facilitated by the transmembrane protein PRM1. Here, we report that mutants affected in the biosynthesis of ergosterol, the major sterol in most fungal species, show distinct defects during germing fusion. Deletion of erg-2, which encodes an enzyme mediating the last step in the pathway, strongly impairs both directed growth and cell fusion. Interestingly, both MAK-2 and SO mislocalize at the tips of interacting Derg-2 germings. In contrast, the absence of ERG-10a and ERG-10b, two enzymes with redundant function that act upstream of ERG-2, does not affect cell-to-cell communication. However, Derg-10a Derg-10b germing pairs show DPRml-like deficiencies in plasma membrane merger. By relating the sterol composition and fusion competence of several erg mutants, we find that not the absence of ergosterol but the accumulation of sterol intermediates specifically impairs distinct steps of germing fusion. While the presence of two double bonds in the sterol side chain provokes Derg-2-like deficiencies, an altered double bond arrangement in the sterol ring system causes DPRml-like defects. During sexual development, cell fusion precedes the fertilization of fruiting bodies. Unlike the defects during germing fusion, female and male mating partners of Derg-2 and Derg-10a Derg-10b efficiently fuse, suggesting that alterations in the sterol composition specifically impair signaling mechanisms mediating vegetative cell fusion. These data suggest that specific structural features of sterols differentially affect membrane properties and functions, such as the membrane recruitment of proteins, the assembly of signaling complexes, and plasma membrane fusion.

180. The role of NADPH oxidases in *Neurospora crassa* cell fusion. Nallely Cano-Dominguez, Ernestina Casto-Longoria, Jesus Aguirre. 1) Departamento de Microbiologia, CICESE, Ensenada, Baja California, Mexico; 2) Departamento de Biologia Celular y Desarrollo. Instituto de Fisiologia Celular UNAM, Mexico City, D.F. Mexico.

Hansberg and Aguirre proposed that reactive oxygen species (ROS) play essential roles in cell differentiation in microorganisms. ROS are generated mainly during mitochondrial electron transport and by the action of certain enzymes. The NADPH oxidases (NOX) are enzymes that catalyze the production
of superoxide by transferring electrons from NADPH to oxygen. *Neurospora crassa* contains the NADPH oxidases NOX-1 and NOX-2 and a common regulatory subunit NOR-1. NOX-2 is essential for ascospore germination, while NOX-1 is required for sexual and asexual development, polar growth and cell fusion. NOR-1 is essential for all these NOX functions. We have found that a functional NOR-1::GFP fusion is localized throughout the cytoplasm, enriched at the hyphal tip and sometimes in aggregates. This suggests that the functional NOX complexes are probably not localized at the plasma membrane. Up to now NOX function in fungi has been evaluated in mutants that completely lack NOX proteins. We generated nox-1 alleles that result in NOX-1 proteins carrying substitutions of proline 382 by histidine or cysteine 542 by arginine, which affect NADPH-binding. Equivalent mutations in phagocytic Nox2/gp91phox do not affect protein stability but completely lack oxidase activity. P382H and CS42R mutants did not produce sexual fruiting bodies and showed a decreased growth and differentiation of aerial mycelia, without affecting production of conidia, these results indicate that sexual development depends on ROS production by NOX-1, whereas during asexual differentiation NOX-1 plays an important role independently of its catalytic activity. Dnox-1, Dnor-1, P382H NOX-1 and CS42R NOX-1 mutants were all able to produce some conidial anastomosis tubes (CATs) but they were unable to complete cell-fusion. All these mutants are also impaired in vegetative hyphae- hyphae fusion, which might explain the growth defects in Dnox-1 and Dnor-1 strains. CATs production is delayed in the presence of antioxidant N- acetyl cysteine (NAC) and Dnox-1 strains show an increase in CATs fusions. The results suggest that some ROS may be implicated in signaling CATs homing and vegetative fusion.


Polarized growth in filamentous fungus requires an excellent and precise machinery to select specific sites where multiple protein complexes assemble to ensure the generation of highly polarized hyphae. One of these protein complexes is the Cdc42p/Bud1p-Bud2p-Bud5p module, which, in *Saccharomyces cerevisiae*, has the function of selecting the proper site of budding. In this study, we characterized the intracellular localization and dynamics of protein homologues for Bud1p, Bud2p and Bud5p in the filamentous fungus *Neurospora crassa*. Preliminary results of *in vivo* confocal microscopy analysis shows that both Bud-2 and Bud-5 display distinct localization patterns in both mature hyphae and germings. In mature hyphae, Bud-2 localization is confined to the apical cytosol, occupying the core of the Spitzenkörper (Spk), while Bud-5 was observed in the apical region of the cells as a bright spot with higher intensity at the outer base adopting a hand fan shape, partially colocalizing with the Spk. In contrast, Bud-2 in germings was associated with the cell membrane and organized as a cap shape covering the apex of the cells, while Bud-5 localization was observed in three different ways: as a bright spot at the apex of germinating spores, then as a cytosolic crescent-shape in longer germ tubes and finally adopting a similar localization pattern as in mature hyphae. Bud-2 and Bud-5 also display distinct localization patterns during branching and septum formation. Bud-2 participates in septum formation while Bud-5 was only involved during the initiation of lateral branches. The distinct cellular localization patterns of Bud-2 and Bud-5 suggest that although both proteins may be important for cell polarity establishment, they also participate in other morphogenetic processes in *N. crassa*.

182. The role of calcium and calmodulin during cell fusion and colony initiation in *Neurospora crassa*. Chia-Chen Chang, Nick Read. Fungal Cell Biology Group, Institute of Cell Biology, University of Edinburgh, Edinburgh EH9 3HJ.

Calcium is an ubiquitous signalling molecule which regulates many important processes in filamentous fungi including spore germination, hyphal growth, mechanosensitive stress responses, circadian rhythms, and virulence. Transient increases in cytosolic free calcium ([Ca$^{2+}$]$_{cyt}$) act as intracellular signals. As the primary intracellular Ca$^{2+}$ receptor, calmodulin (CaM) converts these Ca$^{2+}$ signals into responses by regulating the activity of numerous target proteins. We have found that both Ca$^{2+}$-free medium and two CaM antagonists (calmidazolium and trifluoperazine) selectively inhibit a form of cell fusion called conidial anastomosis tube (CAT) fusion that occurs during colony initiation in the fungal model *Neurospora crassa*. GFP labelled CaM localized as dynamic particles associated with the plasma membrane and moved around within the cytoplasm in both germ tubes and CATs. In particular, CaM showed a dynamic accumulation at two growing tips of CATs that exhibit chemoattraction towards each other. CaM also localized at developing septa in germ tubes. The tubulin inhibitor, benomyl, reduced the movement of CaM in the cytoplasm. Moreover, the absence of extracellular Ca$^{2+}$ inhibited the recruitment of CaM to CAT tips as well as inhibiting CAT chemoattraction. The deletion of the *myosin-5* (myo-5) gene caused the mis-localization of CaM in tips of growing germ tube and CATs. This suggests that the movement of cytoplasmic CaM involves transport along microtubules, and the recruitment of CaM to tips involves myosin-5 along F-actin and is dependent on extracellular Ca$^{2+}$.


*Neurospora crassa* has two putative cyclic nucleotide phosphodiesterases, *PDE-1* (NCU00237) and *PDE-2/ACON-2* (NCU00478). The *pde-2* disruptants showed the normal mycelial growth but lacked the ability to produce conidia, these phenotypes resembled those of the *hah* mutant which has a point mutation in the *PKA* (protein kinase A) regulatory subunit gene. The phenotypes of double mutants, *pde-2:pkac-1* and *hah:pkac-1* mutants, resembled those of the *pkac-1* mutant which shows slow growth and hyperconidiation. In contrast, hyperconidiation of the adenyl cyclase *cr-1* mutant was suppressed by the *hah* mutation but not by the *pde-2* mutation. These results indicate that *PDE-2* acts as a major *CAMP* phosphodiesterase in *CAMP*-PKA pathway, its deletion leads to the hyper-activation of this pathway. Any mutants in *CAMP*-PKA pathway including *pde-2* and *hah* mutants, did not show osmotic sensitivity. However, both *pde-2* and *hah* mutations caused the enhanced osmotic sensitivity in *os-1* (histidine kinase) and *os-2* (MAP kinase) mutants, suggesting of cross-talk between *CAMP*-PKA pathway and OS-2 MAP kinase pathway.

184. Genetic analysis of *GNB-1* and *CPC-2* with the G alpha subunits in Heterotrimeric G protein signaling in *Neurospora crassa*. AMruta Garud, Plant Pathology, UC, Riverside, Riverside, CA.

Heterotrimeric G protein signaling is mediated by Gbalpha subunits. *Neurospora crassa* has three Ga subunits (GNA-1, GNA-2 and GNA-3), one Gb (GBN-1) and one Gg (GGN-1). The GNB-1 protein contains seven tryptophan-aspartate (WD) repeats, suggesting it assumes a beta propeller form. Genetic epistasis has been demonstrated between gnb-1 and the three Ga subunit genes. gna-3 is epistatic to gnb-1 for submerged culture conidiation, while gna-1 and gna-2 are epistatic to gnb-1 during aerial conidiation. In contrast, gnb-1 is epistatic to gna-2 and gna-3 during aerial hyphal development. Additional proteins that have a 7-WD repeat structure have been implicated as Gb subunits in other fungi. The Cross Pathway Control (CPC-2) protein has a seven WD repeat structure, and shares 70% similarity to the mammalian protein RACK-1. In *Neurospora*, CPC-2 was previously shown to play a role in general amino acid control. Genetic epistasis with CPC-2 and the Ga proteins is being studied, using strains lacking CPC-2 and one Ga gene, as well as cpc-2 deletion mutants carrying constitutively activated, GTase-deficient Ga alleles. It is seen that gna-3 is epistatic to cpc-2 during apical extension, aerial hyphal height and asexual sporulation in submerged cultures. gna-1 and gna-2 demonstrate some functional independence. Yeast two hybrid assays show that CPC-2 interacts with GNA-1 and GNA-3. Additional interactions are being examined using additional in vivo and in vitro methods to validate whether CPC-2 acts
185. Communication Interference during Cell Fusion in *Neurospora crassa* is controlled by a Region under Balancing Selection in the Heterokaryon Incompatibility Locus *het-c*. Jens Heller, Javier Palma-Guerrero, N. Louise Glass. Department of Plant and Microbial Biology, University of California at Berkeley, Berkeley, CA.

Vegetative hyphal fusion events are associated with establishment of a fungal colony. However, non-self recognition during fusion events is important to prevent hybrids between genetically dissimilar individuals that might spread mycoviruses, debilitated organelles, and others throughout a fungal population. In filamentous fungi, the ability of two individuals to form a productive heterokaryon via hyphal fusion is controlled by specific loci termed *het* loci. Stable heterokaryons will only form if the individuals involved have identical alleles at all *het* loci. Accordingly, heterokaryotic cells formed between strains that differ in allelic specificity at one or more *het* loci are rapidly destroyed (programmed cell death) or strongly inhibited in their growth. In *Neurospora crassa*, three allelic specificity groups were identified for *het-c*, which is one of the eleven genetically identified *het* loci in this species. We observed that strains with different haplotypes at *het-c* not only show heterokaryon incompatibility (HI) after cell fusion, but also show reduced chemotrophic interactions and cell fusion between conidial germlings (communication interference). These data indicate that *N. crassa* germlings can distinguish both self and nonself at a distance, and which presumably involves diffusible ligands. Two regions of the glycine-rich single-pass plasma membrane protein HET-C were shown to be under balancing selection and both have different functions. By analyzing different chimeras of *het-c*, we demonstrate that the HET-C specificity domain (amino acids 194-236; region I), is required for inducing programmed cell death during HI, but does not affect communication interference during germling fusion. In contrast, the second region of *het-c* that is also under balancing selection (amino acids 521-599; region II) is responsible for communication interference during germling fusion. To understand the mechanism underlying communication interference, we are identifying which amino acids in HET-C region II are responsible for this trait. In addition, we are determining the cellular localization of HET-C during germling fusion and whether the HET-C region II is a processed form, resulting in a diffusible peptide that is responsible for communication interference during chemotrophic interactions and cell fusion of conidial germlings.


The bud emergence (BEM) 46 proteins are evolutionarily conserved members of the a/b-hydrolase super family. The exact function(s) of the protein remain unknown. Vegetative hyphae, perithecia and ascospores of *Neurospora crassa* RNAi and over-expressing transformatants develop normally, but hyphal germination from ascospores is impaired. These results indicate a role BEM46 in maintaining cell type-specific polarity in *N. crassa*. In an attempt to further analyse BEM46 function, alternative splicing was observed in the bem46 RNAi line. We present evidence that alternative splice products impair ascospore germination. The BEM46 protein is localized in the perinuclear endoplasmatic reticulum and also forms spots near to the plasma membrane (Mercker et al. 2009). The use of Lifeact-TagRFP (Lichius & Read pers. comm.) and Bem46-eGFP in heterokaryons of *N. crassa* indicated that the Bem46 protein is not interacting with actin. Likewise, the use of the lipid raft-stainer TexasRedTM showed no co-localization with Bem46-eGFP. We analyzed the potential co-localization of Bem46 with the eisosomal protein LSP1. To that end we cloned the corresponding *N. crassa* ortholog of *lsp1* and fused it to RFP. Indeed we were able to demonstrate a co-localization of LSP1 and BEM46. A yeast two-hybrid approach was undertaken using a previously established *N. crassa* two-hybrid library (Seiler pers. comm.). We identified one interacting protein, the anthranilate synthase component II (Walker & DeMoss 1986). Further investigation showed that the BEM46 protein is likely to interact with the F domain of that protein, which is a (S'-phosphorybosyl)-anthranilate isomerase. The interaction was confirmed in *vivo* by employing bimolecular fluorescence complementation assays.

References:

187. The alternative oxidative induction pathway is involved in senescence associated with over-replication of a mitochondrial plasmid in *Neurospora crassa*. Niccolite Dutkken, Jonathon Gutzeit, Maze Ndonwi, John Kennell. Biology, Saint Louis University, St Louis, MO.

Senescence in *Neurospora crassa* is caused by dysfunctional mitochondria, which is most often due to the effects of mitochondrial plasmids. Variant forms of the Maurerische plasmid cause senescence by integrating into the mitochondrial genome or by over-replicating, disrupting essential mitochondrial genes or their synthesis. Genetic analysis of plasmid-containing strains that escape senescence indicate that two nuclear mutations are required for longevity. One of the mutations associated with a long lived (LL) strain involves the regulation of Alternative Oxidase (AOX). AOX is induced by mitochondrial dysfunction and is among several nuclear encoded genes involved in mitochondrial function and/or biogenesis that are upregulated during senescence. A model of senescence is proposed in which dysfunctional mitochondria stimulate mitochondrial biogenesis resulting in an accumulation of defective mitochondria. Here we show that the LL strain fails to induce AOX due to a mutation in *aad-2* that encodes a zinc cluster transcriptional regulator of the structural alternative oxidase gene, *aad-1*. Surprisingly, a functional AOX is not required for senescence. This implies that other genes controlled by the AOX induction pathway play a critical role in mitochondrial function in *N. crassa*. Homologs of AOD2 in other fungal species have been shown to regulate glucocorticosterone as well as genes involved in mitochondrial function including subunits of the electron transport chain. Mutations in the AOX induction pathway are not sufficient to overcome plasmid induced senescence and a second nuclear mutation is required. This mutation interferes with the integrative form of senescence and is hypothesized to be associated with either mitochondrial recombination or the selection of mitochondrial rearrangements. The studies of senescence in *N. crassa* provide insights into how fungi respond to mitochondrial damage.

188. Relationship among mutagen sensitivity, senescence and mitochondrial morphology in the ultraviolet sensitive-5 mutant of *Neurospora crassa*. Kiminori Kurashima, Michael Chae, Hirokazu Inoue, Shin Hatakeyama, Shuuitsu Tanaka. Laboratory of Genetics, Saitama University, Saitama, Japan.

The *uvs*-5 mutant of *Neurospora crassa* had been isolated that showed high sensitivity to mutagens (Schroeder, 1970 Mol. Gen. Genet. 107:291-304). This mutant also has phenotypes such as senescent, i.e. shortened life span, and progressive accumulation of mitochondrial DNA deletions (Hausner et al., 1995 Fungal Genet. News 42A: 59). These phenotypes were quite similar to the *mus*-10 mutant that we reported previously (Kato et al., 2010 Genetics 185:1257-1269). Further, *mus*-10 and aged *uvs*-5 strains showed fragmented mitochondrial feature although tubular shape was observed in wild type strain. Since the *uvs*-5 mutant had been mapped very closely to *fzo1*, which encoded homologue of dynamin-like GTPase mitofusin, the sequence of the *fzo1* gene in the *uvs*-5 mutant was determined. A single mutation was found as a deducing amino acid substitution of Gin to Arg in the 386th position locating in the conserved GTPase domain. Forced expression of wild-type FZO1 in the *uvs*-5 strain suppressed the defect in mitochondrial morphology and the mutagen sensitivity, but did not in the case of expressing mutated FZO1. Moreover, introduction of this mutation into the endogenous *fzo1* gene of the wild-type strain resulted in showing phenotypes of the *uvs*-5 mutant. Thus, we concluded that the responsible gene of *uvs*-5...
is {\it fzo1}. Attempting to disruption the {\it fzo1} gene was failed, so the {\it fzo1} gene were suggested to be essential for viability as showing in almost all eukaryotes except for yeast. Therefore the mutation of {\it uvs-S} may be a useful model for studying the relation between mitochondrial fusion and early senescence in higher eukaryotes.

189. Localization of EGL-1 and EGL-2, two GPI anchored cell wall b (1-3) endoglucanases, at hyphal apices and septa, and in interconidial septa in Neurospora crassa. Leonora Martinez, Meritxell Riquelme. Microbiology Department, Center for Scientific Research and Higher Education at Ensenada, Baja California.

The unitary model of cell wall growth suggests that the polarized extension of hyphae in filamentous fungi results from the coordinated synthesis and discharge of new cell wall polymers, the action ofhydrolytic enzymes that provide plasticity to the wall and turgor pressure to drive cell expansion. Currently, there is limited information on enzymes capable of hydrolyzing cell wall polymers and that could be contributing to cell wall remodeling. EGL-1 and EGL-2 are putative b (1-3) endoglucanases in Neurospora crassa, with potential binding sites for a glucosyl phosphatidylinositol group (GPI), which would allow them to get anchored into the plasma membrane. To investigate whether these proteins participate in key morphogenetic events during the development of N. crassa, EGL-1 and EGL-2 were labeled with the green fluorescent protein (GFP). For egl-1, the {\it gfp} gene was inserted within the egl-1 encoding sequence, just after the signal peptide sequence. For egl-2, the insertion took place right before the GPI-binding site. Both endoglucanases were localized in the hyphal apical plasma membrane and in septa, however, EGL-2-GFP was strongly and more definite localized at the apical dome and EGL-1-GFP showed less intensity with increasing fluorescence from the subapex to the tip. EGL-1-GFP was mostly found at hyphal septa and interconidial septa and EGL-2-GFP was faintly present in a few old septa. Our results suggest that lytic activity of enzymes, such as the endoglucanases EGL-1 and EGL-2 in N. crassa, is present in critical areas during vegetative morphogenesis, where these enzymes probably play a role in cell wall remodeling, as postulated by the unitary model of cell wall growth.

190. Stability of a G protein alpha subunit in genetic backgrounds lacking the G beta subunit or a cytosolic guanine nucleotide exchange factor. Alexander V. Michkov, Katherine A. Borkovich. Plant Pathology and Microbiology, University of California, Riverside, Riverside, CA.

Heterotrimeric G proteins consist of alpha, beta and gamma subunits. Regulation is accomplished through the alternation between binding of GDP (inactive form) and GTP (active form) by the alpha subunit and dissociation of the alpha subunit and beta-gamma dimer. GDP/GTP exchange is facilitated by both cell surface G protein coupled receptors and cytosolic guanine nucleotide exchange factors (GEFs), such as RIC8. Neurospora crassa has three G alpha subunits (GNA-1, GNA-2 and GNA-3), one G beta (GNB-1), and one G gamma (GNG-1). Interestingly, mutants lacking gnb-1 or the cytosolic GEF ric8 exhibit some defects in common with the gna-1 deletion mutant, which may be explained by the reduced GNA-1 protein levels observed in these mutants. Previous studies in our laboratory showed that levels of gna-1 mRNA are similar in wild type and mutants lacking gnb-1 or ric8, consistent with a post-transcriptional mechanism. Using genetic and biochemical approaches, this study investigated the mechanism underlying regulation of GNA-1 stability in regards to GTP/GDP bound state and amount of protein (normal or overexpressed). The results demonstrate that levels of GNA-1 protein are not visibly reduced over 36 hours in a wild-type background after halting translation using cycloheximide, suggesting GNA-1 is very stable in wild type. To check stability of GDP or GTP bound GNA-1 in different backgrounds, we transformed mutants lacking the gna-1 gene and gnb-1 or ric8 with a wild type (gna-1{\it{167}}) or constitutively active, GTPase-deficient gna-1 allele (gna-1{\it{204L}}). Overexpressing gna-1{\it{204L}} (GDP bound) in a wild-type background increased the level of GNA-1 protein ~3 fold, while overexpression in a gnb-1 mutant gave a nominal increase (~1.6x). Overexpressing gna-1{\it{204L}} (GTP bound) in the Dgnb-1 or Dric8 backgrounds led to ~2 fold higher levels of GNA-1 compared to wild type. In summary, GNA-1 is very stable in wild type, but stability decreases dramatically in gnb-1 and ric8 deletion mutants. The GTP-bound G alpha protein is more stable in a gnb-1 mutant background than GDP-bound GNA-1 protein.

191. Functional analysis the Saccharomyces cerevisiae Ste20, Cla4 homologue in Neurospora crassa. Yuhei Nagami, Makoto Fujimura, Akihiko Ichishi. Faculty of Life Sciences, Toyo University, ORA-GUN, GUNMA, Japan.

Signal transduction pathways are important for a variety of features of fungal development. Small GTPases of Rho family act as molecular switches regulating cell signalling, cytoskeletal organization and vesicle trafficking in eukaryotic cells. The Rho family GTPase Ced42 was first identified in the yeast Saccharomyces cerevisiae, where it is essential for initiation of bud formation and the subsequent switch from apical to isotropic growth. The activation of Ced42 is catalyzed by Cdc24 guanine nucleotide exchange factors (GEFs), which convert Ced42 from an inactive GDP-bound form to the active GTP bound form. Bem1 functions as a scaffold connecting Ced42 with its activator Cdc24. The GDP-bound Ced42 can activate p21-activated kinase (PAK), Ste20 and Cla4. Neurospora crassa has two PAK family kinases Cla4 and Ste20 homologs. We have knowledge of the function of their PAK kinases in N. crassa. In this study, we performed functional analysis of stk-4 (Ste20 homolog) and vel (Cla4 homolog) in N. crassa. The stk-4 deletion mutant showed slow growth than wild type strain, and vel deletion mutant showed more severe growth defects. To determine the subcellular localization and dynamics of STK-4 protein, we constructed GFP-STK-4 fusion constructs. The gfp encoding sequence was fused to the 3’ end of the stk-4 open reading frame. We also constructed the GFP-BEM-1 fusion protein. These constructs were introduced into his-3 locus, and observed using confocal fluorescence microscopy (LSM-510). Both fusion proteins were accumulated at growing hyphal tips and septa. From these results, we consider that STK-4 and BEM-1 are function at the same site.


Due to their capacity to secrete large amounts of proteins, particularly hydrolytic enzymes, filamentous fungi are of great interest for high-level protein production in various industries, such as the textile, pharmaceutical, and biofuel industries. Although the basic components of the eukaryotic secretion pathway are also conserved in filamentous fungi, the highly polarized and compartmentalized growth mode of filamentous fungal hyphae mandates pathways of secretion that are specific to these fungi. While certain aspects of filamentous fungal secretion are under active study, a basic characterization of the entire pathway from start to finish remains to be performed. Such a characterization might provide insights into how filamentous fungi are able to secrete large amounts of enzymes and how these fungi can be engineered to produce even more enzymes in the future. This is particularly of interest to the process of biofuels production, in which the inexpensive production of large amounts of cellulases is a major bottleneck to the efficient and cost-effective production of cellulosic biofuels. In nature the model fungus Neurospora crassa secretes a host of cellulases to allow it to grow on burnt vegetation. The tractability of N. crassa makes it an excellent model to characterize protein secretion in filamentous fungi, particularly the secretion of industrially relevant cellulases. To achieve this goal we are characterizing the cellulase secretion pathway in N. crassa by following the trafficking of fluorescently tagged Endoglucanase 2 (EG-2), a major secreted endocellulase. To determine the compartments through which cellulases traffic we are co-localizing EG-2-GFP with fluorescently-tagged markers of the ER, Golgi, endosomes, and the Spitzenkorper and are assaying the...
consequences to EG-2-GFP trafficking of blocks to secretion imposed by pharmacological or mutational insults. Our initial results indicate that EG-2-GFP shows localization to the ER and is mostly absent from the Spitzkenkorper, suggesting trafficking through a classical ER to Golgi secretory pathway and terminal secretion along lateral hyphae walls. Additionally, targeted blocks to the secretory pathway indicate a potential role of endosomes in EG-2-GFP trafficking.

193. Towards understanding the endoplasmic reticulum associated degradation process of misfolded glycoproteins in Neurospora crassa. Georgios Tripplep1, Hiroto Hirayama2, Tadashi Suzuki1, Akira Hosomi1, Mukesh Dubey3, Magnus Karlsson1. 1) Uppsala BioCenter, Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Box 7026, 75007, Uppsala, Sweden; 2) Glycometabolome Team, Systems Glycobiology Research Group, RIKEN Advanced Science Institute, 2-1 Hirosawa, Wako Saitama 351-0198, Japan.

N-glycosylation is an important post-translational modification of proteins, which occurs in the Endoplasmic Reticulum (ER). These N-linked glycans are reported to play an important role in correct protein folding. Glycoproteins that are unable to fold properly are subjected to destruction by an ER-associated degradation process (ERAD). Degradation of these glycoproteins generates free oligosaccharides (fOs). In animal and plant cells mainly three types of hydrolytic enzymes are involved in the ERAD pathway. First, PNGases which cleave the sugar chain from the protein releasing fOs with N,N'-diacetylchitobiose moieties (fOs-GN2). Secondly, ENGases which catalyse the glycosidic bonds in N,N'-diacetylchitobiose moieties, generating fOs with a single N-acetylglucosamine at their reducing ends (fOs-GN1), and thirdly, a-mannosidases responsible for trimming the mannose chains before final degradation in lysosomes. The existence of this pathway in filamentous ascomycetes is unknown. In this study we investigate the function of ENGases in N. crassa by analysing the phenotype of deletion strain Dgh18-10 and quantifying the content and type of fOs (fOs-GN1 or fOs-GN2), using dual gradient high performance liquid chromatography. Since cytosolic PNGase is enzymatically inactive in N. crassa, ENGases possibly have a crucial role in the ERAD pathway. We found that deletion of an intracellular ENGase results in severe phenotypic effects. This deletion strain shows significantly slower growth rate in carbon-rich media but grows faster in abiotic stress conditions, indicating a more resistant cell wall. Moreover, the conidiation rate is higher in Dgh18-10 compared to WT. Sexual reproduction is also affected, since no ascospores were observed in Dgh18-10. Additionally, the total amount of extracellular proteins was significantly lower in this deletion strain compared to WT. Finally, this mutation causes repression of three chitin synthase genes in N. crassa. Similar results were also observed in the mycoparasitic ascomycete Trichoderma atroviride. These data may suggest that deletion of cytosolic ENGase leads to accumulation of misfolded glycoproteins in the fungal cytosol, which somehow affects its protein secretion/structure of cell wall. This is the first study of the ERAD pathway in filamentous ascomycetes.

194. Saccharomyces cerevisiae spore development and protection against reactive oxygen species. Steve Gorsich, Tricia Stokes, Michelle Steidemann, Kyle Kern. Dept Biol, Central Michigan Univ, Mt Pleasant, MI.

The generation of spores in S. cerevisiae is essential for sexual reproduction and survival of the organism. When diploid S. cerevisiae cells undergo meiotic division to produce four spores it is important for each spore to not only get a haploid copy of nuclear chromosomes, but also a complete complement of organelles and potentially RNP granules. For instance mitochondria undergo temporally regulated fusion and fission events to assure that mitochondria are represented equally in each of the resulting spores. When this network is not maintained, due to mutations in mitochondrial fission genes (e.g. dnm1/dnm1), it has been shown that fewer spores survive and the ones that do survive have reduced respiratory fitness. In addition to mutations affecting spore production we hypothesized that environmental factors could also influence spore development. In the present study, we demonstrated that hydrogen peroxide can photocopy the mitochondrial fission mutant’s phenotypes. Wild-type S. cerevisiae exposed to hydrogen peroxide have mitochondrial morphology and distribution defects during spore development, reduced spore viability, and decreased respiratory competency just as seen in dnm1/dnm1 fission mutants. We next hypothesized that the phenotypes associated with dnm1/dnm1 mitochondrial fission mutants were caused by increased sensitivity to reactive oxygen species (ROS). To support this we demonstrated that dnm1/dnm1 mutants have an increase in ROS during spore development. In addition, sporulation defects associated with dnm1/dnm1 or wild-type cells exposed to hydrogen peroxide were rescued when we overexpressed oxidative stress protection genes. These findings suggest that the ability of S. cerevisiae to produce optimal numbers of fit spores is heavily influenced by their ability to protect themselves from exogenous or endogenous ROS.

195. Genetic analysis of the role of pexosomes in the virulence and survival in Fusarium graminearum. K. Min1, H. Son2, J. Lee2, G. J. Choi3, J.-C. Kim1, Y.-W. Lee2. 1) Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea; 2) Department of Applied Biology, Dong-A University, Busan 604-714, Republic of Korea; 3) Eco-friendly New Materials Research Group, Research Center for Biobased Chemistry, Division of Convergence Chemistry, Korea Research Institute of Chemical Technology, Daejeon 305-343, Republic of Korea.

Pexosomes are single-membrane-bound organelles that are required for diverse biochemical processes, including b-oxidation of fatty acids and detoxification of reactive oxygen species (ROS). In this study, the role of pexosomes was examined in Fusarium graminearum by functional analysis of three genes (PEX5, PEX6, and PEX7) encoding peroxin proteins required for pexosomal protein import. PEX5 and PEX7 deletion mutants failed to localize the fluorescently-tagged pexosomal targeting signal type 1 (PTS1)- and PTS2-containing proteins to pexosomes, respectively, whereas the PEX6 mutant were unable to localize both fluorescent proteins. Deletion of PEX5 and PEX6 triggered reduced growth on long chain fatty acids and butyrate, while the PEX7 deletion mutants utilized fatty acids other than butyrate. Virulence on wheat heads was greatly reduced in the PEX5 and PEX6 deletion mutants, because they were impaired in both spreading from inoculated florets to the adjacent spikelets through rachis. Disruption of PEX5 and PEX6 dropped survivability of aged cells in planta and in vitro due to the accumulation of ROS followed by necrotic cell death. We suggest that PTS1-type pexosomal catalases are responsible for ROS scavenging. These results demonstrate the functions of pexosomes in survival and ROS detoxification of filamentous fungi.


The ascomycete fungus Magnaporthe oryzae causes rice blast disease. Germination and development of its infection structure, the appressorium on the host surface is orchestrated by a complex set of signals from within the fungus, and later between the fungus and resistant or susceptible plant that, respectively, either triggers host defence or leads to infection. Host defences involve localised production of reactive oxygen species (ROS), which either kill the pathogen directly or block fungal invasion by oxidative cross-linking of cell wall glycoproteins. By contrast, infection suggests that the invading fungus can tolerate or, indeed, bypass such defences. Here, we report rice blast fungus’ capacity to withstand transient oxidative stress during early development. We determine the intrinsic cytoplasmic cell glutathione (GSH) concentration by confocal imaging of monochlorobimane, which becomes fluorescent when conjugated to GSH. The redox poise of the glutathione pool was measured by 4-D confocal excitation ratio imaging of GRX-roGFP2. We reveal that this fungus has an extraordinary ability to tolerate severe insults of H2O2, with rapid recovery of its reduced GSH pool and thence continued
growth. Exploring in vivo responses during infection of susceptible (S) and resistant (R) gene host plants reveals that pathogen penetration and proliferation is hugely restricted in the R plants, but surprisingly, there is no change in the roGFP ratio in planta. Thus the sparse infection hyphae within R plants maintain a highly reduced cytoplasm at all times. This questions whether production of ROS by the host is the primary mechanism responsible for restricting pathogen growth in resistant plants.


Fungal dimorphism evolved in multiple fungal lineages. Many pathogenic fungi are dimorphic, for example, switching between yeast and filamentous states. This switch alters host-pathogen interactions and is critical for pathogenicity. However, in pathogenic zygomycetes, whether dimorphism contributes to pathogenesis is a central unanswered question. The pathogenic zygomycete Mucor circinelloides exhibits multi-budded yeast growth under anaerobic/high CO₂ growth conditions, which Louis Pasteur discovered (Etudes sur la Bierre. 1876). Interestingly, we found that in the presence of the calcineurin inhibitor FK506, Mucor exhibits multi-budded yeast growth. We discovered that Mucor encodes three calcineurin catalytic subunits (CnaA, CnaB, and CnaC) and one calcineurin regulatory subunit (CnrB). Disruption of the cnaA gene results in mutants locked in yeast phase growth. These results reveal that the calcineurin pathway governs the dimorphic transition from yeast to hyphae. In virulence tests, we found that the cnbR yeast-locked mutants are less virulent than wild-type in a heterologous host system, providing evidence that hyphae are a more virulent form of this fungus. Protein kinase A activity was elevated during yeast growth under anaerobic conditions, in the presence of FK506, or in the yeast-locked cnbR mutants, indicating a novel connection between PKA and calcineurin. The cnaA mutants are hypersensitive to calcineurin inhibitors and display a hyphal polarity defect. The mutants produce spores that are larger than wild-type. Notably, we found spore size is virulence in previous studies (Li et al. PLoS Pathogens. 2011). Interestingly, the cnaA mutants were found to be more virulent than wild-type. We also observed that the cnaA mutants germinate earlier inside macrophages, providing a possible explanation for the greater virulence of the mutants. Another pathogenic zygomycete, Rhizopus delemar has three cna genes. Phylogenetic analysis revealed that the duplicated cna genes might result from a whole genome and/or segmental gene duplications. Our results demonstrate that the calcineurin pathway orchestrates the dimorphic transition, spore size dimorphism, virulence, and hyphal polarity in Mucor, and the calcineurin pathway elements have been adapted in zygomycetes via variation in their evolutionary trajectory.

198. Genetic analysis of the components of the ime-2 mediated signaling events during nonself recognition and programmed cell death (PCD) in **Neurospora crassa**. **Joanna A. Bueche¹, Elizabeth A. Hutchison²,³, N. Louise Glass¹. 1) Plant and Microbial Biology, UC Berkeley, Berkeley, CA, 94720; 2) Cornell University Microbiology Department, Ithaca, NY 14853.

Recently, we revealed genetic and functional differences in meiotic initiation machinery between Neurospora crassa and Saccharomyces cerevisiae. While N. crassa is missing some meiotic genes identified in yeast, it has three homologs of the whole meiotic transcriptional regulator, Ndt80. None of the NDT80 homologs are required for meiosis in N. crassa. One of the NDT80 homologs, vib-1 is essential for heterokaryon incompatibility (HI) in N. crassa, a nonself recognition mechanism in filamentous fungi. Mutations in vib-1 suppress cell death caused by HI as well as secretion of the extracellular proteases during the nitrogen starvation. Furthermore, deletion of a IME2 (a kinase involved in initiation of meiosis in S. cerevisiae) homolog in N. crassa, ime-2, does not affect sexual development, results in a significant elevation of secreted proteases in response to nitrogen starvation. Moreover, a Dwb-1 Dime-2 mutant restored wild-type levels of cell death during the HI and normal production of extracellular proteases; a deletion of ime-2 suppressed these vib-1 phenotypes. Based on the evidence, we hypothesize that IME-2 negatively regulates a cell death pathway that functions in parallel to the VIB-1 HI pathway and a protease secretion pathway positively regulated by VIB-1. We used a widely modified yeast consensus sequence for ime2 phosphorylation to scan (Scansite) the entire N. crassa genome for possible targets and obtained a list of 30 candidates including VIB-1. All targets were assessed for secretion of the extracellular proteases in absence of nitrogen. Strains containing deletions of 13 of the 30 genes identified in the screen were significantly affected in protease secretion. Mutations in these candidate genes will be tested for the ability to alleviate cell death and Heterokaryon Incompatibility (HI) in the presence and absence of ime-2 and vib-1 hence assessing their role in the parallel HI/PCD pathway redundant with VIB-1.

199. PRO45 is a component of the conserved STRIPAK complex in **Sordaria macrospora**. **Steffen Nordzieke¹, Benjamin Fränzel¹, Sandra Bloemendal¹, Dirk Wolters¹, Ines Teichert¹, Ulrich Kück¹. 1) General and Molecular Botany, Ruhr-University Bochum; 2) Analytical Chemistry, Ruhr-University Bochum, Universitätsstr. 150, 44801 Bochum, Germany.

The complex formation of three-dimensional fruiting bodies in Sordaria macrospora is mediated by an interaction between developmental proteins and conserved signaling cascades and thus an excellent system for developmental biology. We recently have characterized a STRIPAK complex in Sordaria macrospora that is involved in the regulation of fruiting body development. This complex contains striatin (PRO11), a striatin interacting protein (PRO22), the scaffolding subunit of protein phosphatase 2A (SmPP2AA) and a phocein homologue (SmMOB3) [1, 2].

Here we describe PRO45, a novel subunit of the STRIPAK complex in filamentous fungi which is a homolog of the human sarcosomal membrane associated protein (SLMAP). We also present the functional characterization of PRO45: Strains lacking the gene for PRO45 show sterility together with a severe defect in hyphal fusion and vegetative growth rate. The primary structure of PRO45 contains a forhead-associated (FHA) as well as a transmembrane domain. Complementation studies showed that a lack of the FHA domain is responsible for the described defects, whereas a missing transmembrane domain does not affect development. Tandem affinity purification (TAP) followed by mass spectrometry and coimmunoprecipitation (Co-IP) showed subunits of the STRIPAK-complex as interaction partners, confirming the homology of human and fungal STRIPAK. Further microscopic studies provide evidence for a localization of PRO45 in the ER as well as in the nuclear envelope. Integrating these observations, we propose that PRO45 has a function in the physical and signaling connection of STRIPAK-complex and nucleus.


200. Molecular Determinants of Sporulation in **Ashbya gossypii**. **Jurgen W. Wendland, Lisa Wasserstroem, Klaus Lengeler, Andrea Walther. Yeast Genetics, Carlsberg Laboratory, Copenhagen V, Copenhagen V, Denmark.**

Previously we have analysed the pheromone response MAPK signal transduction cascade in A. gossypii. The major findings were (i) deletion of both pheromone receptor genes STE2 and STE3 did not inhibit sporulation whereas (ii) deletion of the transcription factor STE12 resulted in hypersporulation (Wendland et al. 2011). Here we present our analysis of key A. gossypii homologs of Saccharomyces cerevisiae sporulation specific genes. We show that
mutants in IME1, IME2, KAR4, and ND780 are blocked in sporulation. Mutants in IME4, KAR4, and UME6 also confer a vegetative growth defect. IME4 expression was found during vegetative growth while IME2 was not detected under these conditions. We performed transcriptional profiling of non-sporulating strains and determined a core set of about 50 down-regulated sporulation-specific genes in these mutants. Interestingly, this set of down-regulated genes is upregulated in the A. gossypii ste12 mutant providing regulatory evidence of the hypersporulation phenotype of this mutant. Other genes identified in the RNAseq data indicated that during development of sporangia metabolic genes for nutrient uptake are active. Therefore we performed Return-To-Growth assays with mutants inhibited in the sporulation pathway. These strains were kept under conditions in which the wild type initiates sporulation. This lead to induction of sporangium formation, a stage at which these strains remained. Supply of new nutrients resulted in hyphal outgrowth in all mutants indicating that after initiation of the sporulation program A. gossypii can revert to vegetative growth at different stages. In addition we identified differential regulation of two endoglucanases encoded by ENG1 and ENG2. While ENG1 was not differentially regulated, ENG2 was down-regulated in e.g. ime1 but strongly up-regulated in ste12. Deletion analysis of ENG2 showed that Eng2 is required for hyphal fragmentation into individual sporangia. We can thus provide a detailed overview of the genetic regulation of sporulation in A. gossypii. A comparison with S. cerevisiae highlights the role of Kar4 in sporulation upstream of IME1. Finally, our study provides further evidence that the pheromone signaling response MAPK-cascade in A. gossypii has a regulatory control function over sporulation as well as regulation of sporulation by nutritional cues.

201. VELVET is regulated by ENV1 and impacts development of Trichoderma reesei. Hoda Razafkan1, Doris Tirsch2, Monika Schmoll1. 1) Health & Environment - Bioresources, AIT Austrian Institute of Technology GmbH, Tulln, Austria; 2) Vienna University of Technology, Institute of Chemical Engineering, Vienna.

In Trichoderma reesei (teleomorph Hypocrea jecorina), light is a crucial environmental factor for initiation of sexual development and considerably influences expression of glycoside hydrolases. In both processes, the light regulatory protein ENV1 plays a key role. Transcriptome analysis revealed that vel1 (encoding the VELVET orthologue) transcription is regulated by the carbon source in the medium. Moreover, ENV1 negatively regulates vel1 in light. Genes coregulated with vel1 are enriched in functions of amino acid metabolism as well as carbon metabolism and include three non ribosomal peptide synthases (NRPS). This regulatory pattern supports a connection of vel1 with primary and secondary metabolism also in T. reesei. VELVET is known to be a regulator of sexual and asexual development in fungi. Also for T. reesei a function in development was likely, as several genes involved in sexual development are coregulated with vel1. Investigation of strains lacking the T. reesei orthologue vel1 under various nutritional, light- and temperature conditions showed that VEL1 is essential for conidiation and growth of aerial hyphae. Moreover, in crossing assays, sexual development with strains lacking vel1 was delayed and in contrast to the wild type never occurred in constant darkness. When vel1 was missing in both mating partners, no fruiting bodies were formed. Although male fertility was intact, female fertility was found to be dependent on the presence of vel1. Strains lacking the light regulator gene env1 are able to undergo sexual development with wild-type strains, but in crosses of two strains lacking env1, no fruiting bodies are formed in light. This defect is assumed to be caused by a deregulation of the pheromone system in these strains. Interestingly, also strains lacking vel1 are able to mate with env1 deletion mutants in light. Together with the regulatory connection between these genes, these findings support a function of vel1 in the same pathway as env1. We conclude that VEL1 in T. reesei regulates sexual and asexual development and is connected to the light response pathway via ENV1.


Penicillium chrysogenum is a filamentous fungus of major medical and historical importance, being the original and present day industrial source of the antibiotic penicillin with a world market value of about 600 million € per year. The species has been considered asexual for over 100 years and despite concerted efforts it has not been possible to induce sexual reproduction. However, we recently were able to detect mating type loci in different strains, indicating a sexual lifecycle. Isolates, carrying opposite mating types, were found in near-equal proportion in nature and we observed transcriptional expression of mating type loci as well as pheromone and pheromone receptor genes [1]. Utilising knowledge of mating-type (MAT) gene organization we now describe conditions under which a sexual cycle can be induced leading to the production of cleistothecia and meiotic ascospores, which were similar to those described recently for Eupenicillium crustaceum [2]. Evidence of recombination was obtained using both molecular and phenotypic markers. The newly identified heterothallic sexual cycle was used for strain development purposes, generating offspring with novel combinations of traits relevant to penicillin production.

Furthermore, the MAT1-1-1 mating-type gene, known primarily for a role in governing sexual identity, was also found to control transcription of a wide range of genes including those regulating penicillin production, hyphal morphology and conidial formation, all traits of biotechnological relevance. For functional characterization MAT1-1-1 knockout and overexpression strains were generated and analyzed. These discoveries of a sexual cycle and MAT gene function are likely to be of broad relevance for manipulation of other assexual fungi of economic importance.


The filamentous fungus S. commune is a model organism for sexual development of basidiomycetes. Numerous studies revealed the importance of two gene loci, A and B, responsible for tetrapolar mating and sexual development. Both occur in multiallelic subloci leading to a large number of different specificities in nature (9 to 32 depending on locus), which then control compatibility or abortion of mating. While A codes for homeodomain transcription factors, B codes for a pheromone/receptor system. The B-receptors (Ste3-like, seven transmembrane domains, G-protein coupled) recognize pheromones of non-self specificity and induce signal transduction pathways and specific gene regulation. After sequencing of strain H4-8 four new Ste3-like GPCRs, homologous to the known B-specific ones, were found. Three of the four B-receptor like genes (brls) are located close to the B-locus. Their function is unknown, because a B-locus defective strain without any interactions seen in B-dependent development still contains those four GPCRs, which obviously do not respond to any wild type pheromone. However, our results indicate their importance since sequence identity - analyzed by PCR and sequencing - between unrelated strains was found arguing for conservation of these genes. Gene expression was first observed by Reverse Transcriptase PCR as well as Microarray analyses, which disproved the theory that brls are pseudo genes. Expression was then investigated by quantitative Real Time PCR during mating interaction and in monokaryotic strains, which showed comparable results only between gene brl4 and the true mating receptor bar2. Also RNA
204. Characterization of new STRIPAK complex interaction partners in the filamentous ascomycete Sordaria macrospora. Britta Herzig, Yasmine Bernhards, Berit Habling, Eva Reschka, Sabine Riedel, Stefanie Pöggeler. Institute of Microbiology and Genetics, Department of Genetics of Eukaryotic Microorganisms, Georg-August-University Göttingen, Germany.

Using Sordaria macrospora as model organism we investigate the complex process of fruiting-body development and involved proteins in this filamentous ascomycete. This differentiation process is regulated by more than 100 developmental genes. Recently, we have shown that a homologue of the human STRIPAK (striatin-interacting phosphatase and kinase) complex engages a crucial role in sexual development in fungi. The S. macrospora striatin homologue PRO11 and its interaction partner SmMOB3 are key components of this complex (Bloemen et al., 2012). PRO11 contains a conserved WD40 repeat domain and is supposed to function as scaffolding protein linking signaling and eukaryotic endocytosis (Pöggeler and Kück, 2004). SmMOB3 (phocein) is a member of the MOB family (Bernhards and Pöggeler, 2011). Beside their important role in multicellular development and hyphal fusion both proteins seem to be involved in vesicular trafficking and endocytosis.

By means of yeast two-hybrid screens and GFP-Trap analysis we identified several new interaction partners of PRO11 and SmMOB3. Similar to PRO11 and SmMOB3, a multitude of them are predicted to be involved in vesicular trafficking and are localized to the ER or to the Golgi. Here, we show the results of a detailed analysis of the new STRIPAK complex interaction partners. Initially, we isolated the cDNA of the genes confirmed and the interaction by yeast two-hybrid. For further characterization and to get knowledge about their cellular functions we created knock-out strains and analyzed their morphological phenotypes. For localization and expression studies we constructed EGFP-tagged fusion proteins and expressed them in S. macrospora.

Bernhards and Pöggeler, 2011; Curr Genet 57 (2): 133-49.
Bloemen et al., 2012; Mol Microbiol 84 (2): 310-23.


Hypocrea jecorina is the sexual form of Trichoderma reesei, an industrially important cellulolytic filamentous fungus. We report that H. jecorina meiosis utilizes a novel Ku70-dependent dupilication mechanism to generate segmentally aneuploid progenies, thus increasing the diversity of genotypes and ensuring more efficient xylan degradation. H. jecorina sexual reproduction yields hexadecacis with 16 linearly arranged ascospores. Our results indicate that these ascospores are generated via two rounds of postmeiotic mitosis following the two meiotic divisions. Remarkably, the hexadecacis ascis frequently (>90%) contain four or eight inviable ascospores with an equal number of viable segmentally aneuploid ascospores. Array-based comparative genomic hybridization revealed that all the viable segmental aneuploid progenies have a large chromosomal duplication (~0.5Mbp). Deletion of the nonhomologous end-joining gene ku70 restores canonical meiosis and 16 viable euploid ascospores. Segmental duplication contains genes involved in xylan degradation and enhances expression of several carbohydrate-active enzymes, particularly cell wall degrading hemicellulases.


Sequencing of the Ceratostictis fimbriata genome has made it possible to consider the long standing question as to how uni-directional mating type switching functions in this fungal pathogen and its relatives. Uni-directional mating type switching was first observed in the homothallic ascomycete C. fimbriata in the 1960’s. Two forms of progeny arise after meiosis, some self-fertile and thus not requiring an opposite mating partner to complete the sexual cycle. Other isolates are self-sterile and unable to reproduce sexually. This loss of self-fertility has been shown to be associated with the loss of a fragment of the mating specific gene, MAT1-2-1, in self-sterile strains. The aim of this study was interrogate the full genome sequence of C. fimbriata to determine whether the full MAT1-2-1 gene is deleted and whether other MAT genes are affected during mating type switching. We were able to determine that C. fimbriata has both the MAT1-2-1 gene and the MAT1-1 genes (MAT1-1-1 and MAT1-1-2). The self-sterile isolates had only lost the MAT1-2-1 gene and one copy of a 230 base pair perfect repeat which flanks this gene in the self fertile isolates. The loss of the entire MAT1-2-1 gene explains the loss of fertility and the repeats are suggestive of the involvement of recombination during the deletion event. This study illustrates a unique mating strategy in the fungi, not previously understood at the molecular level. The newly gained knowledge will also make it possible to consider the mechanisms underpinning uni-directional switching in other species of Ceratostictis.

207. Mannitol is essential for the development of stress resistant ascospores in Neosartorya fischeri. Timon T. Wyatt1, M.R. van Leeuwen1, H.A.B. Wöstien2, J. Dijkstraheur2. 1) Applied and Industrial Mycology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; 2) Microbiology, Utrecht University, Utrecht, the Netherlands.

The sugar alcohol mannitol is one of the main compatible solutes in Neosartorya fischeri and accumulates especially in conidia and ascospores. In fungi, mannitol has been implicated in a wide variety of functions including carbon storage, maintaining reduction potential, water absorption, heat stress protection, protection against oxidative stress, and tolerance against osmotic stress. Biosynthesis of mannitol in ascomycota mainly depends on mannitol 1-phosphate dehydrogenase (MPD). In our study a functional analysis was performed of the MPD encoding gene mpdA of N. fischeri. The fluorescence proteins GFP and DTomato were put under control of the mpdA promoter. Expression of mpdA was observed in aerial hyphae and conidioblasts, but was especially high in ascotama and ascospores. Disruption of mpdA reduced mannitol as much as 85% of the wild type and increased trehalose levels to more than 400%. Decreased mannitol accumulation had no obvious effect on mycelium growth when exposed to temperature and oxidative stress, while an increased stress sensitivity of conidia against heat and oxidative stress was observed. The most distinct phenotype of mpdA disruption was the complete absence of ascospores. Formation of fruiting bodies (ascotama) and asci was not affected but the developmental defect was shown to occur after meiosis. Similar results were obtained by adding the MPD inhibitor nitrophenol to the wild-type strain. Our result suggest a role of mannitol as carbon storage molecule during sexual development, but also its role as scavenger of hydroxyl radicals can be of importance for the formation of sexual spores. Mannitol might regulate the Reactive Oxygen Species (ROS) levels induced by Nox (NADPH oxidases) family enzymes during sexual development. Taken together,
208. A small lipopeptide pheromone with limited proline substitutions can still be active. Thomas J. Fowler, Stephanie L. Link. Department of Biological Sciences, Southern Illinois University Edwardsville, Edwardsville, IL.

Mating in many fungi involves communication with lipopeptide pheromones. These signaling molecules activate G protein-coupled receptors located on the surface of a compatible mating partner and initiate a mating response. Some of the mushroom fungi code for scores of different lipopeptide pheromones among the mating types. Despite their small predicted size of approximately eleven amino acids, these pheromones have very specific pheromone receptor targets for mate discrimination. In past heterologous expression and mating studies in Saccharomyces cerevisiae, we have made random amino acid substitutions in one pheromone, Bbp2(4), from the mushroom fungus Schizophyllum commune, and site-directed mutations in a closely related pheromone, Bbp2(7). These studies indicated that the peptide portion of the pheromones can be more variable than expected. Within a random mutagenesis study of Bbp2(4), it was noted that the amino acid proline could be substituted for several of the natural residues and an active mutant pheromone was still produced. In this study, the heterologous mating assay was employed to test the extent that proline residues might be substituted into a pheromone before activity was no longer detected. Mature Bbp2(4) is predicted to be eleven amino acids with a farnesyl tail (DSPDGFGGCYC-farnesyl). Single substitutions of proline at several non-natural positions did not stop production of active pheromone, but substitutions with proline at several previously identified critical amino acid positions led to negative results in the mating assays. Among the substitutions that do not disrupt all activity are DSPPGYGCGYC-farnesyl and DSPDGFGPCYC-farnesyl. The three-dimensional conformations of proline-substituted peptides in solution were predicted with PEP-FOLD and viewed with JMOL. The conformational differences of small pheromones tolerated by one receptor are surprising. Substitution of two or more prolines at adjacent non-natural positions in a single pheromone does inhibit production of an active pheromone in the heterologous mating assay. At present, it cannot be determined if multiple proline substitutions inhibit pheromone processing, pheromone transport, or interaction with the receptor.

209. Function of Ras proteins in fungal morphogenesis of Schizophyllum commune. E-M. Jung, N. Knabe, E. Kothe. Department of Microbiology, Friedrich Schiller University, Jena, Germany.

The white rot basidiomycete Schizophyllum commune has been used as a model organism to study mating and sexual development as well as analysis of cell development. Subsequent to nutrient and pheromone recognition, intracellular signal transduction was regulated by different pathways and MAPK signalling cascades. The S. commune genome encodes more than 30 putative signal transduction proteins of the Ras superfamily containing the Ras, Rh, Rab, Ran and Arf subfamilies. Phylogenetic investigation of Ras proteins from various basidiomycetes show that they cluster in two main groups. High sequence similarities between these proteins in basidiomycetes suggest an ancient duplication event. To investigate the function of the small G-proteins Ras1 and Ras2 mutants with constitutively active ras1 alleles as well as a DrasGap1 mutant were analyzed. They show phenotypes with disoriented growth pattern, reduced growth rates and hyperbranching effects. The fungal cytoskeleton, composed of actin and microtubules has been investigated by immunofluorescence microscopy to reveal whether Ras signaling influences the formation of cytokinesis. The second Ras protein, Ras2, was detected by genome analysis. Its function is analysed in current studies.

210. The developmental PRO40/SOFT protein participates in signaling via the MIK1/MEK1/MAK1 module in Sordaria macrospora. Ines Teichert1, Eva Steffens1, Nicole Schnab1, Benjamin Fränzel2, Christoph Krisp2, Dirk A. Wolters3, Ulrich Küch4. 1) General & Molecular Botany, Ruhr University Bochum, Bochum, Germany; 2) Analytical Chemistry, Ruhr University Bochum, Bochum, Germany.

Filamentous fungi are able to differentiate multicellular structures like conidiophores and fruiting bodies. Using the homothallic ascomycete Sordaria macrospora as a model system, we have identified a number of developmental proteins essential for perithecium formation. One is PRO40 [1], a member of the cell wall integrity (CWI) pathway, one of three MAP kinase modules present in S. macrospora. The S. macrospora CWI pathway consists of MAP kinase kinase kinase (MAPKKK) MIK1, MAPKK MEK1 and MAP kinase (MAPK) MAK1, with additional upstream components, protein kinase C (PKC1) and RHO GTPase RH01. Data from tandem affinity purification - MS experiments with PRO40 and MEK1 as bait indicate that PRO40 forms a complex with components of the CWI pathway. Analysis of single and double knockout mutants shows that PRO40, MIK1, MEK1 and MAK1 are involved in the transition from protoperithecia to perithecia, hyphal fusion, vegetative growth, and cell wall stress response. Differential phosphorylation of MAPKs in a pro40 knockout strain was detected by Western blot analysis. We propose that PRO40 modulates signaling through the CWI module in a development-dependent manner. Further interaction studies and complementation analyses with PRO40 derivatives provide mechanistic insight into the function of PRO40 domains during fungal development. [1] Engh et al. (2007) Eukaryot Cell 6:831-843.

211. Map-based identification of the mad photosensing genes of Phycycomyces blakesleeanus. Silvia Polaino Orts1, Suman Chaudhary2, Viplendra Shakya1, Alejandro Miralles-Durán3, Luis Corrochano3, Alexander Idrum1. 1) Cell Biology & Biophysics, University of Missouri-KC, Kansas City, MO; 2) Departamento de Genética, Universidad de Sevilla, Spain.

Phycycomyces blakesleeanus is a filamentous fungus, a member of the subphylum Mucoromycotina. The main reason for the presence of Phycycomyces in laboratories is its sensitivity to light. The fruiting bodies phototropism of Phycycomyces has served as a model of response to blue light in fungi. In 1967, in the laboratory of Nobel laureate Max Delbrück, the first sensory mutants were isolated. Analysis on these strains has enabled a proposed sensory transduction pathway that describes the flow of information from the sensors to the effectors. There are ten mutants, called mad mutants, divided into two classes: those of type 1 are madA, madB, madC and madI, which are altered only in phototropism but not in others tropisms of the sporangiophere. The mutants in the madA and madB genes are altered in all phototropes (phototropism, photoclinotropism, photomorphogenesis, phorocarotenogenesis and phototropism). These two mad genes are the only ones that have been identified and their corresponding proteins interact to form the Mad complex, the main photoreceptor complex of Phycycomyces. The mutants altered in the madC gene are only affected in the phototropism. The remaining mad mutants are called type 2 and are altered in the phototropism and other responses of the sporangiophere, like gravitropism and avoidance. Phycycomyces cannot be stability transformed with DNA. To identify the eight unknown mad mutants, a positional cloning approach was taken coupled to Illumina sequence information. A genetic map was constructed between two wild type parents, and then mad mutants crossed to one of these parents. Through mapping, we have identified candidates for the madC, madD, madI, madF and madG genes, with greatest follow up characterization in madC. The madC gene encodes a Ras GTPase-activating protein, implicating Ras in the light signal transduction pathway in fungi.
212. The C2H2 transcription factor hgrA promotes hyphal growth in the dimorphic pathogen *Penicillium marneffei*. Hayley E. Bugeja, Michael J. Hynes, Alex Andrianopoulos. Department of Genetics, University of Melbourne, Parkville, VIC, Australia.

*Penicillium marneffei* (recently renamed *Talaromyces marneffei*) is well placed as a model experimental system for investigating fungal growth processes and their contribution to pathogenicity. An opportunistic pathogen of humans, *P. marneffei* is a dimorphic fungus that displays multicellular hyphal growth and asexual development (conidiation) in the environment at 25°C and unicellular fission yeast growth in macrophages at 37°C. We have characterised the transcription factor hgrA (hyphal growth regulator), which contains a C2H2 DNA binding domain closely related to that of the stress-response regulators Msn2/4 of *Sacharomyces cerevisiae*. hgrA is not required for controlling yeast growth in response to the host environment, nor does it appear to have a key role in response to stress agents, but is both necessary and sufficient to drive the hyphal growth program. hgrA expression is specific to hyphal growth and its deletion affects multiple aspects of hyphal morphogenesis and the dimorphic transition from yeast cells to hyphae. Loss of HgrA also causes cell wall defects, reduced expression of cell wall biosynthetic enzymes and increased sensitivity to cell wall, oxidative, but not osmotic stress agents. As well as causing apical hyperbranching during hyphal growth, overexpression of hgrA prevents conidiation and yeast growth, even in the presence of inductive cues. HgrA is a strong inducer of hyphal growth and its activity must be appropriately regulated to allow alternative developmental programs to occur in this dimorphic pathogen.

213. Involvement of a specific ubiquitin ligase in the assembly of the dynein motor. Ryan Elsenseter, Robert Schnittker, Michael Plamann. Sch Biological Sci, Univ Missouri, Kansas City, Kansas City, MO.

Cytoplasmic dynein is a large, microtubule-associated motor complex that facilitates minus-end-directed transport of various cargos. The dynein heavy chain (DHC) is >4000 residues in length, with the last two-thirds of the heavy chain forming the motor head. Six domains within the dynein motor exhibit varying degrees of homology to the AAA+ superfamily of ATPases. These domains form a ring-like structure from which a microtubule-binding domain protrudes. Using a genetic assay, we have isolated over 30 DHC mutants of Neurospora that produce full-length proteins that are defective in function. To explore the mechanism by which mutations in the C-terminal region of the DHC affect function, we have identified both intragenic and extragenic suppressors. Interestingly, analysis of the extragenic suppressors revealed that loss of function for a putative E3 ubiquitin ligase restored dynein function in a select set of C-terminal DHC mutants. Our results suggest that these C-terminal DHC mutations block assembly of the dynein motor and loss of activity of a specific E3 ubiquitin ligase restores dynein assembly.


Eukaryotic cells use the microtubule-based motors dynein and kinesin to transport a wide variety of cargos. Cytoplasmic dynein is responsible for minus-end-directed microtubule transport (from the cell periphery towards the nucleus), while kinesins-1, -2 and -3 move cytoplasmic cargo in the opposite direction. While much is known about how these motors work *in vitro*, many questions regarding the mechanism and regulation of microtubule-based cargo transport in cells remain. To identify novel alleles and genes required for microtubule-based transport, we have performed a genetic screen in the filamentous fungus, *Aspergillus nidulans*. We fluoresceingly-labeled three different organelle populations that are known to be cargo of dynein and kinesin in *Aspergillus*: nuclei, endosomes, and peroxisomes. After mutagenesis we used a fluorescence microscopy-based screen to identify mutants with defects in the distribution or motility of these organelles. Here, we report the identification and characterization of new alleles of kinesin, dynein and the dynein regulatory factors, Lis1 and Arp1 (a component of the dynactin complex). In vivo analysis of new dynein alleles revealed that mutations in two of dynein’s nucleotide binding sites (termed AAA1 and AAA3), led to the accumulation of endosomes and peroxisomes at the hyphal tip, with more subtle defects on nuclear distribution compared to dynein null alleles. *In vitro* studies of the AAA3 motor mutation showed dramatic reduction in velocity and prolonged binding to the microtubules in single molecule motility assays.


Ustilago maydis is a dimorphic basidiomycete that infects maize. In this fungus virulence and sexual development are intricately interconnected. Induction of pathogenicity program requires that two haploid compatible cells fuse and form an infective filament after pheromone signaling. The pheromone signal is transmitted by a well-known MAPK cascade. Interestingly, *Sacharomyces cerevisiae* and *Ustilago maydis* use a similar MAPK cascade which contains a C-terminal MAPKK of the cascade (using a constitutively activated allele of *fuz7*, the MAPKK of the cascade) and found that two main G2/M regulators Hsl1, a kinase involved in downregulation of Wee1 and Clb2, the mitotic cyclin, and its deletion affects multiple aspects of hyphal morphogenesis and the dimorphic transition from yeast cells to hyphae. We propose that microtubule-associated cargo transport, which are downregulated at transcriptional level, are regulated by Cdk1. We fluorescently labeled two different cell cycle regulators (using a constitutively activated allele of *hgrA*, a specific E3 ubiquitin ligase). Our results indicate that microtubule-associated motor complexes required for microtubule-based transport of nuclei, endosomes, and peroxisomes in *U. maydis*.

216. Microtubule-dependent mRNA transport and mitochondrial protein import in Ustilago maydis. T. Langner1, T. Pohlmann1, C. Haar1, J. Koepke2, V. Geiser1, M. Feldbrügge1. 1) Institute for Microbiology, Heinrich-Heine University, Düsseldorf, Northrhine-Westfalia, Germany; 2) MARA, Philipps-University, Marburg, Hesse, Germany.

Transport, subcellular localization, and local translation of mRNAs constitute a very important mechanism to ensure correct targeting of proteins to distinct subcellular domains. Although mRNA transport is well studied in various organisms, its function in regulating specific cellular processes like mitochondrial protein import is still ambiguous. We use the con pathogen Ustilago maydis as a model system to study microtubule-dependent mRNA transport during formation of infectious filaments. The key RNA-binding protein Rrm4 is an integral part of this long-distance transport machinery. Combining proteomics, in vivo UV cross-linking, and biochemical approaches, we uncovered that Rrm4 plays a crucial role in active transport of mRNAs encoding mitochondrial proteins. In Rrm4 loss-of-function mutants, mitochondrial proteins are altered in expression and localization, which correlates with impaired production of reactive oxygen species (ROS). We propose that microtubule-dependent mRNA transport and local translation are crucial for correct import of mitochondrial proteins. This work is funded by iGRAD-plant graduate school (German research council, DFG/ GK1525).
174. The vacuole of Neurospora crassa may be composed of multiple compartments with different structures and functions. Barry J. Bowman, Emma Jean Bowman, Robert Schnittker, Michael Plaman. 1) MCD Biology, University of California, Santa Cruz, CA; 2) Department of Biology, University of Missouri, Kansas City, KA.

The structure of the “vacuole” in Neurospora crassa and other filamentous fungi is highly variable with cell type and position in the hypha. Large spherical vacuoles are typically observed in older hyphal compartments, but approximately 100 microns behind the hyphal tip, vacuolar markers are seen in a dynamic network of thin tubules. At the edge of this network nearest the tip, a few distinct round organelles of relatively uniform size (2–3 microns) have been observed (Bowman et al. Eukaryotic Cell 10:654). The function of these round organelles is unknown, although the vacuolar ATPase and a vacuolar calcium transporter are strongly localized there. To help identify organelles we have tagged SNARE proteins and Rab GTPases with GFP and RFP. Several of these tagged proteins (sec-22, rab-7, rab-8) appear in the tubular vacuolar network and in the membrane of the round organelles. A unique aspect of the round organelles is their association with dynin and dynactin (Sivagurunathan et al. Cytoskeleton, 69:613). In strains with mutations in the tail domain of the dynene heavy chain the dynein is often seen in clumps. This aggregated dynein appears to be tightly associated with (and possibly inside) the round organelles, but not in the tubular vacuolar network. Further analysis of the location of SNARE and Rab proteins may help to identify the function of the round organelles.


The A mating type locus in Coprinopsis cinerea controls defined steps in the formation of a dikaryotic mycelium after mating of two compatible monokaryons as well as fruiting body formation on the established dikaryon. Usually, three paralogous pairs of divergently transcribed genes for two distinct types of homeodomain transcription factors (termed HD1 and HD2) are found in the multiple alleles of the A locus. For regulation of sexual development, heterodimerization of HD1 and HD2 proteins coming from allelic gene pairs is required. In some A loci found in nature, alleles of gene pairs are not complete or one of the two genes have been made inactive. Functional redundancy allows the system still to work as long as an HD1 gene in one and an HD2 gene in the other allele of one gene pair are operative (Casselon and Kües 2007). Here, we present the structures of two completely sequenced A loci, A42 (this study) and A43 (Stajich et al. 2010). Evidences for gene duplications, deletions and inactivations are found. The loci differ in the number of potential gene pairs (five versus three), in genes that have been duplicated in evolution, in genes that have been lost in evolution and in genes that are still present but have been made inactive. Furthermore, self-compatible mutants of the A loci are found that due to fusions of an HD1 and an HD2 gene can carry out sexual reproduction without mating with another compatible strain. The products of the fusion genes can take over the regulatory functions normally executed by heterodimers of HD1 and HD2 proteins that come from different nuclei. In this study, we present a sequenced fusion gene from a mutant A43 locus. The 5'-half of an HD2 gene was fused in frame to a complete HD1 gene through a linker made up from former promoter sequence. An earlier described fusion protein (Kües et al. 1994) similarly contains the 5'-half of an HD2 gene that however was fused to the 3'-half of an HD1 gene. Comparison between the resulting fusion proteins indicates that presence of the HD2 homeodomain and the NLSs (nuclear localization signals) from the HD1 protein are likely essential for the function of the fusion proteins. Other domains required for function in the wild type proteins (such as for heterodimerization) are dispensable for fusion proteins that mediate a self-compatible phenotype.

191. Transformation of an NACHT-NTPase gene NWD2 suppresses the pkn1 defect in fruiting body initiation of the Coprinopsis cinerea mutant Proteo159. Yidong Yu, Pierre-Henri Clerget, Gwenael Ruprich-Robert, Markus Aebi, Ursula Kües. 1) Molecular Wood Biotechnology + Technical Mycology, University of Goettingen, Goettingen, Germany; 2) Department of Botany, Stockholm University, Stockholm, Sweden; 3) Institute of Genetics and Microbiology, University Paris-Sud, Orsay, France; 4) Institute of Microbiology, ETH Zurich, Zurich, Switzerland.

Homokaryon AmutBmut is a self-compatible strain of the mushroom Coprinopsis cinerea which can carry out sexual reproduction without fusing with another compatible strain. Due to its single nucleus, this strain allows easy induction of mutations in fruiting body formation. One such mutant is the strain Proteo159, which is defective in the first step of fruiting body initiation (primary hyphal knot formation; pkn1). This mutant has been isolated after protoplasting and regeneration of oidia (Granado et al. 1997). It has a reduced growth speed and a reduced rate of oidiation (asexual spore formation) compared to the wild type AmutBmut. In addition, with age, the mycelium of Proteo159 produces a dark-brown pigment that diffuses into the medium. This pigmentation is not found in AmutBmut. Proteo159 never makes any sclerotium nor initiates formation of any fruiting structure. Complementation tests have been made through transformations with a cosmide bank of the wild type AmutBmut (Bottoli et al. 1999) and the defect has been complemented after transformation with the wild type gene NWD2. This gene codes for a NACHT-NTPase (signal transduction protein with a NACHT domain which is found in animal, fungal and bacterial proteins and named after four different types of P-loop NTPases NAI, CIITA, HET-E and TP1). However, sequencing of this gene in the mutant Proteo159 did not reveal any point mutations, deletions or insertions within this gene. One possibility to explain the pkn1 defect in mutant Proteo159 in connection with the transformation data is that insertion of further copies of gene NWD2 into the genome of mutant Proteo159 has a suppressor effect on the defect in the yet unknown gene pkn1. This situation is reminiscent to findings in Schizosaccharomyces pombe where formation of fusion proteins has been induced in monokaryons upon transformation with the gene Frt1 (Horton and Raper 1991). Gene Frtn1 encodes another type of P-loop NTPase (Horton and Raper 1995) than NWD2. However, the proteins share a novel short motif of amino acid similarity at their C-terminal ends.


The actin cytoskeleton is conserved among all eukaryotes and plays essential roles during many cellular processes. It forms an internal framework in cells that is both dynamic and well organised. The plethora of functions ranges from facilitating cytoplasmic streaming, muscle contraction, formation of contractile rings, nuclear segregation, endocytosis and facilitating apical cell expansions. Oomycetes are filamentous organisms that resemble Fungi but are not related to Fungi. The two groups show significant structural, biochemical and genetic differences. One prominent lineage within the class of oomycetes is the genus Phytophthora. This genus comprises over 100 species that are all devastating plant pathogens threatening agriculture and natural environments. The potato late blight pathogen Phytophthora infestans was responsible for the Irish potato famine and remains a major threat today. Previous the actin organization has been studied in several oomycetes. Next to the common F-actin filaments and cables, cortical F-actin containing patches or plaques have been observed as in Fungi. However, only a static view was obtained. Here, we use an in vivo actin binding moiety labelled to a fluorescent group to investigate the actin cytoskeleton dynamics in hyphae of P. infestans. Our results provide the first visualisation of the dynamic reorganization of the actin cytoskeleton in oomycetes. In the future, this line will provide insight in the role of the actin cytoskeleton during infection.
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221. A novel approach for functional analysis of genes in the rice blast fungus. Sook-Young Park1, Jaehyuk Choi1, Seongbeom Kim1, Jongbum Jeon1, Jaeyoung Choi1, Seomun Kwon1, Dayoung Lee1, Aram Huh1, Miho Shin1, Junhyun Jeon1, Seochan Kang1, Yong-Hwan Lee1. 1) Dept. of Agricultural Biotechnology, Seoul National University, Seoul 151-921, South Korea; 2) Dept. of Plant Pathology & Environmental Microbiology, The Pennsylvania State University, University Park, PA 16802, USA.

Null mutants generated by targeted gene replacement are frequently used to reveal function of the genes in fungi. However, targeted gene deletions may be difficult to obtain or it may not be applicable, such as in the case of redundant or lethal genes. Constitutive expression system could be an alternative to avoid these difficulties and to provide new platform in fungal functional genomics research. Here we developed a novel platform for functional analysis of genes in Magnaporthe oryzae by constitutive expression under a strong promoter. Employing a binary vector (pGOF), carrying EF1b promoter, we generated a total of 4,432 transformants by Agrobacterium tumefaciens-mediated transformation. We have analyzed a subset of 54 transformants that have the vector inserted in the promoter region of individual genes, at distances ranging from 44 to 1,479 bp. These transformants showed increased transcript levels of the genes that are found immediately adjacent to the vector, compared to those of wild type. Ten transformants showed higher levels of expression relative to the wild type not only in mycelial stage but also during infection-related development. Two transformants that T-DNA was inserted in the promoter regions of putative lethal genes, MoRPT4 and MoDBPS, showed decreased conidiation and pathogenicity, respectively. We also characterized two transformants that T-DNA was inserted in functionally redundant genes encoding alpha-glucosidase and alpha-mannosidase. These transformants also showed decreased mycelial growth and pathogenicity, implying successful application of this platform in functional analysis of the genes. Our data also demonstrated that comparative phenotypic analysis under over-expression and suppression of gene expression could prove a highly efficient system for functional analysis of the genes. Our over-expressed transformant library would be a valuable resource for functional characterization of the redundant or lethal genes in M. oryzae and this system may be applicable in other fungi.

222. Distribution and evolution of transposable elements in the Magnaporthe oryzae/grisea clade. Joelle Amsalem1,2, Ludovic Mallet1,2, Helene Chiapello2,3, Cyprien Guerin1, Marc-Henri Lebrun1, Didier Tharreau1, Elisabeth Fournier+. 1) INRA, URGI, Versailles, France; 2) INRA, UMR BIOGER, Thiverval-Gignon, France; 3) INRA, UR MIG, Jouy-en-Josas, France; 4) INRA, URB IA, Castanet-Tolosan, France; 5) CIRED, UMR BGPI, Montpellier, France; 6) INRA, UMR BGPI, Montpellier, France.

Magnaporthe oryzae is a successful pathogen of crop plants and a major threat for food production. This species gathers pathogens of different Poaceae, and causes the main fungal disease of rice worldwide and severe epidemics on wheat in South America. The evolutionary genomics of Magnaporthe oryzae project aims at characterizing genomic determinants and evolutionary events involved in the adaptation of fungus to different host plants. Such evolution may rely on variations in Transposable Elements (TEs) and gene content as well as modification of coding and regulatory sequences. Indeed, TEs are essential for shaping genomes and are a source of mutations and genome re-organizations. We performed a comparative analysis of TEs in 9 isolates from the M. oryzae/grisea clade differing in their host specificity using a reference TEs consensus library (Mg7015_Refs_TE) made from M. grisea 70-15 reference genome. We used REPET pipelines (http://urgi.versailles.inra.fr/Tools/REPET) to detect ab initio and classify TEs in M. grisea 70-15 according to functional features (LTR, ITR, RT, transposase, etc.). After manual curation on consensus provided by the TEdenovo pipeline, we used the resulting consensus of TEs families (Mg7015_Refs_TE) to annotate the 9 genome copies including nested and degenerated ones using TEannot pipeline. We will present results obtained for Mg7015_Refs_TE classification, their annotation, distribution along the genome and preliminary results provided by comparison in M. oryzae/grisea species studied regarding correlation with phylogeny and host specificity.

223. Alternative structural annotation of Aspergillus oryzae and Aspergillus nidulans based on RNA-Seq evidence. Gustavo C. Cerqueira1, Brian Haas1, Marcus Chibucos2, Martha Arnaud1, Christopher Sibthorp3, Mark X Cadick1, Kazuhiro Iwashita1, Gavin Sherlock1, Jennifer Wortman1. 1) Broad Institute, Boston, MA; 2) Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, USA; 3) Department of Genetics, Stanford University Medical School, Stanford, USA; 4) School of Biological Sciences, University of Liverpool, Liverpool, United Kingdom; 5) National Research Institute of Brewing, Hiroshima, Japan.

The correct structural annotation of genes is fundamental to downstream functional genomics approaches. Genes undetected by gene prediction algorithms, incorrect gene boundaries, misplaced or missing exons and wrongly merged genes can jeopardize attempts to produce a comprehensive catalog of an organism’s metabolic capabilities. We are currently working toward generating alternative and improved structural annotation of Aspergillus oryzae and Aspergillus nidulans. Our approach consists of assembling partial transcript sequences from RNA-Seq data, aligning transcript assemblies to their respective genomic loci and finally adjusting the gene models according to the new transcript evidence. Novel putative genes were defined based on transcriptionally active regions containing splice junctions and open reading frames. Gene loci having transcripts suggesting alternative splicing variants were reported. The nucleotide composition in the vicinity of splicing sites was re-evaluated in the light of the newly defined exons-introns boundaries. The modified structural annotation was compared to the original structural annotation of these genomes and alternative gene models derived from approaches similar to those presented here. The improved gene models are available through the Aspergillus genome database (http://www.aspergillusgenome.org).


The opportunistic fungal pathogen, Candida albicans, is a significant medical threat, especially for immunocompromised patients. Experimental research has focused on specific areas of C. albicans biology with the goal of understanding the multiple factors that contribute to its pathogenic potential. Some of these factors include cell adhesion, invasive or filamentous growth and the formation of drug resistant biofilms. The Candida Genome Database (CGD, http://www.candidagenome.org/) is an internet-based resource that provides centralized access to genomic sequence data and manually curated functional information about genes and proteins of the fungal pathogen Candida albicans and other Candida species. The Gene Ontology (GO; www.geneontology.org) is a standardized vocabulary that the Candida Genome Database (CGD; www.candidagenome.org) and other groups use to describe the function of gene products. To improve the breadth and accuracy of pathogenicity-related gene product descriptions and to facilitate the description of as-yet uncharacterized but potential pathogenicity-related genes in Candida species, CGD has undertaken a three-part project: first, the addition of terms to the Biological Process branch of the GO to improve the description of fungal-related processes; second, manual recuration of gene product annotations in CGD to use the improved GO vocabulary; and third, computational ortholog-based transfer of GO annotations from experimentally
characterized gene products using these new terms to uncharacterized orthologs in other *Candida* species. Through genome annotation and analysis, we identified candidate pathogenicity genes in seven non-albicans *Candida* species and in one additional *C. albicans* strain, WO-1. We also defined a set of the *C. albicans* genes at the intersection of biofilm formation, filamentous growth, pathogenesis and phenotypic switching and now, finger and tentacle development, of this opportunistic fungal pathogen, which provide a compelling list of candidates for further experimentation.

225. Genome sequencing of *Verticillium albo-atrum* pathotypes in order to understand wilt disease in hop production. J. Jakes, G. Rot, V. Jelen, S. Radisek, S. Mandelc, A. Majer, B. Zupan, B. Javornik. 1) Agronomy Department, Biotechnical faculty, University of Ljubljana, Ljubljana, Slovenia; 2) Bioinformatics Laboratory, Faculty of Computer and Information Science, University of Ljubljana, Ljubljana, Slovenia; 3) Slovenian Institute of Hop Research and Brewing, Zalec, Slovenia.

Verticillium wilt of hop is a vascular disease caused by *V. albo-atrum*, outbreaks of the lethal strains of which threaten current hop production in Europe. Fungal isolates differ in aggressiveness and have been classified by pathogenicity tests into mild and lethal pathotypes. In general, the mild strain infection varies in intensity from year to year and rarely causes the death of the whole plant, whereas lethal strain infection causes very severe symptoms, with rapid plant withering and dieback. Lethal strains with increased virulence in hop were first reported in the UK in 1933, followed by outbreaks in Slovenia in 1997 and in Germany in 2005. Sequencing the genomes of mild and lethal *V. albo-atrum* hop isolates aimed at the dissection of the pathotype genomes, in order to provide an insight into their genomic structure, which might explain the increased virulence of the lethal strain, enable the detection of virulence-associated factors and elucidate the pathogenicity in *Verticillium spp*. Genomes of three mild and three lethal strains from three different geographic regions were sequenced by illumina technology. The reference lethal strain, with a larger genome than the mild strains, as confirmed by flow cytometry, was sequenced using three different length libraries producing a total of 76.3 M reads. From 4.8 to 11.5 M reads were obtained for the other five strains. Additionally, 38.3 M RNA-seq reads of mild and lethal strain transcriptomes were produced for annotation of the transcripted regions. Bioinformatics analyses included de-novo assembly of the reference genome, followed by mapping of the other genomes for comparison of mild and lethal strains to determine specific regions of the strains. The reference genome was assembled into 715 contigs, with a total length of 33.59 Mb. Comparison of lethal versus mild strains revealed that 0.5 Mb of DNA was only present in the lethal strains. Gene prediction tools supported by RNA-seq analysis revealed 9858 gene models, 91 of which were present in the lethal unique region. Analysis of repetitive DNA based on previously published models masked 1.53% of the assembled genome, while de-novo identification of repeats masked 5.86% of the genome. The presented sequencing study established a new genomic resource for non-alalfa *V. albo-atrum* strains and will enable their virulence to be studied.

226. *Aegerolysin* proteins from *Aspergillus species*. Nada Krasevec, Kristina Sepcic, Sasa Rezonic, Nina Sluga, Peter Macelj, Gregor Anderluh. 1) L11 Laboratory for Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Ljubljana, Slovenia; 2) Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia.

Currently, *Aegerolysin* family (Pfam06355) comprises over 300 proteins, mostly assigned as putative hemolysins, however, their function and biological role is unknown. Some of them, i.e. *aegerolysin*, *ostreolysin*, *pleurotolysin A*, *erylysin A* from the Basidiomycota mushrooms (*Agrocybe aegerita*, *Pleurotus ostreatus* and *P. eryngii*), and their orthologues, *Asp-hemolysin* from the human pathogens *Aspergillus fumigatus* (*Eurotiales*, Ascomycota) and PA0122 (raHU) from *Pseudomonas aeruginosa* (*Proteobacteria*), have been characterized as lipid- or membrane-binding proteins. *Aegerolysins* are specifically distributed among certain fungal species belonging to both Ascomycota and Basidiomycota taxa, however, they could be also found in bacteria and plants. In 2004, it was reported that in addition to the *aegerolysin* component A (pleurotolysin A, PlyA), a 59 kDa component B (pleurotolysin B, PlyB) is obligatory for the observed hemolytic activity of these proteins. In contrast to *aegerolysins* (component A), that appear widely distributed among different organisms, initial bioinformatical search of component B homologues results in a much lower number of similar sequences. The gene products of these proteins have a conserved hemolytic activity, but the role is unknown. Some of them, i.e. *aegerolysin*, *ostreolysin*, *pleurotolysin A*, *erylysin A* from the Basidiomycota mushrooms (*Agrocybe aegerita*, *Pleurotus ostreatus* and *P. eryngii*), and their orthologues, *Asp-hemolysin* from the human pathogens *Aspergillus fumigatus* (*Eurotiales*, Ascomycota) and PA0122 (raHU) from *Pseudomonas aeruginosa* (*Proteobacteria*), have been characterized as lipid- or membrane-binding proteins. *Aegerolysins* are specifically distributed among certain fungal species belonging to both Ascomycota and Basidiomycota taxa, however, they could be also found in bacteria and plants. In 2004, it was reported that in addition to the *aegerolysin* component A (pleurotolysin A, PlyA), a 59 kDa component B (pleurotolysin B, PlyB) is obligatory for the observed hemolytic activity of these proteins. In contrast to *aegerolysins* (component A), that appear widely distributed among different organisms, initial bioinformatical search of component B homologues results in a much lower number of similar putative proteins, even more, both components combined could be found in a few of fungal species only. *Aspergillus* species (A. *tubingenensis*, A. *brasiliensis*, A. *acidus*, A. *glaucus*, A. *versicolor*, A. *sydowi*, A. *wentii* and A. *zonatus*) as a result of community sequencing proposal (CSP2011). The genome sequences are available at MycoCosm (*http://genome.jgi.doe.gov/programs/fungi/index.jsf*) and at the Aspergillus Genome Database (AspGD) (*http://www.aspgd.org/*). The task of EUFGEN (EURotiales Functional GENomics consortium, *http://www.eufgen.org/*) is to complement these genome sequences to those already available for the *Aspergillus* species. The strains were provided by CBS-KNAW fungal biodiversity center (*http://www.cbs.knaw.nl//collection/AboutCollections.aspx*). Our aim is to clarify experimentally the relation between the genome context for the two components and their presumed hemolytic activity.

227. Assembly, Annotation, and Analysis of Multiple Mycorrhizal Fungal Genomes. Alan Kuo, Igor Grigoriev, Annegret Kohler, Francis Martin. Mycorrhizal Genomics Initiative (MGI) Consortium. 1) Fungal Genomics Program, DOE Joint Genome Institute, 2800 Mitchell Dr., Walnut Creek, CA, 94598 USA; 2) Lab of Excellence ARBRE, Department of Tree-Microbe Interactions, INRA-Nancy, 54280 Champenoux, France.

Mycorrhizal fungi play critical roles in host plant health, soil community structure and chemistry, and carbon and nutrient cycling, all areas of intense interest to the US Dept. of Energy (DOE) Joint Genome Institute (JGI). To this end we are building on our earlier sequencing of the Laccaria bicolor genome by partnering with INRA-Nancy and the mycorrhizal research community in the MGI to sequence and analyze dozens of mycorrhizal genomes of all Basidiomycota and Ascomycota orders and multiple ecological types (ericoid, orchid, and ectomycorrhizal). JGI has developed and deployed high-throughput sequencing techniques, and Assembly, RNASeq, and Annotation Pipelines. In 2012 alone we sequenced, assembled, and annotated 12 draft or improved genomes of mycorrhizae, and predicted ~232831 genes and ~15011 multigene families, All of this data is publicly available on JGI MycoCosm (*http://jgi.doe.gov/fungl/*), which provides access to both the genome data and tools with which to analyze the data. Preliminary comparisons of the current total of 14 public mycorrhizal genomes suggest that 1) short secreted proteins potentially involved in symbiosis are more enriched in some orders than in others amongst the mycorrhizal Agaricomycetes, 2) there are wide ranges of numbers of genes involved in certain functional categories, such as signal transduction and post-translational modification, and 3) novel gene families are specific to some ecological types.

228. Comparative reannotation of 21 Aspergillus genomes. Asaf A. Salamov, Robert Riley, Igor Grigoriev. DOE Joint Genome Inst, Walnut Creek, CA.

We used comparative gene modeling to reannotate 21 Aspergillus genomes from MycoCosm and AspGD. Initial automatic annotation of individual genomes may contain some errors of different nature, for example, missing genes, incorrect exon-intron structures, ‘chimeras’, which fuse 2 or more genes, or splitting genes into 2 or more models. The main premise behind the comparative modeling approach is that for closely related genomes most orthologous families have the same conserved gene structure. The algorithm maps all gene models predicted in all individual Aspergillus genomes to each genome and for each locus selects among the potentially many competing models the one, which most closely resembles the orthologous genes from other genomes. This procedure is iterated until no change in gene models will be observed. For the 21 Aspergillus genomes we predicted a total of 4503 new gene models (~2% per genome), supported by comparative analysis, additionally correcting ~18% of oldgene models. This resulted in total of 4065
more genes with annotated PFAM domains (~3% increase per genome). Analysis of few genomes with transcriptomics data shows that new annotation sets also have a higher number of EST-supported splice sites at exon-intron boundaries.

229. Using the phenotypic information in the PHI-base database to explore pathogen genomes, transcriptomes and proteomes. Martin Urban¹, John Antoniw², Natalia Martins³, Artem Lysenko², Jacek Grzebyta³, Elzbieta Janowska-Sedja², Manoos Sarjip³, Kim Hammond-Kosack³. ¹ Plant Biology and Crop Science, Rothamsted Research, Harpenden, Hertfordshire, United Kingdom; ² Computational and Systems Biology, Rothamsted Research, Harpenden, Hertfordshire, United Kingdom; ³ Embrapa - Genetic Resources and Biotechnology, Brasilia, Brazil.

The Pathogen-Host Interactions database (www.phi-base.org), called PHI-base, stores expertly curated molecular and biological information on genes for which the effect on pathogen-host interactions has been tested experimentally. Fungal, oomycete and bacterial pathogens which infect animal, plant, fish, insect and/or fungal hosts are included. Information is also given on the target sites of some anti-infective chemistries. This database, available since 2005, is used to analyse effectively the growing number of verified genes that mediate an organism’s ability to cause disease and/or to trigger host responses. PHI-base is also used as a valuable resource for the functional annotation of novel genomes (http://phytopathdb.org), in comparative genomics studies and for the discovery of candidate targets in medically and agronomically important microbial pathogens for intervention with synthetic chemistries and natural products (fungicides). Each curated entry in PHI-base is checked by individual species experts and is supported by strong experimental evidence (e.g. gene deletion, complementation experiments) and literature references. This extensive manual curation aims to position PHI-base as a ‘gold standard’ for researchers in the pathogen-host biology community. Genes are annotated using controlled vocabularies (Gene Ontology terms, EC Numbers, etc.), and links to other external data sources (for example, NCBI taxonomy, EMBL and Uniprot) are provided. Here we describe a significant update of PHI-base (Version 3.4) in which the data content has more than doubled. PHI-base now provides information on more than 2,200 genes described in 3000 pathogen-host interactions, which are associated with more than 106 pathogenic species. A Fusarium species case study is presented, where the database content has been used in an integrated network analysis (combining information from gene co-expression, predicted protein-protein interactions and sequence similarity) to predict proteins in Fusarium graminearum that may be involved in pathogenicity. This approach has identified 215 candidates including 29 proteins currently annotated as ‘hypothetical’. As the content of PHI-base grows, we expect this database to be an important resource for exploring conserved and species-specific themes in pathogenicity.

230. RNA-Seq analysis reveals new gene models and alternative splicing in Fusarium graminearum. Chunzhuo Zhao¹,², Cees Waalwijk², Pierre Wit³, Dingzhong Tang⁴, Theo vander Lee⁴. ¹ Wageningen-UR, Wageningen, Gelderland, Netherlands; ² State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China.

The genome of Fusarium graminearum has been sequenced and annotated, but correct gene annotation remains a challenge. In addition, posttranscriptional regulations, such as alternative splicing and RNA editing, are poorly understood in F. graminearum. Here we took advantage of RNA-Seq to improve gene annotations and to identify alternative splicing and RNA editing in F. graminearum. In total, 25,720,650 reads were generated from RNA-Seq. Transcripts were detected for 84% of the genes predicted by machine annotation in the Broad database. Of these reads, 74.8% matched to exonic regions, 10.6% to untranslated regions (UTRs), 12.9% to intergenic regions and only 1.7% to intronic regions. We identified and revised 655 incorrectly predicted gene models (10% of the gene models that could be tested), including revisions of intron predictions, intron splice sites and prediction of novel introns. In addition, we identified 231 genes with two or more alternative splice variants, mostly due to intron retention. The analysis showed that the majority of these alternatively spliced transcripts lead to premature termination codons, PTCs. Apart from PTC isoforms, some alternatively spliced transcripts encoding proteins with diverse lengths were identified. The effects of the diversity in the transcript length on the biological function of proteins are still unknown, but several functions including binding properties, intracellular localization, enzymatic activity or stability may be affected. Interestingly, the expression ratios between different transcript isoforms appeared to be developmentally regulated. Surprisingly, no RNA editing was identified in F. graminearum. Moreover, 2459 novel transcriptionally active regions (nTARs) were identified and our analysis indicates that many of these could be genes that were missed in the automated annotation. A number of representative novel gene models and alternatively spliced genes were validated by reverse transcription polymerase chain reaction and sequencing of the generated amplicons. Our results demonstrate that posttranscriptional regulation can be studied efficiently using our developed RNA-Seq analysis pipeline and may be important in adaptation of F. graminearum to changing environmental conditions that occur during different growth stages.

231. Comparison of transcriptome technologies in the MpkA deletion mutant of Aspergillus fumigatus. Clara Baldin¹,², Sebastian Mueller³, Marco Groth³, Konrad Gruetzmann³, Reinhard Guthke³, Olaf Kniemeyer³, Axel Brakhage³, Vito Valiante³. ¹ Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Beutenbergstr. 11a, 07745 Jena, Germany; ² Department of Systems Biology / Bioinformatics, Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Beutenbergstr. 11a, 07745 Jena, Germany; ³ Department of Microbiology and Molecular Biology, Friedrich Schiller University Jena, Beutenbergstrasse 11a, 07743 Jena, Germany; ⁴ Genome Analysis, Leibniz Institute for Age Research - Fritz Lipmann Institute, Beutenbergstr. 11, 07743 Jena, Germany; ⁵ Department of Bioinformatics, Friedrich Schiller University Jena, Ernst-Abbe-Platz 2, 07743 Jena, Germany.

RNA deep sequencing techniques are rising as powerful strategy to analyze the transcriptome profile of different organisms. Especially, this approach will be very helpful whenever a microarray platform has not been established yet or when different platforms show low reproducibility of the generated data. In the present study, the expression profile of Aspergillus fumigatus has been analysed via different transcriptome analysis approaches. A. fumigatus is a saprophytic fungus that is emerging as one of the most important airborne fungal pathogens. The adaptation of this fungus to different environments stimulated research on the regulation of the cell-wall integrity pathway, which is mediated by the Mitogen Activated Protein Kinase (MAPK) MpkA. Previous microarray analyses showed that MpkA is involved not only in the regulation of genes responsible for cell wall maintenance, but also in protection against reactive oxygen species, iron starvation response and secondary metabolites production (Jain et al., Mol. Microbiol. 2011). Using the same strains and lab conditions, we performed a transcriptome study using RNA deep sequencing to directly compare different transcriptome analysis techniques. The RNA-seq technique was found to be more sensitive than microarray analyses giving us the possibility to gain new insight into the role of MpkA. We were able to identify a substantial number of novel transcripts, to detect new exons, untranslated regions, thousands of new splice junctions, and found evidence for widespread alternative splicing events. We could also identify a large group of genes belonging to known and unknown gene clusters, which are normally involved in secondary metabolite production. They are differentially regulated in the DmpkA mutant strain. Moreover, the transcriptome data were compared to proteome data. Comparison between these two biological levels contributes to a better understanding of transcript stability and of post-transcriptional regulatory mechanisms, giving a more global overview about MpkA regulatory circuits (Müller, Baldin et al., BMC 2012).
232. Spliceosome twintrons ("swintrons") revealed by fungal nuclear genomes. Michel Fillipi, Erzsébet Fekete, Claudio Scazzocchio, Levente Karaffa. 1) Department of Biochemical Engineering, University of Debrecen, H-4010, Debrecen, Hungary; 2) Department of Microbiology, Imperial College London, London SW7 ZAZ, UK.

The spliceosome is an RNA/protein complex, responsible for internal intron excision in eukaryotic genes. In mitochondria and plastids intron excision does not involve the spliceosome. For a class of chloroplast introns (II and III) "introns within introns" (twintrons) have been described. The removal of the internal intron is necessary for the excision of the external intron, and thus RNA maturation. Analogous structures have not been described for spliceosomal introns. We have predicted four putative instances of "introns within introns" in nuclear genes of fungi. We call these "swintrons" for "spliceosomal twin introns". Putative swintrons show a variable phylogenetic distribution. The presence of the internal intron predicts specific splicing intermediates. We have experimentally confirmed the existence of the predicted intermediate for the splicing of an RNA encoding a putative cyclic imidine-hydrolase of Fusarium verticillioides (Sordariomycetes, Hypocreales), where the internal intron interrupts the donor sequence between the first and second nucleotide and predicted an analogous structure for a gene encoding a sugar transporter in two Magnaporthea. In the bioDA gene (encoding an enzyme catalysing two steps of biotin biosynthesis of the Sordariales, an internal intron, predicted to interrupt a donor sequence of an intron between the second and third nucleotide has been confirmed by isolation of the splicing intermediate. In the fourth instance the putative internal intron disrupts the donor sequence between the fourth and fifth nucleotide of the 5′ sequence. In this instance, the presence of the internal intron was disproved, revealing an unsuspected case of alternative splicing.

233. NGS data revealed that the NSDA sterile mutant contains a mutation in the SCF ubiquitin ligase subunit gene, culC, in Aspergillus nidulans. Dong-Soon Oh, Dong-Min Han, Masayuki Machida, Kap-Hoon Han. 1) Dept Pharmaceutical Engineering, Woosuk Univ, Wanjoo, Korea; 2) Division of Life Science, Wonkwang University, Iksan, Korea; 3) Bioproduction Research Institute, Hokkaido Center, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo, Japan.

Sexual development and fruiting body production of fungi play pivotal roles in production of ascospores by meiosis as well as adaptation of various environmental changes. In a homothallic fungus Aspergillus nidulans, many environmental factors and genes affecting sexual development have been elucidated. One of the first and important attempts for understanding the sexual development of A. nidulans was isolation of NSD, BSD and ASD mutants, which are defective in the process. Among them, NSD mutants are divided into four different complementation groups, NSDA, NSDB, and two of the mutants, NSDC and NSSD, have already been characterized about the responsible genes, nsdC and nsdD and their functions. However, nsdA and nsdB mutations are remained to be unveiled. Since classical complementation experiments were not successful, we analyzed the whole genome sequence of NSDA mutant obtained from Next Generation Sequencing (NGS) to identify the nsdA4 mutation. As a result, we found three NSDA mutant-specific mutations and confirmed the mutations by PCR followed by sequencing analysis. One of the mutations was found in AN3939 locus which encodes SCF ubiquitin ligase subunit CulC. The mutation was G to T transversion, making D468Y amino acid residue change. Since the COP9 signalosome and ubiquitin ligase play important roles in fungal development, this mutation could be the correct nsdA4 mutation responsible for the sterile NSDA mutant phenotype. However, since two more mutant-specific mutations were also found in NSDA, detailed genetic characterization and mutation analyses will have to be performed.

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234. Whole genome sequencing of two Aspergillus oryzae strains isolated from Meju, a traditional brick of dried fermented soybean, in Korea. Dong-Soon Oh, Seung-Bum Hong, Jung-Hwa Kim, Goro Terai, Hiroko Hagihara, Masayuki Machida, Kap-Hoon Han. 1) Dept of Pharmaceutical Engineering, Woosuk Univ, Wanjoo, Korea; 2) Korean Agricultural Culture Collection, NIAB, Korea; 3) Bioproduction Research Institute, Hokkaido Center, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo, Japan.

In Korea, various Aspergillus oryzae-like fungi are generally regarded as one of causal agents of Korean Meju, a soybean brick for soybean paste, fermentation. Since the fungal strain plays important roles in Japanese fermented food, A. oryzae type strain of Japan, RIB40, has been sequenced and analyzed in detail. Despite the importance of the A. oryzae strains in Korean fermented food, not many fungal strains have been isolated from fermented foods as well as Meju and the characteristics of the fungi isolated from Meju have not been elucidated so far, especially in molecular genetics and genomics level. In this study, we tried to reveal the differences between Japanese and Korean A. oryzae strains by characterizing the whole genome structure and their features. The whole genome sequence of two A. oryzae-like fungi, which were isolated from Korean Meju by Korean Agricultural Culture Collection (KACC), were obtained by Next Generation Sequencing. The genome sequences of these strains were analyzed using Oregon State University genome database and further selected the genes that are conserved among filamentous fungi. Most of these genes were widely conserved among other Aspergillus species and filamentous fungi, but not found in other organisms. Moreover, several genome array analysis revealed that some of these genes were highly expressed in various conditions, such as liquid or solid-state cultivations. In this work, we designated these genes as cff (Conserved among Filamentous fungi and Function unknown genes) genes. The analysis of the functions of these cff genes will be important to reveal the novel molecular mechanisms which conserved among filamentous fungi. In this context, we constructed cff genes disruptants library and analyzed the phenotype of these cff disruptants to examine the function of the genes and to identified new drug or breeding target genes. First of all, we isolated function unknown genes according to KOG category of A. oryzae genome database and further selected the genes that are conserved among 14 filamentous fungi as the candidate genes. Then we further examined several database, such as Swiss plot, AspGD etc., to verify the function-unknown then decided cff genes. From these cff genes, we performed the disruption of the highly expressed cff 147 genes and obtained 130 cff genes disruptants including 9 heterokaryon type disruptants. We observed the morphological phenotype of these cff genes disruptants on the minimal medium and natural medium using three serial powder plates, as a model assay of industrial conditions. As the result, some disruptants showed characteristic phenotypes in the hyphae growth and the conidiation. Furthermore, we examine the drug sensitivity of these disruptants using hydroxurea, camptothecin, mifafungin et. al. As the results, significant growth inhibition was observed in some disruptants, while some disruptant shown slight drug resistant. Now we are going to examine stress responses and second metabolite productions. We will further analyses the detail.
molecular function of the genes which shown significant phenotype in these analysis.

236. *Penicillium purpurogenum* degrades lignocellulose. What can we learn of this process by analyzing the genome, transcriptome and secretome of the fungus? Wladimir Mardones1, Eduardo Callegari1, Jaime Eyzaguirre1. 1) Department of Biology, Universidad Andres Bello, Santiago, Chile; 2) Sanford School of Medicine, Division of Basic Biomedical Sciences, University of South Dakota, Vermillion, SD.

*Penicillium purpurogenum* grows on a variety of natural carbon sources and secretes to the medium numerous cellulolytic and hemicellulolytic enzymes. Although some information on the lignocellulose biodegradation process has been obtained by the study of individual enzymes, a more comprehensive approach has been attempted by analysis of the genome, transcriptome and secretome of the fungus. A genome sequence draft has been attained by means of illumina Hi-Seq 2000 analysis followed by assembly (Allpaths-LG) and partial annotation (MAKER pipeline): 36 Mb total length, 579 scaffolds, 5038 Kbp, 8984 genes predicted. Using the same sequencing technology and the Trinity assembler, a transcriptome of the fungus grown on sugar beet pulp (50% pectin, 20% cellulose) has been obtained. It includes 7,172 ESTs with mean length of 307 bp; 7195 ESTs were significantly identified in the genome. The secretome of the sugar beet pulp culture was analyzed by shotgun mass spectrometry (2D-Nano-LC MS/MS) and 53 proteins were identified by MASCOT. An analysis of the genome draft for genes related to lignocellulose biodegradation enzymes (using dbCAN) showed 347 genes of putative CAZymes (38 carbohydrate esterases, 245 glycosyl hydrolases, 56 glycosyl transferases, 6 polysaccharide lyases and 2 carbohydrate binding modules). The transcriptome data (using BLASTX) showed that 111 CAZy genes were transcribed. In addition, 46 putative CAZymes were identified in the secretome. Among the 46 recognized, 6 are cellulosases and 19 are pectinases, directly related to the degradation of sugar beet pulp. This is the first *Penicillium* genome sequence technology and annotated for its lignocellulose biodegradation enzyme genes. Most of the identified genes correspond to putative non-characterized enzymes. This information will be of value for a better understanding of the lignocellulose biodegradation by filamentous fungi. Support: FONDECYT 1100084; UNAB DI-61-12/R.

237. Functional genomics of lignocellulose degradation in the Basidiomycte white rot *Schizophyllum commune*. Robin A. Ohm1, Martin Tegelaar2, Han A. B. Wösten2, Igor V. Grigoriev1, Luis G. Lugones1. 1) US DOE Joint Genome Institute, Walnut Creek, CA, USA; 2) Department of Microbiology and Kluiver Centre for Genomics of Industrial Fermentations, Utrecht University, Utrecht, The Netherlands.

White and brown rot fungi are among the most important wood decayers in nature. Although more than 50 genomes of Basidiomycyte white and brown rot have been sequenced by the Joint Genome Institute, there is still a lot to learn about how these fungi degrade the tough polymers present in wood. In particular, very little is known about how these fungi regulate the expression of genes involved in lignocellulose degradation. In Ascomycetes, several conserved transcription factors involved in regulation of complex carbon source degradation have been identified, but there are no homologs of these in Basidiomycetes. Few Basidiomycete white or brown rot genes are generally amenable, hindering a functional genomics approach to the study of lignocellulose degradation. A notable exception is *Schizophyllum commune*, for which numerous genetic tools are available. *S. commune* was grown on several carbon sources (glucose, cellulose, lignin or beech wood) and gene expression was analyzed. Numerous genes are strongly up-regulated on the complex carbon sources, compared to on glucose. As expected, many of these encode CAZymes (notably glycoside hydrolase family 61) and FOLymes, but also several well conserved proteins with unknown function. Interestingly, three transcription factor genes are up-regulated during growth on complex carbon sources, suggesting they may be involved in regulating this process. These transcription factors are highly conserved in Basidiomycetes, but not in Ascomycetes. The two laccase genes of *S. commune* are very lowly expressed on complex carbon sources, suggesting that their function in lignocellulose degradation is limited. A promoter analysis of up-regulated genes reveals a conserved putative transcription factor binding site, which is also present in related fungi. Experiments to validate these findings, as well as a proteomics analysis during growth on complex carbon sources, are currently in progress.

238. Functional characterization of genes expressed in early infection stages by the phytopathogenic fungus Botrytis cinerea. J. Espino, N. Temme, A. Viefhues, B. Oeser, P. Tudzynski. Institut of Plant Biology and Biotechnology, Westf. Wilhelms University, Schlossplatz 8, 48143 Munenster, Germany.

Botrytis cinerea is a phytopathogenic fungus that causes important economic losses in the agricultural field, due to its aggressiveness and ability to produce the “grey mould disease” in more than 200 plant species. Nowadays, the main strategy of control consists in the use of fungicides, although some strains are becoming resistant to these chemicals. Therefore, the knowledge of the molecular mechanisms during host-plant interaction could be a useful tool to develop new effective treatments against this organism. In microarray studies, we have identified more than 150 genes which are expressed during the early stages of infection, but not in conidia, suggesting an important role during fungal germination and penetration. Most of these genes codify for proteins with unknown function. By means of bioinformatic analyses, transmembrane domains and signal peptides were identified in some of these proteins, suggesting a possible role in signaling pathways of the plant. We are currently focusing on 18 of these genes, and we validated their expression by real time PCR. In all cases the expression pattern observed in the microarrays studies could be confirmed by quantitative PCR results. Some of them showed an expression at 12 hours post inoculation even 10,000-fold compared to the expression in conidia. In order to elucidate the possible role of these genes, we have generated knock-out mutants of 9 single genes and 9 genes located in clusters. Pathogenicity studies as well as further characterization of the different deletion mutants are now in progress.

239. Regulation of biofilm formation in Candida parapsilosis. Linda Holland, Leona Connolly, Denise Lynch, Geraldine Butler. School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin, Dublin, Ireland.

Candida parapsilosis is a major cause of infection in premature neonates, particularly because of its tendency to grow as biofilms on indwelling medical devices. The biofilm architecture of *C. parapsilosis* biofilms is substantially different to that of Candida albicans, in part due to regulation of biofilm formation in either *C. albicans* or *C. parapsilosis*. The collection was assayed for changes in biofilm formation using 24 deletion strains, efg1, czf1, mkc1, gzf3, ume6, ace2, cph2 and bcr1 having been deleted using the PCR method originally developed for *C. albicans* to construct gene deletions in the type strain *C. parapsilosis* CLIB214 (1). To date, we have generated 100 homozygous deletion strains. We selected predicted protein kinase genes, transcription factors and also genes that are known to be important for biofilm formation in either *C. albicans* or *C. parapsilosis*. The collection was assayed for changes in biofilm formation using 24-well Nunc polystyrene plates and by measurement of the dry weight of mature biofilm. Eight deletion strains, efg1, czf1, mkc1, gzf3, ume6, ace2, cph2 and bcr1 have a defect in biofilm development. Only efg1 and bcr1 deletions of *C. albicans* have similar defects. *C. albicans* and *C. parapsilosis* therefore share some key regulators of biofilm formation, but there are also substantial differences. References: (1) Noble SM, French S, Kohn LA, Chev V, Johnson A. D Nat Genet. 2010 Jul;42(7):590-8. Systematic screens of a Candida albicans homozygous deletion library decouple morphogenetic switching and pathogenicity.

240. Functional analysis of the Mps1 MAP kinase pathway in the rice blast fungus Magnaporthe oryzae. E. Grund1, M.-J. Gagey1, V. Toquin3, R. Beffa3, N. Poussetreau1, M.-H. Lebrun1. 1) MAP CNRS-UCB-INSA-Bayer CropScience, Lyon, France; 2) Biochemistry Dept, Bayer CropScience, Lyon, France; 3) Bayer CropScience AG, Frankfurt/Main, Germany; 4) BIOGER INRA, Thiverval-Grignon, France.
FULL POSTER SESSION ABSTRACTS

Signaling pathways are important in coordinating fungal cellular processes required for stress resistance, development and pathogenicity. The Mps1 MAP kinase pathway of Magnaporthe oryzae is involved in cell wall integrity, sporulation and pathogenicity. Dmps1 mutants displayed an abnormal mycelial growth (reduced hyphae and melanisation), did not sporulate and were non-pathogenic on plants as reported (a). Sensitivity of M. oryzae to cell wall degrading enzymes (CWDE) and cell wall inhibitors (CWI) was found to be dependent on pH. Indeed, M. oryzae cell walls display a resistance to enzymatic degradation at pH 5, while they are sensitive at pH 6. Dmps1 loses this pH 5 induced cell wall resistance, while it is as sensitive to CWDE as wild type at pH 6. M. oryzae is highly resistant to calcfluor (cell wall disorganizing agent) at pH 5 (10x) compared to pH 6. Dmps1 loses this pH 5 induced calcfluor resistance, while it is as sensitive as wild type at pH 6. M. oryzae is more sensitive (20x) to Nikkomycin Z (chinone synthase inhibitor) at pH 5 than pH 6, while sensitivity to Aculaein (glucan synthase inhibitor) is independent of the pH. However, Dmps1 is as sensitive as wild type to these inhibitors at both pH. We conclude that the pH 5 induced resistance of fungal cells walls to CWDE and calcfluor requires the Mps1 pathway. This also suggests that the Mps1 pathway is strongly activated at pH 5 compared to pH 6. To test this hypothesis, we are assaying the phosphorylation status of Mps1 at different pH as well as under several stress conditions and developmental stages to know when this pathway is activated. Additionally we constructed an activated allele of Mps1, the MAPK upstream of Mps1, placed under the control of either its own promoter (b) or the repressible PIA1 promoter. These transformants will be used to assess the effect of controlled activation of the Mps1 pathway on M. oryzae cellular functions. The different conditions of Mps1 pathway activation will be used for a comparative transcriptomic analysis of wild type and Dmps1 mutants.


241. A RNA-Seq directed functional genomics screen to identify novel cell wall genes in the hyphal tip of Neurospora crassa. Diwy A. Sain, Lorena Rivera, Jason Stajich. Plant Pathology & Microbiology, University of California, Riverside, Riverside, CA. The cell wall is one of the most important organelles of the fungal cell and differentiates pathogenic fungi from the plants and animals they infect. This makes cell wall biosynthesis an excellent target for anti-fungal drugs. To identify new targets we employed a functional genomics approach informed by gene expression patterns based on RNA-Seq data of the filamentous fungus Neurospora crassa. The growth tips of fungal hyphae are enriched for cell wall biosynthesis activity proteins and transcripts (1-2). Based on this idea we sequenced RNA from the tip (1 hr growth) and colony interior (20 hr growth) of vegetative growing culture of N. crassa. 70 genes were up-regulated in the tip (at least 5 fold) and we supplemented this list with 42 tip expressed genes from a study of N. crassa colony development using microarrays, where mRNAs transcripts in the colony tips were enriched in functional categories related to cell wall growth and morphogenesis (2). We used the N. crassa knockout collection (3) to identify developmental phenotypes and under chemical stress conditions to expose sensitivity in cell wall and growth defects. Almost 60 percent of the genes were found to be sensitive to cell wall stress agents, Caspofungin (cell wall integrity inhibition) and SDS (cell wall disruption) suggesting that our gene-set was enriched for genes having a cell wall defect. We tested these genes for defects in the hyper-osmolar stress (NaCl & Glycerol) and oxidative stress pathways as well as sexual development pathway. We found 20 knockout strains having defects in all or nearly all of these pathways suggesting these cell wall genes are involved in multiple pathways of growth and development of filamentous fungi. This includes Zn-Cys transcription factors (NCU04866 & NCU04663), Glycoside Hydroxase 13 family proteins (NCU08131 & NCU08132) and genes with no annotated function (NCU04826 & NCU01254). All of these genes possess homologs in other Peziomycotina fungi. Hence our approach using gene expression selected a candidate gene-set enriched for growth processes that may be useful as targets for anti-fungal drug development against filamentous pathogenic fungi. 1) Bartnicki-Garcia & Lippman. Science 1969; 165(3890):302-4. 2) Kasuga & Glass. Euk Cell 2008; 7(9):1549-64. 3) Colot et al. PNAS 2006; 103(27):10352-7.

242. Identification of centromeres in the plant pathogen Zymoseptoria tritici (synonym Mycosphaerella graminicola). Klaas Schotanus1, Lanelle R. Connolly2, Kristina M. Smith2, Michael Freitag2, Eva H. Stukenbrock1. 1) MPFR Fungal Biodiversity, Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany; 2) Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR, USA.

Several plant pathogenic fungi contain small, apparently dispensable chromosomes, and in several cases pathogenicity genes have been identified on these chromosomes. The ascomycete Zymoseptoria tritici has up to eight dispensable chromosomes in addition to thirteen “core” chromosomes. During meiosis dispensable chromosomes are lost at elevated rates, resulting in progeny with distinct novel chromosome sets. So far little is known about the role of these chromosomes and their evolutionary dynamics. We hypothesize that loss of dispensable chromosomes during meiosis may be correlated to unstable centromeres. Thus, one goal was to identify and characterize centromeric regions on core and dispensable chromosomes to allow us to understand the underlying genetics of chromosome instability. Both core and dispensable chromosomes in the Z. tritici reference isolate IPO323 have been sequenced from telomere to telomere, yielding a unique opportunity to identify the centromeric regions influencing genome. We tagged the Z. tritici centromere-specific histone 3 (CenH3) with GFP and confirmed correct insertion by Southern analyses. We demonstrate expression of GFP-tagged CenH3 by western blot and epifluorescence microscopy. ZtCenH3-GFP was localized in discrete foci in interphase nuclei, but in contrast to other fungi (e.g., Neurospora, Fusarium, Saccharomyces and Schizosaccharomyces) there are several foci per nucleus instead of a single chromocenter. We also performed chromatin immunoprecipitation followed by DNA sequence (ChIP-seq) on the CenH3-GFP strains. To confirm the CenH3-GFP results, we tagged two additional centromere proteins (CEN-B and CEN-S) with GFP. To assess stability of centromeres during mitosis and meiosis, we obtained evolved asexual progeny of IPO323 after 50 and 100 generations and progeny from a cross. Comparison of centromeric positions in the genome of the founder strain (IPO323) versus sexual and asexual progeny will allow us to infer dynamics of centromeres on core and dispensable chromosomes, and aid in our understanding of the evolutionary dynamics of dispensable chromosomes in fungal plant pathogens.


Loss of RNAi in VGGI Cryptococcus gattii sheds light on RNAi roles in Cryptococcus neoformsans R. Blake Billmyre, Xuying Wang, Marrianna Feretazki, and Joseph Heitman RNAi is a broadly conserved homology-dependent silencing mechanism which functions to defend the genome by silencing transposons and viral elements. The opportunistic human pathogen C. neoformsans, utilizes an RNAI-dependent process to robustly silence repetitive elements during the sexual cycle. Interestingly, RNAI components have been widely lost from the VGGI subtype of the closely related sister species C. gattii. We have taken a comparative genomics approach to compare the RNAI deficient genome of VGGI C. gattii with the RNAI proficient genomes of VGI C. gattii and serotypes A and D C. neoformsans. This approach has identified a total of fourteen gene losses or truncations of otherwise conserved genes in VGI, including three of the known canonical RNAI components. Two of the remaining eleven genes have been shown to have a role in the sex-induced silencing pathway in C. neoformsans var. grubii, despite a lack of homology with previously identified RNAI components in other organisms. One of these genes CPR2, was previously studied in our lab as a constitutively active G-protein coupled pheromone receptor. CPR2 also confers a moderate defect in sex-induced silencing, but no defect in silencing during vegetative growth. Similarly, the zinc finger factor ZnF3 was previously identified in our lab and here was
unexpectedly found to be important for both sex-induced and vegetative silencing. The nine remaining missing genes are being tested for roles in both sex-induced and mitotic silencing (SIS, MIS).


The systematic profiling of the impact of small molecules on the growth rate of gene deletion mutants is termed chemogenomic profiling. This approach has been extensively used in model organisms, primarily baker's yeast, to functionally annotate genes and to obtain insights into mode-of-action (MOA) for chemical compounds (1). Here we describe the application of systematic chemical-genetics a significant human pathogen. Cryptococcus neoformans is an opportunistic basidiomycetes pathogen responsible for lethal meningitis in immunocompromised patients. Current therapies are inadequate due to a paucity of drugs and a poor understanding of pathogenesis. Our laboratory previously constructed a partial gene deletion collection and used it to identify numerous genes required for infection as well as for the production of virulence factors (2). This work identified numerous novel infectivity genes, but many did not have an identifiable molecular function. We have now used chemogenomic profiling to both bridge this gap in gene annotation and to obtain insights into drug MOA. To accomplish this goal, we identified and utilized over 200 diverse chemical compounds that impact pathogen growth to create a unique phenotypic signature for ~1500 C. neoformans gene deletion strains. We used colony arrays, robotics, automated image analysis, and extensive data normalization algorithms to analyze several million phenotypic measurements. We used these data to identify clusters of genes and compounds with related patterns of chemical-genetic interactions. Our analysis identified virulence genes that act through related mechanisms. For example, one gene set involves a number of predicted to be involved in histone modification. Members of a second set of genes are required in production of the C. neoformans polysaccharide capsule, a well-established virulence factor. We have also obtained new insights into the MOA of several antifungal compounds. The Cryptococcus chemical-genetic map will be a valuable resource for functional annotation of the genome of this meningitis pathogen, characterization of new drug targets, and the identification lead compounds for antifungal drug development.


245. Whole genome sequencing of high-mortality and low-mortality strains of Cryptococcus neoformans var. grubii to discover genetic determinants of virulence. Tami R. McDonald, Kirsten Nielsen. Department of Microbiology, University of Minnesota, Minneapolis, MN.

In sub-Saharan Africa, meningitis caused by the fungus Cryptococcus neoformans var. grubii is a major cause of AIDS-related mortality. To investigate the role of fungal genotype in clinical disease, we sequenced 8 genes for 503 clinical isolates of Cryptococcus neoformans var. grubii. A phylogenetic analysis of these strains demonstrated that 501 isolates were VNI strains. Haplotype network analysis revealed three major groups (BURST groups 1 - 3). Patient mortality was associated with fungal strain genotype, with strains in BURST group 3 demonstrating low mortality. Whole genome sequencing of 13 representative genotypes revealed SNPs unique to the high mortality strains, and SNPs unique to the low-mortality strains, pointing to possible targets for future gene deletion and allelic swap experiments to determine the role of the genes in pathogenesis.

246. Identification of high temperature-regulated genes controlled by Sch9 through comparative transcriptome analysis in Cryptococcus neoformans. Dong-Hoon Yang1, Kwang-Woo Jung1, Jang-Won Lee1, Min-Hee Song1, Anna Floyd2, Joseph Heitman1, Yong-Sun Bahn1, 1) Biotechnology Department, Yonsei University, Seoul, South Korea; 2) Departments of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA; 3) Departments of Medicine, and Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC, USA.

Adaptation to temperature changes is one of crucial virulence factors for Cryptococcus neoformans during host infection. In the human fungal pathogen, diverse signal transduction pathways, such as Ras/Cdc24, calmodulin/calciineurin, Mpk1 and Hog1 MAPK pathways, are involved in the temperature adaptation process. In addition to the pathways, the Sch9 protein kinase has been implicated in thermotolerance of C. neoformans, but its regulatory mechanism remains elusive. In this study we aimed to identify Sch9-dependent or -independent temperature-regulated genes in a genome scale and to elucidate the regulatory mechanism of Sch9 in thermotolerance of C. neoformans. For this purpose, we performed comparative transcriptome analysis with the wild type serotype A H99 strain and sch9D mutant during temperature upshift from 25°C to 37°C or 40°C. The temperature upshift caused a global scale of remodeling in gene expression profiles (1872 genes, P<0.05) in the wild type strain of C. neoformans. Gene expression patterns shown during temperature shifting from 25°C to 40°C were generally similar, albeit with the different induction or repression levels, to those from 25°C to 37°C. Especially the expression levels of the genes encoding chaperone, heat shock protein, or the proteins related to ergosterol biosynthesis were differentially regulated by temperature shifting. The basal expression levels of a number of the temperature-regulated genes were modulated by Sch9 protein kinase.

Interestingly the basal expression levels of the genes related to unfolded protein response (UPR) pathways including IRE1, HXL1, and KAR2 were also regulated by Sch9 protein kinase, implying the potential link between Sch9 and the UPR pathway. Supporting this finding, the sch9D mutant showed increased resistance to ER stress agents, including tunicamycin and DTT. This study not only provides further insight into the regulatory mechanism of thermotolerance in C. neoformans, but also elucidates the regulatory mechanism of Sch9 in thermotolerance through a genome-scale identification of the Sch9-dependent genes.

247. Genome-wide analysis of eleven white- and brown-rot Polyporales provides insight into mechanisms of wood decay. Chiaki Honjo1,2, Kiyohiko Igarashi1, David Hibbett3, Bernard Henrissat2, Masahiro Samejima2, Dan Cullen1. 1) Graduate School of Agricultural and Life sciences, University of Tokyo, Tokyo, Japan; 2) Forest Products Laboratory, USDA, Madison, WI; 3) Biology Department, Clark University, Worcester, MA; 4) CNRS, Marseille, France.

Many efficient wood decay fungi belong to the Polyporales, and these can be categorized as white-rot fungi or brown-rot fungi, based on decay patterns. White-rot fungi degrade cell wall polysaccharides such as cellulose and hemicellulose as well as the more recalcitrant phenylpropanoid polymer, lignin. In contrast, brown-rot fungi depolymerize the polysaccharides but the modified lignin remains in the wood. Comparative analysis of white- and brown-rot gene repertoires and expression profiles have revealed substantial variation but considerable uncertainty persists with respect to precise mechanisms. Addressing this issue, we performed genome-wide analysis of carbohydrate-active enzymes (CAZy) and some oxidative enzymes related to polysaccharides degradation in eleven white- and brown-rot fungi. This analysis included classifying and enumerating genes from three recently sequenced polyporales Bjerkandera adusta, Ganoderma sp. and Phlebia brevispora. Furthermore, secretome analysis of seven Polyporales grown on wood culture were conducted. Summarizing, the average number of genes coding CAZy in the genomes of white-rot fungi was 373, significantly more than the 283 observed in brown-rot fungi. Notably, white-rot fungi have genes encoding cellulase and hemicellulase such as those belonging to glycoside hydrolase (GH) families 6, 7, 9 and 74, whereas these are lacking in genomes of brown-rot polyporales. White-rot genes encoding oxidative enzymes potentially related to cellulose degradation such as cellobiose dehydrogenase (CDH), polysaccharides monoxygenase (PMO, formerly GH61), cytochrome b562 with cellulose-
binding module, are also increased relative to brown-rot fungi. Indeed, secretomeric analysis identified GH6, GH7, CDH and PMO peptides only in white-rot fungi. Overall, these results show that, relative to brown rot fungi, white rot polypeptides maintain greater enzymatic diversity supporting lignocellulose attack.

248. Genomic context and distribution of effector genes in *Fusarium oxysporum*. Sarah Maria Schmidt1, Peter van Dam2, Petra M. Houterman3, Ines Schreiber4, Lison Ma5, Stephan Amyotte6, Biju Chellappan7, Sjef Boeren8, Frank L.W. Takken8, Martijn Rep1. 1) Molecular Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, the Netherlands; 2) Fachgebiet Medizinische Biotechnologie, Institut für Biotechnologie, Technische Universität Berlin, Gustav-Meyer-Allee 25, Germany; 3) Department of Plant Pathology, University of Kentucky, 201F Plant Science Building, 1405 Veterans Drive, Lexington, KY 40546-0312, USA; 4) Laboratory for Biochemistry, Wageningen University, Dreijenlaan 3, 6703HA, Wageningen, the Netherlands. 

Strains of the *Fusarium oxysporum* species complex (FOSC) are able to infect a wide range of mono- and dicotyledonedous plants. Based on the host specificity of individual strains, the FOSC is divided into various *formae speciales*. All strains share a common core genome and possess additional lineage-specific (LS) chromosomes. The fungus secretes effector proteins into the host vascular system that presumably manipulate the host to promote infection. In the tomato pathogen *F. oxysporum* f. sp. *lycopersici* (Fol) these effectors are encoded by SIX (Secreted In Xylem) genes. Interestingly, all SIX genes are present on a single LS chromosome that can be transferred horizontally to a previously non-pathogenic *Fot* strain, resulting in gain of pathogenicity towards tomato. Upon close inspection of this tomato pathogenicity chromosome we discovered that a non-autonomous miniature transposable element (mite) is present in the promoters of all SIX genes. Promoter deletion analysis at two different SIX gene loci did not reveal a direct role of the mite for SIX gene expression. However, we were able to use this genomic signature to predict novel effector gene candidates in the *Fol* genome. Expression of several of these novel candidates during infection was confirmed by mass spectrometric analysis of the xylem sap of *Fol*-infected tomato plants. We also discovered a small reservoir of ‘silent’ effector genes that are not expressed during infection. Next, we used our method to predict effector gene candidates in the genomes of several other *formae speciales* and developed a more global picture of the effector gene complement in the FOSC. Effector genes in *Fot* consistently reside in repeat-rich environments. Some strains contain 2 or 3 paralogs of an effector gene. Additionally, many genomes feature truncated effector gene homologs. Overall, the effector gene distribution among different *formae speciales* is patchy, and there is no unique set of effectors that is common to all plant pathogenic strains of the FOSC.

249. Whole genome sequencing reveals new links between diverse plant pathogens; an expanded *AvrLm6*-like gene family in *Venturia* species. Jason Shiller1, Angela van de Wouw2, Dan Jones3, Joanna Bowen4, Carl Mesarchi5, Matthew Templeton5, Kim Plummer6. 1) La Trobe University, Melbourne, Australia; 2) University of Melbourne, Melbourne, Australia; 3) Plant and Food Research, Auckland, New Zealand. 

*Venturia inaequalis* and *V. pirina* are hemi-biotrophic fungi that cause apple scab and pear scab, respectively. These diseases cause significant losses to growers worldwide. In some cases, scab is controlled with resistant cultivars, but fungicides are more commonly used. Resistance to scab follows the gene-for-gene model, whereby a gene coding for a resistance protein in the host will have a cognate gene coding for an avirulence protein (or effector) in the fungus. No *Venturia* effectors have been characterised to date, but work is underway to identify effectors from the whole genome sequences and secreted proteins of *V. inaequalis* and *V. pirina*. Whole genome sequencing of *Venturia inaequalis* and *V. pirina* has revealed predicted proteins with some sequence similarity to *AvrLm6*, a *Leptosphaeria maculans* effector that triggers resistance in *Rlm6* canola. The mechanism of action of *AvrLm6* is unknown. Until recently, *AvrLm6* was thought to be unique to *L. maculans*, with orthologues absent, even in closely related species. *AvrLm6*-like genes from Venturia spp whose genomes are sequenced form large families containing up to 30 members. We have also identified orthologues in *F. oxysporum* (Fo5176) and *C. higginsianum* from public database searches. Gene expansions have also been observed for other effector-like genes in the Venturia genomes. The *AvrLm6*-like predicted protein from *V. inaequalis*, with the highest sequence identity to *AvrLm6*, was unable to trigger a resistance response in *Rlm6* canola. However, this does not preclude the *AvrLm6*-like proteins from being functionally active in the *Malus-Venturia* pathosystem. Transcriptome analyses (RNA-seq) of *in planta* and *in vitro* samples of *V. inaequalis* have revealed that a number of *AvrLm6*-like genes are up-regulated during infection (compared to growth in *vitro*). These results were confirmed with qRT-PCR. The most highly up-regulated predicted protein, *ALVi_149*, was tagged with YFP. YFP expression was observed only in the sub-cutticular stromata (specialised, biotrophic infection structures). RNA silencing is currently underway to determine the role of *ALVi_149* in pathogenicity of *V. inaequalis*. The major question that remains is; what purpose do these genes serve for these diverse fungi and what is driving the gene expansions in Venturia spp?

250. Oömycetes Protein Array Project. Samantha Taylor1, Regina Hanlon2, Mandy Wilson3, Jean Peccoud2, Brett Tyler1. 1) Oregon State University, Corvallis, OR; 2) Virginia Tech, Blacksburg, VA. 

Oömycetes are eukaryotes that outwardly resemble fungi, but are related to brown and golden-brown algae. The most destructive oömycete genus is *Phytophthora*, with over 80 species that collectively attack a wide range of plant species, causing damage to crops that is estimated in billions of dollars annually in the US. The goal of the Oömycetes Protein Array Project is to generate a collection of cloned proteins from 1440 predicted oömycete effector sequences, to use Gateway® technology to facilitate the easy transfer of clones into expression vectors, and to make the resulting clones available to the scientific community for further research. When the project is over, the final collection should include 390 clones from *P. sojae*, 550 clones from *P. infestans*, 370 clones from *P. ramorum*, and 130 clones from *H. arabidopsis*.

251. Extensive chromosomal reshuffling drives evolution of virulence in an asexual pathogen. Ronnie de Jonge1, 2, Melvin Bolton1, Anja Kombrink1, Koste Yadeta1, Grady van den Berg1, Bart Thomsma1. 1) Laboratory of Phytopathology, Wageningen University, Drouweaaldaalsteen 1, 6708 PB Wageningen, The Netherlands; 2) VIB Department of Plant Systems Biology, Ghent University, Bioinformatics and Evolutionary Genomics Division, Technologiepark 927, B-9052 Gent, Belgium; 3) United States Department of Agriculture, Agricultural Research Service, Northern Crop Science Laboratory, Fargo, ND 58102-2765, United States.

Sexual recombination drives genetic diversity in eukaryotic genomes, and fosters adaptation to novel environmental challenges. Although strictly asexual microorganisms are often considered as evolutionary dead ends, they comprise many devastating plant pathogens. Presently, it remains unknown how such asexual pathogens generate the genetic variation that is required for quick adaptation and evolution in the arms race with their hosts. Here we show that extensive chromosomal rearrangements in the strictly asexual plant pathogenic fungus *Verticillium dahliae* establish highly dynamic ‘plastic’ genomic regions that act as a source for genetic variation to mediate aggressiveness. We show that these plastic regions are greatly enriched for *in planta*-expressed effector genes, encoding secreted proteins that enable host colonization including the previously identified race 1-specific effector *Ave1* that activates Ve1-mediated resistance in tomato. The plastic regions occur at the flanks of chromosomal breakpoints and are enriched for repetitive sequence elements, especially retrotransposons. Our results demonstrate that asexual pathogens may evolve by prompting chromosomal rearrangements, enabling
252. Genomic census of transmembrane proteins of the marine fungus, Corollospora maritima. Derek Johnson, Joseph Spatafora. Botany and Plant Pathology, Oregon State University, Corvallis, OR.

The class Sordariomycetes (Ascoscyta) presents a model phylogenetic system to study the genomic evolution of marine fungi, as there have been at least four major independent transitions from terrestrial to marine environments. These marine fungi have adapted to an environment which requires increased control of the movement of water between the cell and the environment due to osmosis. Given this, the greatest adaptive pressure may be found working at the interface of the fungal cell with the environment in the form of transmembrane proteins and in biochemical pathways dealing in osmoregulation. We have initiated a comparative, phylogenomic study of the independent lineages of marine Sordariomycetes with the goal of determining what pathways have been modified in the transition to the marine environment. Here we present preliminary data of the draft genome of the marine fungus Corollospora maritima, which is a member of the largest family of marine fungi, Halosphaericaeae (Microascales). The draft genome of C. maritima is similar to other Sordariomycetes (37 MB, 10269 predicted gene models) but is characterized by an increased number of transmembrane proteins; both in raw number (2017), and as a percentage of protein coding genes (19.64%), than non-marine members of the Sordariomycetes. Thus, it is hypothesized that C. maritima has expanded osmoregulatory protein families and adapted novel transmembrane proteins as a consequence of the transition to the marine environment. Transmembrane proteins belonging to C. maritima along with select species of fungi and Eukaryotes were predicted via hidden markov modeling using the program TMHMM and clustered into orthologous groups using Ortho-MCL. Putative protein identities (e.g., aquaporins, aquaglyceroporins, osmosensors and the sodium efflux ENA ATPases) were assigned to each cluster using the BLAST tool comparing orthologous protein cluster identities to proteins of the model organisms Neurospora crassa and Saccharomyces cerevisiae. Expansions and contractions of transmembrane proteins will be presented in a phylogenetic context, and more complex patterns of evolution, such as more ancient lineage sorting vs. lineage specific expansions, will be tested for each orthologous group of proteins using gene tree/species tree reconciliation analyses.

253. Fungal Calcium Signaling Database (FCSD). Venkatesh Moktali, Bongsoo Park, Seogchan Kang. Penn State University, University Park, PA 16802, USA.

Calcium probably is one of the most versatile elements in biological systems. It serves as a pivotal signal in controlling diverse cellular and developmental processes to ensure the healthy functioning of every organism ranging from microbes to humans. The mechanism of translating external stimuli to specific cellular and developmental responses via changes in calcium ions plays an essential role in the plant-microbe and microbe-environmental interactions. Accordingly, many genes of the calcium-signaling pathway have been found to be virulence factors of fungal pathogens. How this simple and ubiquitous ion has evolved to control so many processes is one of the central questions in biology with many practical implications. Rapid advances in genome sequencing of many fungal and oomycete species have uncovered conserved core calcium signaling genes, as well as lineage-specific features. To support systematic studies on this evolutionary variability in fungi and oomycetes and the functional roles of individual genes, we built the Fungal Calcium Signaling Database (FCSD; http://fcsd.ifungi.org/), an online platform that categorizes and annotates key calcium signaling proteins from more than 120 published fungal and oomycete genomes. The database also archives experimental results from studies on mutants of calcium signaling genes and resulting calcium signatures in both video and picture formats. The calcium signaling genes in FCSD are divided into five major groups namely, calcium-permeable channels, calcium pumps, calcium exchanger/antiporter, calcium signaling regulators, and calcium-binding proteins. Comparison of calcium signaling machineries between fungi and oomycetes has been conducted to identify evolutionary changes that have shaped up this signaling pathway in these kingdoms. The FCSD will greatly support the fungal community in studying and understanding calcium signaling.


The subphylum Pezizomycotina presents a vast diversity of ecological niches and biochemical processes observed in fungal subphyla. Changes in members of the cytochrome P450 (CYP) superfamily appear to have played key roles in fungal niche adaption and evolution. Availability of genomic data from many species in this subphylum has enabled comprehensive phylogenetic studies to understand the taxon-specific genetic changes that potentially underpin the observed functional and ecological diversity. CYPs from 53 Pezizomycotina species were analyzed to study the gene birth and death patterns at the genus level. This analysis revealed niche- and class-specific CYP family expansions and contractions. Putative metabolic functions were assigned to individual CYPs in each species based on sequence similarity to functionally characterized CYP proteins. Also, pathogenic Pezizomycotina fungi were divided into three classes (hemibiotrophs, obligate biotrophs and necrotrophs) to identify CYP family expansions and innovations potentially associated with these classes. Large losses in CYP families were observed among obligate biotrophs whereas hemibiotrophs and necrotrophs showed gene gains as well as functional innovation in the form of species-specific CYP families. Examination of the classes/divisions within Pezizomycotina suggested a number of independent losses and gains in CYP families. These findings shall be presented in the poster.


Many eukaryotes interact with heritable endobacteria to satisfy diverse metabolic needs. Of the characterized fungal-bacterial endosymbioses, the association between Gigasporaceae (Glomeromycota) and Ca. Glomeribacter is one of the best described. Glomeribacter is a member of the Burkholderia lineage of b-proteobacteria, and was shown previously to represent one of the few cases of an ancient, long-term non-essential endosymbiont. In order to further explore what adaptations have taken place to shape this unique bacterial lifestyle, we have sequenced three Glomeribacter lineage specific features. To support our findings we tested for each orthologous group of proteins using gene tree/species tree reconciliation analyses.
256. The Unique family of Telomere-Linked Helicases in Fungi. Olena Novikova1,2, Mark Farman2. 1) Department of Biological Sciences, University at Albany, Albany, NY; 2) Department of Plant Pathology, University of Kentucky, Lexington, KY.

Subtelomeres and telomeres are highly dynamic regions of eukaryotic chromosomes and their maintenance is crucial for cellular function. Several helicases are known to be present in the telomeric DNA, e.g. RecQ-like helicases such as human BLM or WRN helicases. Curiously, RecQ-like helicase genes are found in very close proximity to telomeres in several fungal species. In the present study we performed comprehensive survey of these Telomere-Linked Helicases (TLHs) in 101 fully-sequenced fungal genomes. The TLHs were widely yet sporadically distributed among fungal species being present in 46 species belonging to all investigated groups except Zygomycetes. The TLHs were also unique to fungi. Many of the TLH genes were found either next to telomeres or at the ends of contigs, providing indirect evidence that they are telomere associated. To date, the TLH gene families are the only examples where the chromosomal positions of member genes are absolutely conserved across a kingdom. Despite the seemingly conservative positions of the TLH genes on the chromosomes, the genes themselves are clearly not well conserved because they were found in only in half of the fungal genomes surveyed. The TLHs were highly divergent from one another and demonstrated complex evolutionary histories that reflect recurrent cycles of telomere crisis and recovery. Their telomere association leads us to hypothesize that the TLHs are involved either in telomere maintenance, or in the recovery processes associated with telomere crisis.

257. Evolution of proteins containing intein- and Hedgehog-like Vint domains in Fungi. Olena Novikova, Marlene Belfort. Department of Biological Sciences and RNA Institute, University at Albany, Albany, NY.

Inteins are protein sequences that autocatalytically splice themselves out of the protein precursors - analogous to introns - and ligate the flanking regions into a functional protein. Intein-containing genes are present in all three kingdoms of life. Moreover, it was shown that the C-terminal domain of eukaryotic Hedgehog (Hh) proteins has sequence similarity to inteins. The Hh pathway is one of the fundamental signal transduction pathways in animal development and is also involved in stem-cell maintenance and carcinogenesis. Two distinct domains can be found in Hh - the N-terminal 'Hedgehog' domain (HhN), and the intein-like C-terminal 'Hog' or 'Hind' domain (HhC). The Hedgehog pathway is absent from fungi. However, other families of proteins were found containing Hog/Hind-like domains. Representatives from one of these families carry the von Willebrand factor type A (vWA) domain in addition to the Hog/Hind-like domains. These domains are called Vint (von Willebrand Hint-like). Vint-containing proteins were initially reported for plants, fungi and some metazoans. We explored the diversity of Vint-containing proteins, their distribution and evolutionary history in fungi. Vint-containing proteins are widely distributed among fungal lineages; however, they are absent from some of the fungal species and entire fungal groups (e.g. Saccharomyces). Based on the evolutionary pattern, we propose a modular model of the evolution for Vint-containing proteins. While the vWA domain seems to be the core functional unit, an additional domain, the U-box, is a recent acquisition. The vWA and U-box combination was found exclusively in fungi. The Vint domain is highly conserved and is likely under purifying selection. The functional role of Vint-containing proteins and the Vint domain in particular is the subject for further studies. This research has been supported by NIH grant GM044844.

258. The genome and development-dependent transcriptome of Pyronema confluens: a window into fungal evolution. Stefanie Traeger1, Jason Stajich2, Stefanie Pöggeler1, Minou Nowroussian1. 1) Department of General & Molecular Botany, Ruhr University Bochum, 44780 Bochum, Germany; 2) Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA; 3) Institute of Microbiology and Genetics, Department of Genetics of Eukaryotic Microorganisms, Georg-August University, 37077 Göttingen, Germany.

In the last decade, genomes of many filamentous ascomycetes have been sequenced and are invaluable for the analysis of the evolution of species and for understanding their physiological and morphological properties. However, while there are at least ten genome sequences available for each of the more derived groups of filamentous ascomycetes (Sordariomycetes, Leotiomycetes, Eurotiomycetes, Dothideomycetes), only one genome from the basal group of Pezizomycetes has been sequenced, namely that the black truffle, a fungus with a specialized life-style and fruiting body. Therefore, we sequenced the genome and transcriptome of the Pezizomycete Pyronema confluens, a saprobe with typical apothecia as fruiting bodies. The genome was assembled from a combination of Roche/454 and Illumina/Solexa reads. It has a size of 50 Mb, and a predicted 13369 protein-coding genes. P. confluens is homothallic, and we found two MAT loci that are not fused or in close proximity, and encode an alpha domain and an HMG domain transcription factor. The MAT1-2 locus is flanked by the conserved apn2 gene, whereas both MAT loci are flanked by a pair of paralogous genes not found in this location in other ascomycetes. Thus, the P. confluens MAT loci might reflect an evolutionary transition state on the way towards the relatively conserved genomic arrangement of MAT loci in higher ascomycetes. Sexual development in P. confluens is light-dependent, and qRT-PCR analyses of predicted photoreceptor genes showed that all are upregulated by light. Fruiting body formation is stimulated by part of the visible spectrum, and we are currently investigating the effect of different wavelengths on development and gene expression. For RNA-seq analysis, we used three conditions: growth in the light, and two different conditions allowing only vegetative growth. We analyzed expression levels for genes with different degrees of evolutionary conservation to find out if genes with different lineage-specifics are preferentially expressed under any of the conditions investigated. Interestingly, the highest percentage of genes upregulated during sexual development is found among the P. confluens orphan genes. This might indicate that, similar to the situation in animals, genes associated with sexual reproduction evolve more rapidly than genes with other functions.

259. Genome and transcriptome analysis of the mycoparasite Clonostachys rosea. Kristiina Nygren1, Mikael Brandström Durling1, Chatchai Kosawang2, Dan Funck Jensen1, Magnus Karlsson1. 1) Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden; 2) Department of Plant Biology and Biotechnology, University of Copenhagen, Denmark.

The ascomycete Clonostachys rosea is an efficient antagonist against a range of fungal plant pathogens, presumably as a consequence from its mycoparasitic lifestyle. C. rosea is therefore used as a biological control agent against pathogens threatening agricultural crops. Still, very little is known about the mechanisms behind the mycoparasitism in C. rosea. By comparative genomics using C. rosea and publically available genome sequences from closely related species exhibiting different lifestyles we aim at exploring evolution of genes important for the transition into a mycoparasitic lifestyle. And by analyzing the transcriptome in interactions with different fungal plant pathogens we intend to get a deeper understanding on the gene expression of secreted enzymes during initial attack but also to study potential specialization towards specific fungal prey species. For these purposes we have sequenced the genome of C. rosea strain IK726. In addition we have performed an RNAseq study to investigate the gene expression during interactions with the two plant pathogens Botrytis cinerea and Fusarium graminearum. Our draft genome of C. rosea (strain IK726) reveals that the species has a larger genome than it’s closest sequenced relatives (58 Mb, which is 40 - 75 % larger than the 7 closest sequenced species). Preliminary comparative genome analyses indicate that the mycoparasitic function of C. rosea differs from earlier findings in mycoparasites of the closely related genus Trichoderma. For example, in C. rosea we find a significant expansion of ABC-transporter genes.
260. The mitochondrial genomes of *Fusarium circinatum*, *F. verticillioides* and *F. fujikuroi* are unexpectedly similar. G. Fourie¹, N.A. van der Merwe², B.D. Wingfield², B. Tuzynski³, M.J. Wingfield², E.T. Steenkamp¹. 1) Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; 2) Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; 3) University of Munster, Germany.

The *Gibberella fujikuroi* species complex consists of species that are of considerable agricultural, medical and veterinary importance. Nevertheless, many of the relationships among species in this complex remain largely unresolved, irrespective of the markers employed. In this study, we considered the feasibility of using mitochondrial genomes to resolve the higher level evolutionary relationships of the species in the complex. Because there is limited information available regarding the structure and evolution of mitochondrial genomes in the *G. fujikuroi* species complex, we fully characterized the mitochondrial genomes of the representative species; *Fusarium circinatum*, *F. verticillioides* and *F. fujikuroi*. Overall, the mitochondrial genomes of the three species displayed a high degree of synteny, with all the genes in identical order and orientation. This similarity also extended to the intergenic regions, as well as introns that share similar position within genes. The results show genome similarity beyond the expected characters common to the mitochondrial genomes of the Sordariomycetes. The intergenic regions and introns generally contributed significantly to the size differences and diversity observed among these genomes. Phylogenetic analysis of the concatenated protein-coding data set separated members of the *G. fujikuroi* complex from other *Fusarium* species. The individual mitochondrial gene trees did not always support the phylogeny of the concatenated data set and at least six distinct phylogenetic trees were recovered. This incongruence could arise from biased selection on some genes or recombination among mitochondrial genomes, potentially linked to a hybridization event. The results suggest that using individual genes for phylogenetic inference could mask the true relationships between species in this complex.

261. Comparative pathogenomics: next generation dissection of mechanisms of pathogenesis on plants. Donald M Gardiner¹, Jana Sperschneider², Paula Mooihiuizen³, Matthew Bellgard⁴, Kemal Kazan¹, Jen Taylor¹, John Manners¹. 1) Plant Industry, CSIRO, St Lucia, Queensland, Australia; 2) Plant Industry, CSIRO, Canberra, ACT, Australia; 3) Plant Industry, CSIRO, Perth, WA, Australia; 4) Centre for Comparative Genomics Murdoch University Perth, Western Australia, 6150.

Comparative analyses between fungal plant pathogens that share a common host have revealed important mechanisms of virulence in a number of different systems. We have an interest in understanding how *Fusarium* pathogens of wheat and barley cause head blight and crown rot diseases on these hosts. With modern genome sequencing technologies the power to undertake comparative analyses to assist in the understanding of key molecular mechanisms involved in pathogenesis has undoubtedly increased. We have recently sequenced an additional seven *Fusarium* isolates that are associated with wheat including isolates of *F. equiseti*, *F. acuminatum*, *F. culmorum* and *F. pseudograminearum* and are using these in comparative analyses. Sequence based homology searching between limited numbers of selected species have been particularly powerful in identifying signatures of horizontal transfer between phylogenetically diverse species which have been an important force in the evolution of virulence and examples of these that we have shown to be involved in virulence will be discussed. We are also developing methodologies to predict genes important in virulence that consider more remote homologies between species that may represent structural and/or functional conservation which also consider phylogenetic distributions and enrichment in species with particular lifestyles or shared plant hosts.

262. Characterisation of *stuA* homologue in *Fusarium culmorum*. Matias Pasquali¹, Francesca Spanu², Virgilio Balmas², Barbara Scherm², Kim Hammond Kosack³, Lucien Hoffmann³, Marco Beyer³, Quirico Migheli². 1) Environment and Agriobiotecnology Dept, CRP GABRIEL UPPMANN, Belvaux, Luxembourg; 2) Dipartimento di Agraria - Sezione di Patologia vegetale ed entomologia and Unità di ricerca Istituto Nazionale di Biotecnologia e Biosistemi, Università degli Studi di Sassari, Viale Italia 39, I-07100 Sassari, Italy; 3) Wheat Pathogenomics, Department of Plant Biology and Crop Sciences, Rothamsted Research, Harpenden, Herts AL5 2JQ, UK; 4) Centro interdisciplinare per lo sviluppo della ricerca biotecnologica e per lo studio della biodiversità della Sardegna e dell’area mediterranea, Università degli Studi di Sassari, Viale Italia 39, I-07100 Sassari, Italy.

*Fusarium culmorum* is one of the most harmful pathogens of durum wheat and the causal agent of foot and root rot (FRR) disease. *F. culmorum* produces different trichothecene mycotoxins that are involved in the pathogenic process. The role of the gene *FcStuA*, a *stuA* ortholog protein with an APSES domain sharing 98.5% homology to the FgStuA protein (FGSG10129), was determined by functional characterisation of deletion mutants originating from both strains showed common phenotypic characters including stunted vegetative growth and is a potential target for novel fungistatic / fungicidal molecules.

263. Comparative analysis of noncoding sequences in the Gibberella fujikuroi species complex. Christian Sieber¹, Ulrich Güldener², Martin Münsterkötter³, Karsten Suhré³. 1) Helmholtz-Zentrum München, Neuherberg, Bayern, Germany; 2) Research Center for Environmental Health, Institute of Bioinformatics and Systems Biology, 85764 Neuherberg, Germany; 3) Department of Physiology and Biophysics, Weill Cornell Medical College, Education City, Qatar.

Initially genome analysis methods focused on the coding part and its resulting proteins, therefore the role of noncoding DNA features was widely uninvestigated for a long time. Due to the luxuriance of new sequencing technologies a plurality of genome sequences are now available and many different repeat families could be identified already, which exhibit a diversity of functions such as mRNA stabilization or the control of translation (Khemici 2004, Espelii 2001). Interspersed repeats account for a considerable amount of noncoding sequence in fungal genomes. The proportion of repetitive elements and the compositions of repeat families differ from species to species whereas little is known about their origin and impact on the genomic functionality. The availability of new *Fusarium* genome sequences facilitate an extensive comparative approach across the *Gibberella fujikuroi* species complex. Besides known transposable elements and satellite repeats, a diversity of previously unknown interspersed repeat families are prominent in *F. fujikuroi* and closely related species. While some of them are distributed through the whole complex, others can exclusively be found in only one genome. Interestingly the predicted secondary structures of the elements exhibit a stable fold in terms of free energy and complementary base pairing. Moreover gene-chip and RNA-seq experiments reveal that some elements are part of the transcriptome and still seem to propagate further in the genome. Main questions are: What influence do interspersed repeats have on genome structure and the speciation process of fungi? How can repeats contribute to host-pathogen
interaction and specification?


*Fusarium circinatum* causes pitch canker, an important disease of *Pinus* species worldwide. Little is known of the genetic determinants of virulence in this pathogen although virulence-related genes have been identified in other *Fusarium* species. The purpose of this work was to assess the heritability of virulence in *F. circinatum* and to identify genomic regions associated with virulence. Virulence was altered through a series of sibling crosses pushing the populations towards high or low virulence. Crossing high and low virulence parents from the F1-High and F1-Low generations, respectively, generated progeny (HL-F1) with a nearly continuous distribution of virulence phenotypes. One hundred progeny were evaluated for polymorphic markers segregating for virulence by AFLP-PCR. Four markers were found to have a strong association with the long lesion length phenotypes. The AFLP-PCRs containing the markers were sequenced using Illumina HiSeq and the genomic regions associated with virulence were located on the *F. circinatum* genome. These regions are being studied to determine the changes resulting in loss of virulence.

265. Understanding the remodeling of the wheat grain genome expression during infection, a gate to get new insights on the molecular cross talk controlling the development of the interaction between the wheat and *Fusarium graminearum*. Chetouhi Cheng12, Bonhomme Ludovic12, Cambon Florence12, Lecomte philippe12, Biron G-David1, Langin Thierry12. 1) INRA, UMR 1095 GDEC, F-63093 Clermont-Ferrand cedex 2, France; 2) UBP, UMR 1095 GDEC, F-63100 Clermont-Ferrand, France; 3) CNRS-UBP 6023 LMGE, F-63171 Aubière, France.

Despite numerous progresses in understanding the molecular plant defences against pathogens, the molecular mechanisms used by a fungal pathogen to counter the plant defences and to optimize the host cell environment for fungal growth remain largely unknown. One of the main goals of our group is to better understand resistance and susceptibility mechanisms in wheat to *Fusarium graminearum*. This fungal pathogen represents the main causal agent of Fusarium head blight (FHB), an important worldwide disease in wheat reducing grain yield and quality. Contamination of grain by trichotheccene mycotoxins produced by *F. graminearum* during infection is the primary causes of reduced grain quality. Until now, very little is known on the molecular cross-talk between this fungus and its host during a compatible interaction. In this study, we characterize the impact of *F. graminearum* infection on the wheat cellular processes and identify wheat genes, so called susceptibility genes (S-gene), required for disease development. Until now, very little is known on the transcriptional changes induced by *F. graminearum* in a susceptible wheat cultivar. A whole genome expression analysis was performed on the French susceptible wheat cultivar Réctal challenged with a pathogenic and mycotoxigenic *F. graminearum* strain. Using a microarray analysis, we have identified 1,453 differentially expressed genes while proteome comparative analysis showed 80 differentially regulated proteins between healthy and Fusarium-damaged kernels at different development stages of the grain (flowering at 450 °Cd). These disease-associated genes and proteins belong to three main functional groups including (i) plant defense, (ii) primary, secondary and energy metabolism and (iii) regulation and signaling. These results demonstrate that *F. graminearum* infection strategy associates (i) suppression of plant defense, and (ii) subtle changes in nutrient availability related-processes. These preliminary results strongly suggest that *F. graminearum* manipulates the functioning of wheat kernel cells to optimize its nutrition, and therefore that the disease susceptibility of wheat relies on a parasite manipulation by this pathogenic fungi. This is the first exhaustive study of the molecular mechanisms associated with FHB development in a susceptible wheat cultivar.

266. Genetic and epigenetic changes in *Fusarium graminearum* following serial subculture. Rhaisa Crespo1, Helen Halen-Adams1. 1) Food Science and Technology, Univ of Nebraska-Lincoln, Lincoln, NE; 2) University of Puerto Rico, Mayaguez, Mayaguez, PR.

*Fusarium* isolates are notably unstable in culture and given to degradation unless certain precautions are taken. After only a few rounds of serial subculture, isolates can irreversibly lose the ability to form sporodochia, followed by conidia. The mechanism of these changes is unknown. To understand the nature of *Fusarium* morphological changes in culture, we began subjecting the sequenced strain of *F. graminearum* to serial subculture in July, 2011. Multiple lineages were begun from an initial soil stock, and each lineage is subcultured weekly, and a sample stored under glycerol at -80 C. For this study, we have performed shotgun pyrosequencing using 454 FLX-Plus on five of the lineages from one year after the study began; we have also sequenced one lineage from the beginning of the study (when all lineages should have been identical, and not significantly different from the published *F. graminearum* genome), and compared all to the published genome. Finally, we have used illumina for bisulfite pyrosequencing to obtain methylation profiles.

267. Evolutionary and functional analysis of mitosis-related kinase genes in *Fusarium graminearum*. Huiqian Liu1, Jiwen Ma1, Shijie Zhang1, Dawei Zheng1, Juanyu Zhang1, Chenfang Wang1, Jin-Rong Xu2,3. 1) College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, China; 2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, United States of America.

Eukaryotic cell cycle is a series of recurrences of a defined set of events; during which, nuclear DNA is replicated in the S phase and segregated into two daughter nuclei during mitosis. To date, many protein kinases important for the onset and progression through mitosis have been identified. Most of these mitosis-related kinases are thought to be conserved from yeast to humans. In the model fungi used for cell cycle studies, including Saccharomyces cerevisiae, Schizosaccharomyces pombe, and Aspergillus nidulans, the single copy cdc2 (CDC28) CDK gene is a key regulator of cell cycle essential for growth. However, the filamentous ascomycete Fusarium graminearum, the causal agent of wheat and barley head blight disease, has two putative Cdc2 orthologs. Either one of them is essential but deletion of both may be lethal. Whereas the cdc2 mutant has only minor defects in germination and growth, deletion of cdc2A had no obvious defects in growth but resulted in significant reductions in virulence. Ascosporogenesis but not perithecium formation or ascogenous hyphal growth was blocked in the cdc2A mutant. Mutants deleted of the single CDK kinase gene FgCk1 had similar phenotypes with the cdc2A mutant. Both cdc2A and cdc2B interacted with itself and each other, and with FgCk1. Therefore, cdc2A and cdc2B must have independent and overlapping functions in *F. graminearum*. It is likely that cell cycle regulation involves different cdc2 kinases and CDK activation mechanisms between vegetative and in plant growth. Infectious growth and ascosporogenesis may require only cdc2A, which is activated by FgCk1. In addition, we found that *F. graminearum* has two Aurora protein kinase genes that are orthologous to yeast Ipl1. The two F. graminearum Aurora kinases differ at the amino acid residue that is known to be related to different functions of Aurora A and Aurora B in humans, further indicating that yeast and *F. graminearum* differ in some key protein kinases involved mitosis. In addition, we systematically identified orthologs of other mitosis-related kinase genes in representative fungi. Although most of them are conserved across fungal tree of life, some mitosis-related kinase genes are lost or duplicated in certain lineages.

268. Functional analysis of A MADS-box transcription Mcm1 in *Fusarium graminearum*. Cui Yang1, Guotian Li2, Qian Zheng1, Meigang Liu1, Jin-Rong Xu1,2, Chen Fang Wang1,2. 1) NWAFU-PU Joint Research Center, College of Plant Protection, Northwest A&F University, Yangling, Shanxi, China; 2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, United States of America.
Fusarium head blight, an important disease of wheat and barley is primarily caused by *Fusarium graminearum* in many parts of the world. In this homothallic ascomycete, sexual reproduction and the mating type locus play a critical role in its infection cycle because ascospores are the primary inoculum. In this study we identified and characterized the FgMCM1 gene in *F. graminearum* that is orthologous to yeast MCM1 MADS-box transcription factor. Deletion of FgMCM1 resulted in the loss of perithecium production and pathogenicity. The FgMCM1 mutant rarely produced conidia with abnormal morphology and germination and was defective in response to various stresses. The FgMcm1-GFP fusion proteins localized to the nucleus and fully complemented the FgMcm1 mutant. Interestingly, approximately half of the sub-cultures of the FgMcm1 mutant often were significantly reduced in growth rate. These spontaneously occurred stunted subcultures had similar or more severe defects than the original FgMcm1 mutant in most of the phenotypes. In yeast two-hybrid assays, FgMcm1 interacted with Mat1-1-1, Fst12, and Tup1 but not with Mat1-2-1. The FgMcm1 mat1-1-1 double mutant was stable, suggesting that defects of the FgMcm1 mutant may be related to the interaction of FgMCM1 with the other MAT TF genes. The Fvmctn1 mutants of *F. verticillioides* had similar defects but were not unstable. To further understand the instability of the FgMcm1 mutant and slow growth subcultures, RNA samples isolated from the wild type, original FgMcm1 mutant, and a stunted subculture were sequenced. RNA-seq data and analyses will be presented. Overall, our data indicate that FgMcm1 may interact with MAT locus and other transcription factor genes to regulate cell identity and fungal development and pathogenesis in *F. graminearum*.

269. Genome sequencing of the *Fusarium graminearum* species complex in Korea. Hae Young Jeong1, Ulrich Guldener2, Hee-Kyoun Kim3, Seunghoon Lee4, Theresa Lee5, Sung-Hwan Yun1. 1) Systems & Synthetic Biology Research Center, KRIIBB, Daejeon 305-806, South Korea; 2) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München - German Research Center for Environmental Health, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany; 3) Dept Med Biotech, Soonchunhyang Univ, Asan, Chungnam 336-745, South Korea; 4) Microbial Safety Team, National Academy of Agricultural Science, RDA, Suwon 441-707, South Korea.

The *Fusarium graminearum* (Fg) species complex, the causal agent of Fusarium head blight of small grain cereals, comprises at least 15 lineages, or phylogenetically distinct species. Among these, lineages 6 (*F. asiaticum*) and 7 (*F. graminearum sensu stricto*) are major populations of the Fg complex recovered from rice and corn, respectively in Korea; lineages 3 (*F. boothii*) and 2 (*F. meridionale*) were also recovered from corn. The *F. asiaticum* population is clearly different from the *F. graminearum* population in terms of self-fertility, trichothecene production, and host preference. We have sequenced the genomes of 19 Fg complex isolates belonging to the 4 clades or species found in Korea using 454 pyrosequencing or Illumina HiSeq technologies. As a representative genome sequence for *F. asiaticum*, we reconstructed five linear replicons from the *F. asiaticum* SCK04 strain, which consist of four chromosomes, each corresponding to those of the previously sequenced *F. graminearum* strain PH-1 along with a separated small segment (451kb). By integrating genome models that included the results obtained by ab initio gene prediction tools which incorporated RNA-seq data we manually identified a set of 12,448 protein-coding genes in SCK04. Genome-wise comparison between SCK01 and PH-1 revealed a remarkable level of genomic synteny throughout the four chromosomes, but several rearrangements including inversions being located on chromosomes II and III in SCK04. Interestingly, the 451-kb fragment in SCK01 showed little sequence relatedness with the PH-1 genome. Similarly, we were able to assemble into 7 large contigs from the genome of a representative strain (GWS2-6-3) of *F. boothii*. Using these three representative genomes, we have intensively analyzed and compared the genomes of the Fg complex field isolates to provide insights into understanding of evolutionary relationship among the Fg complex in Korea.

270. Identification and functional analysis of virulence genes in different host-pathogenic forms of *Fusarium oxysporum*. P. van Dam, S.M. Schmidt, M. Rep. Molecular Phytopathology, University of Amsterdam - SfLS, Amsterdam, the Netherlands.

The species complex *Fusarium oxysporum* (Fo) represents one of the most abundant and widespread microbes of the soil microflora, including plant-pathogenic strains that, together, are able to infect a broad host range. In the tomato-pathogen *Fusarium oxysporum* f. sp. lycopersici (*Fo*), 11 small Secreted In Xylem (SIX) effector proteins were identified. These were later shown to be encoded on *Fo*’s mobile ‘pathogenicity-chromosome’ that can be transferred horizontally to non-pathogenic Fo strains, resulting in acquired pathogenicity. The goal of this project is to identify host-specific virulence genes in other *formae speciales* of Fo.

A representative set of isolates from different *formae speciales*, vegetative-compatibility groups and races will be selected for genome sequencing. The development of a comparative genomics bioinformatics pipeline, relying on sequence homology and specific patterns in the promoter regions (e.g. transposable elements) for identification of putative effector genes in newly sequenced *F. oxysporum* genomes takes a central position in our strategy. Using this approach, several putative effector genes were already identified in a strain of *F. oxysporum* melonis, which infests muskmelon.

In combination with functional analysis of candidate effector genes, we want to compare the sets of virulence genes of *formae speciales* that infect members of the Cucurbitaceae and Solanaceae families, such as cucumber, muskmelon, watermelon, tobacco, sweet potato and eggplant. Newly discovered virulence genes can be used as molecular markers for diagnostic purposes. Additionally, phylogenetic analysis of virulence genes across *formae speciales* will help to reconstruct the evolution of host-specific pathogenicity in Fo, and the dynamics of the mobile accessory genome.


A protocol for generating knock-outs of Verticillium albo-atrum, which is a destructive soilborne fungal pathogen that causes vascular wilt diseases, was successfully established. V. albo-atrum, along with V. Dahiae, is a significant source of crop plant disease, since between them they infect a broad spectrum of host species, from ornamental trees to major crops such as potato, tomato, cotton, tobacco and hop. The genome of V. albo-atrum has already been sequenced. Translation of genome sequence information into biological functions is therefore possible. One of the most powerful approaches for dissecting the gene function in phytopathogens is the study of the phenotypes of mutants in which a genomic locus has been altered by insertion (gene disruption) or replacement (gene replacement) with heterologous DNA. This is a high throughput reverse genetics approach, which greatly contributes to understanding the gene function of fungal pathogens. Our protocol for generating knock-outs of the fungal pathogen V. albo-atrum comprises two methods: the creation of knock-out plasmids by the USER Friendly cloning technique and transformation of the fungal pathogen by Agrobacterium tumefaciens-mediated transformation (ATMT). Knock-out strains of V. albo-atrum were made by site directed modifications of the pathogen genome by means of homologous recombination and achieved by introducing a DNA fragment containing two homologous recombination sequences flanking a selection marker. pRH-HU2 plasmid, containing a hygromycin resistance gene, was used for USER Friendly cloning of knock-out plasmids. Two PCR amplons, containing homologous recombination sequences flanking a deletion gene, were inserted into the vector, which was used to transform E. coli cells and isolated plasmids were electroporated into A. tumefaciens. V. albo-atrum knock-outs were generated by ATMT. Knock-out strains for two genes found to be highly expressed at the protein level in the xylem of infected hop plants, were generated. V. albo-atrum knock-out transformants were verified by PCR testing and Southern blot analysis, which confirmed that deletion of the target gene had been successful. This is the first report, to our knowledge, of the creation of V. albo-atrum gene knock-outs. It demonstrates that knock-out transformants of this fungal pathogen can be efficiently made.
272. Functional analysis of catalase-peroxidase encoding genes in the fungal wheat pathogen *Zymoseptoria tritici*. A. Mirzadi Gohari, R. Mehrabi, P. J.G.M. de Wit, G. H.J. Kema. 1) Wageningen University and Research Centre, Plant Research International, P.O. Box, 16, 6700 AA, Wageningen, The Netherlands; 2) Wageningen University, Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands; 3) Department of Plant Protection, College of Agriculture, University of Tehran, Plant Pathology Building, Karaj, Iran.

*Zymoseptoria tritici* is the new name of the foliar wheat pathogen *Mycosphaerella graminicola* (teleomorph)/*Septoria tritici* (anamorph) that causes septoria tritici blotch particularly in regions with high rainfall and moderate temperatures during the production season. Similar to many plant pathogens, *Z. tritici* possesses three catalase-peroxidase genes that are known to detoxify H2O2 accumulated in the foliage during colonization. In the current study, we functionally analysed these three catalase-peroxidase genes and found that *MgCatD-1*, encoding a secreted catalase-peroxidase, plays an important role in the pathogenicity of *Z. tritici*. *MgCatD-2* mutants hardly induced any disease symptoms and expression analysis of *MgCatD-1* in planta revealed that it is up-regulated during pathogenesis, particularly at 8 dpi (days post inoculation). This coincides with an important switch from a biotrophic to a necrotrophic lifestyle during pathogenesis suggesting that this gene is likely required to overcome H2O2-dependant defence responses during colonization. Furthermore, the *MgCatD-1* strain is hypersensitive to H2O2 as the spore germination dropped to 50% at 4mM H2O2 and to complete inhibition at 6 mM H2O2 compared to the WT IPO323 strain. These results show that secreted catalase-peroxidase is an important pathogenicity factor for successful pathogenesis of *Z. tritici*.

273. Efficient recycling of selective marker genes with the Cre-loxP recombination system via anamastosis in *Cryphonectria parasitica*. Dong-Xiu Zhang, Donald Nuss. Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD.

Reverse genetic analysis has played a significant role in advancing fungal biology, but is also limited by the number of available selectable marker genes (SMGs). The Cre-loxP recombination system has been adapted for use in filamentous fungi to overcome this limitation. Expression of the Cre recombinase results in excision of an integrated SMG that is flanked by loxP sites allowing subsequent rounds of transformation with the same SMG. However, current protocols for regulated expression or presentation of Cre require multiple time consuming steps. During efforts to disrupt four independent RNA-dependent RNA polymerase genes in a single strain of the chestnut blight fungus *Cryphonectria parasitica*, we tested whether Cre could successfully excise loxP-flanked SMGs when provided in trans via anamastosis. Stable Cre-producing donor strains were constructed by transformation of wild-type *C. parasitica* strain EP155 with the Cre coding domain under the control of a constitutive promoter. Excision of multiple loxP-flanked SMGs was efficiently achieved by simply pairing the Cre donor strain and the loxP-flanked SMGs transformed recipient strain and recovering mycelia from the recipient colony just above the anamastosis zone. This method was shown to be as efficient as and much less time consuming than excision by transformation-mediated expression of Cre and should be applicable for optimizing reverse genetics analysis in a broad range of filamentous fungi.

274. RNA-seq reveals the pleiotropic regulating functions of the transcription factor XYR1 in *Trichoderma reesei*. Liang Ma, Lei Zhang, Ling Chen, Gen Zou, Chengshu Wang, Zhizhua Zhou. 1) Key Laboratory of Synthetic Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China; 2) Key Laboratory of Insect Developmental and Evolutionary Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China.

*Trichoderma reesei* is a well-known cellulase producer and widely applied in enzyme industry. XYR1 has been demonstrated to be the main transcription activator of cellulase and hemicellulase gene expression in *T. reesei*. To comprehensively investigate the genes regulated by XYR1, RNA-seq was used to determine the transcriptional profile of *T. reesei* Rut-C30 and the *Dxyr1* strain cultured on either cellulose or glucose. A total of 467 distinct genes were classified as differentially expressed comparing parent strain with the *xyr1*-deleted strain when induced by cellulose, among which were mainly those involved in carbohydrate metabolism, lipid metabolism, protein fate and solute transport. Almost all the functional cellulase genes were found to be downregulated in the *Dxyr1* strain while all the differentially expressed protease genes were upregulated. Furthermore, the expression of 84 genes identified as cellulose-induced were significantly impaired when *xyr1* was deleted. In contrast, 281 genes showed expression difference in the *Dxyr1* strain when cultured on glucose as carbon source, and most of these were involved in solute transport, lipid metabolism, secondary metabolism and amino acid metabolism. In addition, we also found that the functional restoration of HYR1 in the *Dxyr1* strain required the homologous insertion of *xyr1* at its original locus. Our study provides the global insight into the genes regulated by XYR1 beyond the hydrolase-encoding genes in *T. reesei* and might aid future studies to improve its cellulase production.

275. Tyrosinase an important enzyme for melanin production in the oomycete *Saprolegnia parasitica*. Maria Saraiva, Irene de Bruijn, Laura Grenville-Briggs, Debra McLagan, Vincent Bulone, Pieter van West. 1) School of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom; 2) Laboratory of Phytopathology, Wageningen University, 6709 PD Wageningen, Netherlands; 3) KTH - Royal Institute of Technology, School of Biotechnology AlbaNova University Center SE-106 91 Stockholm Sweden.

Filamentous fungi possess a pigment that often is considered an indirect virulence factor giving the fungi leverage. The pigment, melanin, acts as a protective agent against several threats and hazards that these organisms may encounter such as oxidants, killing by macrophages, UV and also antimicrobial compounds. In the biosynthetic pathway of melanin Tyrosinase is the first enzyme involved. Tyrosinases are widely distributed in nature, where they have been found in prokaryotes, eukaryotic microorganisms, invertebrates, plants and mammals. Here we describe the first functional characterisation of a tyrosinase (SpTyr) from the fish pathogenic oomycete *Saprolegnia parasitica*. This aquatic water mould infect a wide range of fish, amphibians and crustaceans that are relevant to the aquaculture industry and aquatic ecosystems, causing a disease called *Saprolegniosis*. This disease is responsible for millions of losses in aquaculture. Previously we found that SpTyr is highly expressed in sporulating mycelia of *S. parasitica* and can provide a high-throughput tool for *S. parasitica* functional genomics.

276. Comparative transcriptomics of *Cordyceps bassiana* to understand expression levels of its NRPS related genes. B. Shrestha, J. Oh, M.-W. Hyun, J.-G. Han, H.-W. Kwon, S.-H. Hyun, S. H. Kim, H.-K. Choi, G.-H. Sung. 1) Mushroom Research Division, Rural Development Administration, Suwon 441-707, Republic of Korea; 2) College of Pharmacy, Chung-Ang University, Seoul 156-756, Republic of Korea; 3) Department of Microbiology and Institute of Basic
277. Global analysis of the Colletotrichum gloeosporioides genome and transcriptome reveals a conserved role for pacC pH regulation in fungi. Noam Alkan1, Xiangchun Meng1, Eli Reuveni2, Gilgi Friedlander3, Serenella Sukno1, Michael Thon3, Robert Flühr2, Dov Prusky. 1) Plant Sciences, Weizmann Institute of Science, Rehovot, Israel; 2) Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, the Volcani Center, Bet Dagan, Israel; 3) Fruit Tree Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, China; 4) Department of Microbiology and Genetics, University of Salamanca, Villamayor, Spain.

Colletotrichum gloeosporioides, a widely distributed economically important agent in postharvest fruit disease, thrives by massive secretion of ammonia. The alkalized environment hijacks host response and activates fungal pathogenicity genes. In contrast, other types of fungi acidify the environment for their optimal growth. In both cases the pacC transcription factor, with homologs in yeasts and fungi, exerts critical pH control of gene expression. To explore global aspects of pH-dependent gene expression, we sequenced the C. gloeosporioides genome and compared transcriptomes of WT and a ΔpacC mutant to examine pacC regulation. The 54 Mb C. gloeosporioides genome comprises 16,603 transcripts. Transcriptome analysis of the mutant showed that DpacC regulates more than 5% of the fungal genome including; transporters to maintain cellular homeostasis, cell wall degrading enzymes to optimize pathogenicity and GATA-like transcription factors. The predictions were verified by monitoring gene expression in different media and during fruit infection as well as by pathogenicity assessment of selected deletion mutants. Analyses showed over-representation of pacC binding sites in the promoters of the pacC up-regulated genes. However, in the promoters of the down-regulated genes GATA-like binding sites were dominant. The results suggest duality in global pacC control; direct regulation of alkaline-induced transcripts but indirect regulation, by activation of other transcription factors to down-regulate acid expressed transcripts. To establish the generality of this scenario, conservation of pacC distribution was examined in genes selected by homology from different fungi that have contrasting alkaline or acidifying pathogenicity strategies. The results showed that irrespective of pathogen colonization strategy, the homologs of up-regulated genes had over-representation of pacC binding sites. Significantly, the homologs of down regulated genes revealed cross-genome over-representation of GATA transcription factor binding sites. Thus, regulation by pacC is a phylogenetically conserved fungal mechanism exerting dual pH control for maintaining homeostasis and pathogenicity in changing environments.

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279. Anisogramma anomala: a unique fungus with a huge genome. Guohong Cai, Thomas Molnar, Bradley Hillman. Plant Biology and Pathology, Rutgers The State University of New Jersey, New Brunswick, NJ. Anisogramma anomala (Ascomycota: Diaporthales) is the causal agent of hazelnut eastern filbert blight (EFB). This obligate and biotrophic fungus only reproduces sexually through ascospore. EFB is the most important disease of commercially grown hazelnut (European hazelnut), Corylus avellana, in the United States. It only causes minor disease on its natural host, the American hazel C. americana, which is an understory shrub commonly found in deciduous forests in Northeast region of North America. Despite quarantine efforts, EFB was found in Washington in the late 1960s and reached Oregon’s Willamette Valley, the main hazelnut production area, in 1986. Commercial cultivars carrying a single locus from cultivar Gasaway confer complete resistance to EFB in the west coast. However, certain strains from the east can break the resistance. We sequenced an Oregon strain using Illumina GA IX platform. Approximately 26M 146 bp paired-end reads were used to produce a draft assembly with contig N50 of 10,384 bp and scaffold N50 of 32,987 bp. Excluding gaps, total assembly was 333.6 Mb. This is huge for a fungus with single nucleus. The genome size produced by draft assembly was corroborated by flowcytometry, which measured the genome size at approximately 370 Mb. The genome has low GC ratio of 32%. Approximately 85% of assembled genome is repetitive, and AT-rich sequences are enriched in the repetitive regions. We are exploring the genome sequences looking for answers to the uniqueness of this fungus.

280. Leptosphaeria maculans ‘brassicaceae’: “Transposable Elements changed my life, I feel different now”. Jonathan Grandaubert1, Conrad Schoch2, Hossein Borhan3, Barbara Howlett1, Thierry Rouxel1. 1) INRA-BIOGER, Thiverval-Grignon, France; 2) NCBI, National Institutes of Health, Bethesda, MD, USA; 3) AAF Saskatoon, Canada; 4) School of Botany, University of Melbourne, Australia.

The Dothideomycetes phytopathogens Leptosphaeria maculans and Leptosphaeria biglobosa form a complex of 8 species and putative subspecies suggested to have diverged “recently”. In 2007, the sequencing of an isolate of Leptosphaeria maculans ‘brassicaceae’ (Lmb) provided the first reference genome for this fungus. The 45-Mb genome has an unusual bipartite structure, alternating large GC-equilibrated and AT-rich regions. These AT-rich regions comprise one third of the genome and are mainly composed of mosaics of truncated Transposable Elements (TEs) postulated to have “invasion” the genome 5-10 MYA; they also comprise 5% of the predicted genes of which 20% encode putative effectors. In these regions, both genes and TEs are affected by Repeat Induced Point mutation (RIP). To investigate when and how genome expansion took place in the evolutionary series, and the consequences it had on fungal adaptability and pathogenicity, the genomes of five members of the species complex showing contrasted host range and infection abilities were sequenced. In silico comparison of the reference genome with those of 30-32-Mb genome of L. maculans ‘lepidii’ (Lm), L. biglobosa ‘brassicaceae’, L. biglobosa ‘thlaspii’ and L. biglobosa ‘canadensis’, showed these species have a much more compact genome with a very low amount of TEs.
Genomics of fungal interactions for bioenergy crops. Igor Grigoriev. Fungal Genomics Program, US DOE Joint Genome Institute, Walnut Creek, CA.

Bioenergy crops depend on plant interactions with fungi including pathogens and symbionts. Saprobic fungi determine efficiency of plant material bioconversion. Genomics offers keys to better understand molecular mechanisms of these interactions. The US Department of Energy Joint Genome Institute in collaboration with its user community leads massive genome sequencing and exploration of diverse fungi. Genome and transcriptome data produced for over 200 fungal species along with comparative genomics tools enable us to compare attributes of different lifestyle on genomic level. Comparison of large and diverse groups of fungi with similar lifestyle lead to genomic characteristics of their common traits. Interestingly, plant parasites, symbionts and saprobes reveal similar features encoded in their genomes, variations of which may correlate with their lifestyle. At the same time, expression, regulation, and evolution of these features ultimately determine the lifestyle of a fungus and require functional genomics efforts of the scale similar to that of modern genome sequencing.

Genomic analysis of oleaginous fungi. H. Koike\(^1\), I. Takeda\(^1\), T. Ishii\(^2\), M. Umemura\(^2\), K. Tamano\(^2\), S. E. Baker\(^3\), M. Machida\(^3\). 1) Bioprocess Research Institute, AIST, Tsukuba, Japan; 2) Bioprocess Research Institute, AIST, Sapporo, Japan; 3) Pacific Northwest National Laboratory, WA.

Microbial production of fats and oils is being developed as a means of converting biomass to biofuels. Some fungal species such as Mortierella and Aspergillus species are known to be oleaginous, accumulating fats and related compounds to more than 20% of their biomass. We have performed genome analyses of oleaginous fungal species, intending future genetic engineering of Aspergillus oryzae species as a host for the overproduction of fats and related compounds. We have sequenced and assembled draft genomes of three species (four strains) of lipid overproducing Mucoromycota fungi using SOLID 5500xl sequencer. The gene expression profiles under the lipid producing conditions have been analyzed by RNA-seq. We generated de novo assembly of the Mucoromycota genomes using 50 bp reads generated from mate-paired libraries with 2.5 - 3.0-kb inserts. The assembled scaffolds of 4 species showed N50 values of 58 -477 kb and the maximum scaffold lengths of 247kb - 1.1 Mb. We have identified approximately 10,000 genes in the genome using ALN and GlimmerHMM. Comparative genomic analysis showed their phylogenetic relationships between each other and with other Mucoromycota species whose genomes have been sequenced. The gene expression profiles of Mucoromycota fungi were compared with each other and with those of Aspergillus oryzae to deduce induced and repressed metabolic pathways in terms of fats biosynthesis. We are intending to establish effective methods to over-produce fatty acid, secondary metabolites, and other related compounds useful for biofuel and/or chemical industry using Aspergillus oryzae.

Genome sequencing, assembly and annotation of a marine fungal isolate of Scopulariopsis brevicaulis using three different next generation sequencing technologies. Abhishek Kumar, Frank Kempen. Dept. of Genetics & Mol. Bio, Institute of Botany, CAU Kiel, KIEL, SH, Germany.

Several hundreds of marine fungal species are known which possess unique features, as they have to adopt to the marine environment. The enormous biodiversity of marine fungi is mirrored by the molecular diversity of their secondary metabolites. However, very little is known about marine fungal genetic resources, as they are ignored because they constitute very small fraction of over million of fungal species. Here, we report the first genomic sequence of a marine isolate of Scopulariopsis brevicaulis. S. brevicaulis is known to produce the cyclic peptides Scopularide A and B. This species is common soil saprophyte and has been isolated from a wide variety of substrates. Some species of this genus are reported to cause human diseases. We have established the genomic sequence of a marine isolate of S. brevicaulis using three different next-generation sequencing methods namely, Roche 454, Illumina and Ion-torrent. The assembled genome of S. brevicaulis is ~32 Mb in size with N50 equals to 88 kb and 935 contigs containing 16298 genes with average intron length equals to 129.4. During annotation process, we were able to annotate 9340 genes (57.31 %) while 6958 genes (43.69 %) remained non-annotated in S. brevicaulis genome. This genome has 17 genes encoding for non-ribosomal peptide synthetases (NRPSs), 18 polyketide synthases (PKSs) and one gene is hybrid NRPS-PKS.


Next-generation sequencing (NGS) has rapidly provided large wealth of applications of genomics to biology and medicine. We used NGS to sequence genome of Pestalotiopsis, a marine fungal isolate, known to produce wide variety of secondary metabolites with potentials in cancer treatment. We have established the genomic sequence of this marine isolate using two different next-generation sequencing methods namely, Roche 454 and Illumina HiSeq 2000. The assembled genome size of Pestalotiopsis is ~46 Mb with N50 equals to 71.9 kb and 4186 contigs containing 23492 genes, which is surprisingly very high for a fungus. The average intron length and the average intron per gene are 126.8 and 2.2, respectively. During annotation process, we annotated 72% genes, while 28% genes remained non-annotated. The US Department of Energy funded marine fungi project, these secondary metabolites are further characterized for their roles in cancer treatment.

Genome sequencing, assembly and annotation of a marine fungal isolate of Calcosporium using different next generation sequencing technologies. Abhishek Kumar, Frank Kempen. Dept. of Genetics & Mol. Bio, Institute of Botany, CAU Kiel, KIEL, SH, Germany.

Biology has more sharpen weapon in terms of application of genomics due to rapidly evolving next-generation sequencing (NGS), which made large-scale sequencing affordable to regular laboratory practice. We used NGS to sequence genome of Calcosporium, a marine fungal isolate, known to produce wide variety of secondary metabolites with potentials in cancer treatment. We have established the genomic sequence of this marine isolate using two different next-generation sequencing methods namely, Roche 454 and Illumina HiSeq 2000. The assembled genome size of Calcosporium sp. is about 35 Mb genome with N50 equals to 91.9 kb and 2464 contigs containing 15459 genes. The percentage GC% for this genome is 50.7%. The average intron length and the average intron per gene are 121 and 2.1, respectively. During annotation process, we annotated 72% genes, while 28% genes remained non-annotated for Calcosporium genome with 52 NRPSs, 66 PKSs and 7 hybrid NRPS-PKS genes. Under EU-funded marine fungi project, these secondary metabolites are further characterized for their roles in cancer treatment.

Next-generation sequencing (NGS) techniques have changed the facets of genomics and its application. Until now marine fungal isolated are neglected in fungal genetics, largely due to fact that fungi have enormous diversity on land itself. We aimed to explore marine fungal isolates and their encoding natural products as drugs against cancer under the EU-funded project marine fungi (www.marinefungi.eu). We have taken three marine isolates namely, Scopulariopsis brevicaulis, Pestalotiopsis sp. and Calcarisporium sp. We have established the genomic sequences from these marine isolates of using three different next-generation sequencing methods (Roche 454, Illumina and ion-torrent) and predicted genes are presently in process of validation using illumina based RNA-seq. We are also comparing wild type phenotypes with higher -yielding mutants of these fungi with special interest on specific natural compound. The assembled genome of Scopulariopsis brevicaulis is ≈32 Mb in size with N50 equals to 88 kb and 935 contigs containing 16298 genes with average intron length equals to 129.4. During annotation process, we were able to annotate 9340 genes (57.31 %) while 6958 genes (42.69 %) remained non-annotated in Scopulariopsis brevicaulis genome. This genome has 17 genes encoding for non-ribosomal peptide synthetases (NRPSs), 18 polyketide synthases (PKSs) and one gene is hybrid NRPS-PKS. Similarly, the genome size for Pestalotiopsis sp. is≈46 Mb with N50 equals to 71.9 kb and 1486 contigs containing 23492 genes, which is surprisingly very high for a fungus. The average intron length and the average intron per gene are 126.8 and 2.2, respectively. During annotation process, we annotated 60% genes of Pestalotiopsis genome with 44 NRPSs, 62 PKSs and 7 hybrid NRPS-PKS genes. The assembled genome size of as Calcarisporium sp. is about 35 Mb genome with N50 equals to 91.9 kb and 2464 contigs containing 15459 genes. The percentage GC% for this genome is 50.7%. The average intron length and the average intron per gene are 121 and 2.1, respectively. During annotation process, we annotated 72% genes, while 28% genes remained non-annotated for Calcarisporium genome with 52 NRPSs, 66 PKSs and 7 hybrid NRPS-PKS genes.

287. From Shiitake genomics to comparative mushroom genomics: Mushroomomics. Hoi Shan Kwan, Chang Han Au, Man Chun Wong, Jing Qin, Yung Lee, Kin Sing Wong, Lei Li, Qianli Huang, Wen Yan Nong, Man Kit Cheung, Jinhui Chang, Xuanjin Cheng. School of Life Sciences, The Chinese University of Hong Kong, Hong Kong, China.

The era of mushroom "omics" has come amongst the mushrooming of "omics" in all life science fields. I propose to name "omics" of mushrooms "Mushroomomics". My laboratory has been working on the genomics and transcriptomics of the wood-degrading fungus Lentinula edodes, Shiitake mushroom, one of the most important cultivated mushrooms. We sequenced the genome of the monokaryon LS4A using Roche 454 and ABI SOLID sequencing platforms. Over 13,000 protein-coding genes were predicted. We constructed a high-density genetic linkage map that was useful to link scaffolds into super-scaffolds. The L. edodes genome assembly revealed a genome size of about 40 Mb. We performed RNA-Seq of multiple stages of L. edodes. For comparison, we also analyzed the transcription profiles of different stages of the model mushroom Coprinopsis cinerea using NimbleGen microarrays. Genes differentially expressed during fruiting body initiation and development in these mushrooms were identified. We conducted comparative analyses on publicly available genome sequences of basidiomycetes and ascomycetes, and revealed genes expanded in genomes of mushroom-forming fungi. The expanded genes included specific types of regulators, ubiquitin ligases, protein-binding proteins, protein kinases, and transcription factors. In particular, F-box and paracaspase domain proteins were significantly expanded. The cataloging of the unique composition of plant biomass-degrading enzymes in L. edodes genome revealed lignin-degrading laccases and manganese peroxidases, and multiple polysaccharide-degrading enzyme families, such as glycoside hydrolase families that target beta-glucans and pectin. We compiled the genome sequences of L. edodes and other fungi into an Ensembl-based platform, equipped with a battery of genomic analysis tools, for comparative mushroom genomic analysis. Our works have generated rich resources for the analysis of genomics and transcriptomics of mushroom-forming fungi. Our analysis also provided insights into the molecular mechanisms of fruiting body development in fungi and the evolution of fungal complex multicellularity. Indeed, our works showed that the era of "Mushroomomics" has arrived.

288. Comparative genomics of Ceratocystis polonica and Ophiostoma bicalor, two bark beetle-associated pathogenic fungi. 1) Jieka Lah1, Tom Hsiang2, Colette Breuil3, Joerg Bohlmann3, Radovan Komet2. 1) Lab. for Mol. Biol, National Inst. of Chemistry, Ljubljana, Slovenia; 2) School of Environmental Sciences, University of Guelph, Guelph, Canada; 3) Dept. of Wood Science, Faculty of Forestry, University of British Columbia, Vancouver, Canada; 4) Michael Smith Laboratories, University of British Columbia, Vancouver, Canada; 5) Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Slovenia.

Outbreaks of bark beetles and their pathogenic fungal associates are responsible for killing conifer trees and destroying forests worldwide. For example, in British Columbia, the mountain pine beetle and its major associated fungal pathogen Grosmannia clavigera have destroyed over 17 million hectares of pine forests. The sequenced genome of G. clavigera has provided insight into fungal mechanisms involved in resistance to conifer defense compounds, and into population genomics of this species. In Europe, where outbreaks of the European spruce bark beetle Ips typographus threaten Norway spruce (Picea abies), genomic resources are lacking for pathogenic fungal associates, Ceratocystis polonica and Ophiostoma bicalor. The aim of our work is to create these resources and to compare the genomes of these species with the annotated G. clavigera genome. The sizes of C. polonica and O. bicalor genomes, sequenced using illumina and assembled using Draftus ver. 1.3.4, are 34.7 and 27 Mb, respectively. We identified (with Augustus ver. 2.6.1) and functionally annotated 6405 genes in C. polonica and 7746 in O. bicalor. We report results from preliminary analyses on gene family evolution, and on genes that code for enzymes important in eliminating and modifying conifer defense compounds, and those involved in specialized metabolism. The number of genes coding for ABC-transporters is approximately the same in all three species, while G. clavigera has more cytochrome P450 genes, polyketide synthases and ribosomal peptide synthases than either European bark beetle associate. Our results lead us to hypothesize that despite the fact that these species occupy apparently similar ecological niches, there are differences in their metabolism of host defense compounds and specialized metabolism.

289. The largest fungal mitochondrial genome of a basidiomycete contains signs of genetic flexibility and recombination events. Taina K. Lundell1, Heikki Salavirta1, Ilona Oksanen1, Jaana Kuuskeri2, Miia Mäkelä1, Pia Laine1, Lars Paulin1. 1) Microbiology and Biotechnology, Department of Food and Environmental Sciences, Viikki Campus, University of Helsinki, FI-00014 Helsinki, Finland; 2) Institute of Biotechnology, DNA Sequencing and Genomics Laboratory, Viikki Campus, University of Helsinki, FI-00014 Helsinki, Finland.

Background and results. As the first part of de novo genome sequencing of the biotechnologically important, wood-degrading enzyme producing white-rot Basidiomycota species Phlebia radiata, we first assembled and gene annotated its mitochondrial genome (mtDNA) using 454 sequencing. The P. radiata mtDNA of 157 kb is in the largest mitochondrial genome among fungi sequenced and characterized so far. The genome assembled as a single circular dsDNA molecule containing over 100 open reading frames. However, almost 80% of the mt genome is comprised of non-coding, intergenic and intron sequence regions. Genes for mt SSU and LSU rRNA, 28 tRNAs, and fifteen genes encoding the conserved protein subunits in the mitochondrial
inner membrane complexes I, III, IV and V are identified. Additional protein-coding ORFs (sum 39) are predicted including a gene for ribosomal protein (rps3), a viral RNA-directed DNA polymerase (reverse transcriptase), and a gene for bacterial-originated DNA-directed DNA polymerase II of family B (dpob). Total of 57 intron-homing endonucleases with core LAGLIDADGD and GYI-YIG domains were recognized in over 30 group I and II type introns, up to 3.4 kb in length, in ten of the fifteen conserved genes (cox1,2,3; cob; nad1,2,4,4L,5; rnl). Multigene phylogeny of the conserved proteins confirms current fungal taxonomy and a common, single origin of the mtDNA within Basidiomycota. Conclusions. The exceptionally large mt genome is explained by long intergenic stretches of DNA carrying repetitive and partially overlapping sequence elements, presence of additional open reading frames with unknown function, existence of the 1.6 kb duplication-inversion, and due to frequent intron splicing of the coding sequences. A few of the qualities indicate plasmid or viral origin, such as the dpob, and the cob gene-interrupting long group II intron with reverse-transcriptase ORF. These features together with the duplicated inversion and dense repeat stretches, and the long introns with intron-associated homing endonucleases are indications of genetic flexibility, not previously recognized to such extent in fungal mitochondrial genomes. Thus, it may be concluded that DNA recombination as well as regulation of gene transcription are allowed and on-going events in the P. radiata mt genome.

290. De Novo Assembly of Fungal Genomes and Detection of Structural Variation using Extremely Long Single-Molecule Imaging, Nicholas R. Rhind1, Alex Hastie2, Ernest Lam3, Andy Nguyen1, Han Cao1. 1) Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester MA; 2) BioNano Genomics, San Diego CA.
De novo genome assemblies using only short read data are generally incomplete and highly fragmented due to the intractable complexity found in most genomes. This complexity, consisting mainly of large duplications and repetitive regions, hinders sequence assembly and subsequent comparative analyses. We have overcome these problems in four fungal genomes by assembling whole genome shotgun (WGS) contigs with a high resolution long range physical-mapping strategy. We used a single molecule genome analysis system (Irys) based on NanoChannel Array technology that linearizes extremely long DNA molecules for observation. This high-throughput platform automates the imaging of single molecules of genomic DNA hundreds of kilobases in size to measure sufficient sequence uniqueness for unambiguous assembly of complex genomes. High-resolution genome maps assembled de novo from the extremely long single molecules retain the original context and architecture of the genome, making them extremely useful for structural variation and assembly applications.
We have built full genome assemblies of the four fission yeast genomes: Schizosaccharomyces pombe, S. janicus, S. octosporus and S. cryophilus. The S. pombe assembly is similar to the published finished S. pombe genome, but reveals several complicated genomic features that were mis-assembled by a clone-based approached. In addition, we have resolved centromeric and telomeric repeats that had not been assembled by traditional approaches. The S. janicus and S. octosporus assemblies identify a number of mis-assemblies in the published WGS genomes. The S. cryophilus genome is the first genome-scale assembly of what was a draft assembly of WGS contigs.
Our strategy combining genome map-based scaffolding with deep sequencing offers an integrated pipeline for whole genome de novo assembly solving many of the ambiguities inherent when using sequencing alone. Additionally, genome maps serve as a much-needed orthogonal validation method to WGS assemblies. As a result, genome maps improve contiguity and accuracy of whole genome assemblies, permitting a more comprehensive analysis of functional genome biology and structural variation.

291. Discovering host specificity candidate genes of Sporisorium reilianum by genotyping mixed-variety offspring, T. Wollenberg1,2, J. Donner1, J. Schirawski1,2. 1) Microbial Genetics, RWTH Aachen University, Aachen, Germany; 2) Molecular Biology of Plant-Microbe Interaction, Georg-August-University Göttingen, Göttingen, Germany.
The biotrophic plant pathogenic basidiomycete Sporisorium reilianum exists in two host-specific varieties, S. reilianum f. sp. zeae (SRZ) and S. reilianum f. sp. reilianum (SRS). SRS causes head smut of sorghum and leads to weak symptoms on maize, whereas SRZ causes severe plant damage. The biotypes of SRS and SRZ are the only dimorphic fungal pathogens that form spherules in the host. Furthermore, SRZ exists in two host varieties that are mating competent and are able to infect both maize and sorghum with a very low infection rate. To identify the genetic basis for the difference in host specificity, we analyzed virulent and non-virulent segregants of a mixed-variety (SRZ x SRS) infection both phenotypically and genotypically by a PCR-based approach. We identified twelve chromosomal regions that were associated to the phenotypic behavior of the strains. This shows that host adaptation is a multi-genic trait. To identify associated genes we genotypically analyze a larger set of strains by genome-wide SNP comparison after Illumina re-sequencing.

The mammalian pathogens Coccidioides immitis and C. posadasi are the only dimorphic fungal pathogens that form spherules in the host. Furthermore, all of Coccidioides’ closest known relatives are non-pathogenic. In this project, we are interested in genome changes between the Coccidioides lineage and its relatives, and how these changes compare to recently published comparative and population genomics, and transcriptomics studies in Coccidioides. Coccidioides and its closest sequenced relative, Uncinocarpus reesii, are estimated to have diverged 75-80 million years ago. Here, we have sequenced the genomes of four species more closely related to Coccidioides than U. reesii: Byssynynega catenaphila, Chrysosporium queenslandicum, Amauroascus niger and A. mutatus. For each of these four species, we prepared genomic DNA Illumina sequencing libraries; the resulting genome assemblies ranged from 23-34Mb, with N50 of 90kb-205kb. Predicted genes were confirmed by RNAseq; the total number of genes ranged from 8,179-9,184. We assessed individual gene gain/loss, and family expansion/contraction in Coccidioides using these new genomes and other recently published genomes from the Onygenales order, including the yeast-forming dimorphic pathogens Histoplasma and Paracoccidioides, and the dermatophytes Microsporum and Trichophyton. We have compared these results to genes identified in recently published Coccidioides “omics” studies that show evidence of positive selection, introgression and/or differential expression.

293. The transcriptional response during cell-fusion incompatibility in Podospora anserina. Frédérique Bidart, Sven J. Sauer, Corinne Clavé. IBGC, CNRS, Bordeaux, France.
Heterokaryon incompatibility is a form of non-self recognition common in filamentous fungi that occurs when filaments of different isolates of the same species fuse. Compatibility is controlled by so-called het loci and fusion of strains of unlike het genotype triggers a complex incompatibility reaction that leads to the death of the fusion cell. Herein, we analyze the transcriptional changes during the incompatibility reaction in Podospora anserina. The incompatibility response was found to be associated with a massive transcriptional re-programming: 2248 genes were up-regulated by a factor 2 of more during incompatibility. In turn, 2463 genes were down-regulated. HET and NACHT domains previously found to be involved in the control of heterokaryon
incompatibility were highly enriched in the up-regulated gene set. In addition, incompatibility was characterized by an up-regulation of secondary metabolism clusters, toxins and effector-like proteins, proteolytic and other hydrolytic activities. Genes for ribosome synthesis and energy production were in contrast down regulated. There was a significant overlap between regulated genes during incompatibility in P. anserina and N. crassa indicating communality in the incompatibility responses in these two species. In P. anserina, the up regulated set was found to be enriched for proteins lacking orthologs in other species and chromosomal distribution of the up-regulated genes was uneven with up regulated genes enriched in genomic islands and certain chromosomes. Globally, this study shows that transcriptome changes occurring during cell fusion incompatibility in P. anserina are massive and pleiotropic and in several aspects related to host-pathogen interactions described in other fungal species.

294. Comparative Analysis of Putative Rhodopsins in Early Diverging Fungal Lineages. Steven Ahrends1, Edgar Medina2,3, Jason Stajich1. 1) Plant Pathology & Microbiology, University of California, Riverside, Riverside, CA; 2) Departamento de Ciencias Biológicas, Universidad de Los Andes, Bogotá, Colombia; 3) University Program in Genetics & Genomics and Department of Biology, Duke University, Durham, NC.

Species belonging to the early diverging zoosporic fungal lineages (Blastocladiomycota, Chytridiomycota, Cryptomycota, and Neocallimastigomycota) reproduce via motile unflagellated spores. Previous work has shown that some of these zoosporic fungi are phototactic [1]; however, light sensing in zoosporic fungi has not been fully explored. The opsins are a broad class of photosensitive, seven-transmembrane G-protein coupled receptor proteins. One sub-class of opsin, the type 2 Rhodopsins, has previously only been identified in metazoan lineages [2]. Here we describe the identification and structural/functional analyses of a putative type 2 rhodopsin in several species of recently sequenced zoosporic fungi: *Batrachochytrium dendrobatidis*, *Spizellomyces punctatus*, *Allomyces macrogynus*, *Rozella allomyces*, *Gonapodya prolifera*, and *Homoplaphyctis polyrhiza*. Computational modeling of the B. dendrobatidis and S. punctatus proteins indicates that they both adopt the seven-transmembrane helix conformation typical of GPCRs. Additional observed motifs are the so-called “ion lock” and conservation of the retinal binding pocket. The B. dendrobatidis protein sequence is notably lacking the conserved lysine residue, however this residue is present in the S. punctatus sequence. The number of identified Ga proteins is roughly consistent among the basal lineages, the Zygomycetes, and the Dikarya. Comparative genomics analyses of rhodopsin and flagellar genes in the basal lineages, Zygomycetes, and Dikarya show a correlation of flagellum and rhodopsin presence across the fungi, suggesting an evolutionary linkage between light-sensing and motility during the transition from aquatic to terrestrial lifestyles. [1] Saranak & Foster. Nature. 1997. [2] Spudich et al. Ann. Rev. Cell Dev. Biol. 2000.

295. Molecular Tools to Silence and Confirm Genes in Phytophthora Sojae. Felipe R. Arredondo1, Brett M. Tyler2, Shiv D. Kale1. 1) Botany and Plant Pathology, Oregon State University, Corvallis, OR; 2) Virginia Bioinformatics Institute, Virginia Tech. Blacksburg, VA.

Bioinformatics analysis is a powerful tool that decreases the number of possible leads to confirm the function of an interesting gene. These leads are only virtual and theoretical but still have to be proven correct and accurate. PEG/Protoplast transformation and particle bombardment transient expression have been part of Phytophthora molecular research for many years and valuable tools in gene identification and confirmation of function. Possibly there are many hundreds of genes in Phytophthora species involved during the infection mechanism; the function of many of these virulence genes is largely unknown. Introducing genes into P. sojae via PEG/Protoplast transformation is a reliable tool that can confirm gene function by over-expression or silencing. Another reliable tool is particle bombardment with the PDS1000 to transiently express intact or modified genes into soybean leaves. In this poster I will introduce the mechanics of these powerful tools.

296. Functional Characterization of Transcription Factor Genes, *MoNIT4* and *MoLEU3*, in *Magnaporthe oryzae*. Jaehyuk Choi1, Soyeon Yoo2, Yong-Hwan Lee2,3,4. 1) Center for Fungal Pathogenesis; 2) Department of Agricultural Biotechnology; 3) Center for Fungal Genetic Resources, Plant Genomics and Breeding Institute, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea.

Rice blast disease caused by *Magnaporthe oryzae* is one of the most destructive threats to global rice production. Nitrogen metabolism has shown to play an important role in pathogenicity of this fungus. Here, we characterized two genes encoding putative transcription factors belonging to the Zn(II):Cys6 family, *MoNIT4* and *MoLEU3*, homologs of nit-4 in *Neurospora crassa* and leu3 in *Saccharomyces cerevisiae*, respectively. The D*MoniT* mutant showed reduction in conidiation and mycelial growth under nitrogen starvation compared to the wild-type. Addition of ammonium restored the growth defects in the mutant. The expression of nitrate and nitrite reductase genes was significantly reduced in the mutants, suggesting that these genes are under control of MoNIT4. The D*MoLeu3* mutant exhibits severe defects in conidiation and pathogenicity as well as mycelial growth under nitrogen starvation. Addition of leucine complemented the defects in conidiation and mycelial growth in the D*MoLeu3* mutant. The decreased expression of 3-isopropylmalate dehydrogenase, 3-isopropylmalate dehydrogenase, and NADP-specific glutamate dehydrogenase genes supports that MoLEU3 functions as a regulator for leucine biosynthesis and ammonia assimilation pathways. Taken together, these findings will help to understand the nitrogen metabolism network in *M. oryzae* and its role in the development of the rice blast disease.

297. Comparative genomic analysis of world-wide *Magnaporthe oryzae* isolate collection. Jaeyoung Che1,2,3,4,5, Jong-Won Lee1,2,3,4,5, Soo-Young Park1, Junhyun Jeon2,3,4,5, Jae Hyuk Choi3,4,5, Ki-Tae Kim1,2,3,4,5, Yong-Hwan Lee1,2,3,4,5,1. 1) Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea; 2) Department of Bioinformatics and Life Science, Soongsil University, Seoul 156-743, Korea; 3) Center for Fungal Pathogenesis; 4) Center for Fungal Genetic Resources, Plant Genomics and Breeding Institute, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea.

Since the genome sequence of *Magnaporthe oryzae* 70-15 was released, functional and comparative genomic researches have been extensively carried out. However, characteristics of essential genes in *M. oryzae* are largely unknown. In this study, we sequenced 39 *M. oryzae* isolates collected from world-wide and analyzed to find the set of core genes. The average assembly size and the total number of predicted genes in the 39 *M. oryzae* genomes were 37Mb and 14,517 genes, respectively. We found that a total of 5,148 genes turned out to be conserved at the amino acid level (identity 80%) among the 40 *M. oryzae* genomes including strain 70-15. To investigate a set of the core genes, we applied three different databases: Functional Transcription Factor Database (FTFD; http://ftfd.snu.ac.kr/), Fungal Cytochrome P450 Database (FCPD; http://p450.riceblast.snu.ac.kr/) and Fungal Secretome Database (FSD; http://fsd.snu.ac.kr/). In addition, three in-house databases were used for the prediction of cell wall-degrading enzymes (CWDEs), peroxidases and laccases. As a result, we found a core set of genes in *M. oryzae*: 932 secreted proteins, 269 transcription factors, 33 cytochrome P450 genes, 18 CWDEs, 17 peroxidases and 5 laccases. Furthermore, we discovered an overlap of 38 genes between the above groups which have secretory potential. To archive and manage those newly sequenced genomes, we developed the *Magnaporthe* Atlas (http://www.magnaporthe.org/) as a web-based solution. Our core gene set of *M. oryzae* will facilitate discovery of lineage-specific innovations with implications in coevolution with specific host cultivars.

298. Functional characterization of two genes encoding putative Zn(II):Cys6 transcription factors, *MoC0D1* and *MoC0D2* in *Magnaporthe oryzae*. Hyunyoung Chung1, Sook-Young Park1,2,3,4, Jeahyuk Choi3,4,5, Junhyun Jeon1,2,3,4,5, Yong-Hwan Lee1,2,3,4,5,6. 1) Department of Agricultural Biotechnology; 2) Center for Fungal
Pathogenesis; 3) Center for Fungal Genetic Resources, Plant Genomics and Breeding Institute, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea.

Transcription factors (TFs) play pivotal roles in regulation of gene expression during cellular processes. The rice blast fungus, Magnaporthe oryzae undergoes a series of morphological changes during the infection. To elucidate the roles of TFs in development of rice blast disease, two Zn(II),Cys, TF genes, MocOD1 and MocOD2, were characterized. Both DMocOD1 and DMocOD2 mutants showed defects in conidiation and pathogenicity. Reduced pathogenicity of the DMocOD1 mutant was due to defects in invasive growth while the DMocOD2 mutant exhibited no pathogenicity. Especially, restricted invasive growth and accumulation of dark brown granules around infection hyphae were frequently observed in the DMocOD2 mutant. The granule accumulation is considered as a plant defense response. Genetic complementation with the wild type alleles restored the defects in conidiation and pathogenicity. Taken together, both MocOD1 and MocOD2 are responsible for conidia development and pathogenicity in the rice blast fungus. This is the first report of the involvement of Zn(II),Cys TFs in pathogenesis of fungal plant pathogens.

299. Comparative proteomics of monocolon-al antibody enriched haustoria from three races of Puccinia triticina. Christof Rampitsch, Eva Beimck, Aslihan Guneli, Guus Bakkeren, Tao Fan. 1) Cereal Research Ctr, Agriculture & Agrifood Canada, Winnipeg, Canada; 2) Ahi Evran University, Department of Chemistry, Kirsehir, Turkey; 3) Pacific Agrifood Research Centre, Summerland BC, Canada.

Puccinia triticina (Pt) causes leaf rust on wheat and is a problem in most areas where wheat is grown. The host-pathogen interaction follows the gene-for-gene model, where an interaction between host resistance (R) genes and pathogen avirulence (avr) genes determines whether the plant will remain healthy, or whether the pathogen can complete its life cycle and cause disease. Pt is an obligate parasite which penetrates wheat leaves through stomata, colonizes the apoplastic space and forms haustoria inside host cells. Haustoria mediate nutrient up-take between the host and pathogen thus play a major role in pathology. Purification of milligram quantities of haustoria from Pt to >95% homogeneity, determined visually by calcofluor white staining, has been made possible through the development of specific monocolonal antibodies. The haustoria proteome is of great interest, since it likely contains proteins with potential roles in pathogenesis. We have purified haustoria from races 1, 9 and 161 for a comparative proteome analysis, because complete genomic sequences exist for these. This is an essential requirement for homology-based matching of mass spectral data. Haustoria are surrounded by the host plasma membrane, an extrahaustorial matrix and extrahaustorial membrane. As a result, they are recalcitrant tissues that resist analysis by conventional proteomics approaches. We have designed a strategy for obtaining the maximum yield of tryptic peptides from purified haustoria, suitable for LC-MS analysis. Preliminary results of the comparative proteome will be presented and discussed.

300. RNA-seq analyses of gene expression in the microsclerotia of Verticillium dahliae. Dechassa Duressa, Amy Ancheta, Donquan Chen, Anna Klimes, Katherine F. Dobinson, Maria Garcia-Pedrajas, Steven J. Klosterman. 1) USDA-ARS, Salinas, CA; 2) Comprehensive Cancer Center & Division of Preventive Medicine, University of Alabama at Birmingham, AL; 3) Department of Biology, University of Western Ontario, London, ON, Canada; 4) Agriculture and Agri-Food Canada, London, ON, Canada; 5) Department of Physiological and Biological Science, Western New England University, Springfield, MA; 6) Estación Experimental La Mayora CSIC, Málaga, Spain.

Verticillium dahliae is a soilborne fungus that causes wilt disease in plants. Verticillium wilt is difficult to control because the pathogen is capable of persisting in the soil for 10 to 15 years as melanized microsclerotia, rendering crop rotation strategies for the control of this disease ineffective. The microsclerotia of V. dahliae produce infectious hyphae that give rise to primary infections. As such, the processes of microsclerotia formation, maintenance, and germination are critically important in the disease cycle of V. dahliae. To shed additional light on the molecular processes involved in microsclerotia biogenesis and melanin synthesis in V. dahliae, three replicate RNA-seq libraries were prepared from 10 day-old microsclerotia (MS)-producing cultures of V. dahliae (ave=52.3 million reads), and those not producing microsclerotia (NoMS, ave=50.8 million reads), and analyzed for differential gene expression. The comparisons revealed up-regulation of MS library genes involved in melanogenesis, including tetrahydroxynaphthalene reductase (344-fold increase) and scytalone dehydratase (231-fold increase), and of additional genes located in a 48.8 kilobase melanin biosynthetic cluster. Numerous hypothetical protein-encoding genes were also identified as differentially expressed in the MS library. For confirmation of differential expression, selected genes identified by RNA-seq as up- or down-regulated were analyzed by RT-qPCR of RNA from several MS and NoMS culture types, including MS cultures that were stored for 6 months at 4°C, and seven day old cultures having an intermediate number of melanized MS. These data provide further insight into gene expression during melanin biosynthesis and MS formation in V. dahliae, and the products encoded by these genes may represent alternative disease control targets.

301. Exploring the Genome Diversity of Mycorrhizal Fungi to Understand the Evolution and Functioning of Symbiosis. Francis M. Martin, Mycorrhizal Genome Initiative Consortium. Tree-Microbe Interactions, Lab of Excellence ARBRE, INRA-Nancy, Champenoux, France.

Genomics has introduced an important new dimension into mycorrhizal research by establishing data to serve as a new and fundamental resource for genetics and molecular biology of the symbiosis formation. With the current genomic view of ectomycorrhizal (EM) fungi that we have, a possible scenario suggests that (1) irreversible losses of lignocellulose decomposition pathways play a key role in the evolutionary stability of the ectomycorrhizal mutualisms and (2) that each major EM fungal clade has subsequently and independently designed symbiotic molecular toolboxes each time the mycorrhizal lifecycle has arisen in the tree of life. This hypothesis would predict that symbiotic toolboxes are tailor made for each major fungal clade (e.g., Agaricales, Boletales, Sebacinales) and may be tuned according to specific plant hosts. To further our understanding of the evolution of these symbioses, the Mycorrhizal Genomics Initiative targets a set of 30 fungal mycorrhizal species. Taxa have been selected for (1) their phylogenetic novelty, (2) their ability to establish different types of mycorrhizal symbioses and (3) their taxonomic relationships with already sequenced EM genomes. Several target species are capable of forming different types of intracellular colonizing structures - and this plasticity depends on plant host. Several species are dominant fungi in their ecological settings and others are currently used in the commercial forestry industry to inoculate conifer or hardwood seedlings for lumber, bioenergy and landscape trees. I will discuss how the comparative analysis of mycorrhizal genomes has, and will continue, to shed light on the evolution of mycorrhizal symbioses.


The bud emergence 46-like (BEM46) protein from Neurospora crassa belongs to the alpha/beta-hydrolase superfamily. Recently, we have reported that the BEM46 protein is localized in the perinuclear ER and also forms spots close by the plasma membrane. The protein appears to be required for cell type-specific polarity in Neurospora crassa. Furthermore, initial studies suggested that the BEM46 amino acid sequence is conserved in eukaryotes and is considered to be one of the widespread conserved “known unknown” eukaryotic genes. To unravel origin and molecular evolution of these genes in different eukaryotes, we carried out a comprehensive sequence, structural functional and phylogenetic analyses of BEM46 orthologs. During this study, we
found that all eukaryotes have at least a single copy of a bem46 ortholog. Upon scanning of these proteins from various species, expansions leading into several paralogs in vertebrates and plants were identified. We illustrate insertion/deletions (indels) in the conserved domain of BEM46 protein, which allow differentiating fungal classes such as ascomycetes from basidiomycetes. Furthermore, we analyze several duplicates of this gene in different animal and plant genomes to understand possible mechanisms of evolution after separation from the fungal lineage. In addition, we unravel that BEM46 protein from N. crassa possess a novel endoplasmic-retention signal (PEKK) using GFP-fusion tagging experiments, hinting there is need to re-define the motifs in conserved in various protein sequences as over a million of genome sequences will be available in next decade.


Filamentous fungi are currently the primary source of plant cell wall degrading enzymes for the production of biofuels from lignocellulosic feedstocks. However, despite tremendous improvements of these enzyme cocktails over the last years, they are still rather inflexible and will not work optimally in situations such as complicated with a changing variety of feedstocks. Fungi have evolved with their host plants in a long and intricate relationship, and a detailed understanding of their responses to the various building blocks present in the plant cell wall material will also help to improve the industrial applicability and versatility of these enzyme cocktails. In recent years, the ascomycete Neurospora crassa has been developed as a model system to study cellulose and xylan degradation by filamentous fungi. As a complement to these studies, here we performed a systems analysis of pectin degradation, the third major plant cell wall polysaccharide. A combination of proteomics and transcriptomics was used to define the “toolbox” N.crassa uses to degrade this highly complex heteropolysaccharide, and to identify new components that seem to work both synergistically and antagonistically in this process. Moreover, in combination with the data from two earlier studies, describing the responses to cellulose and xylan, the acquired knowledge allowed for the first time to put the individual responses to each of these three main plant cell wall polysaccharides into perspective. Central to this analysis was the construction of a co-expression matrix covering the most relevant carbon source-related inducing conditions. The applicability of this matrix could be demonstrated by successfully guiding in the functional characterization of an unknown sugar transporter, which was identified to mediate L-arabinose uptake. Only if we understand the building blocks of the carbon-related response pathways we can attempt to put them together into the “bigger picture”. The comparative approach presented here therefore is an important step towards a more profound understanding of the fungal degradation process of complex biomass.


The availability of whole genome sequence allows immediate comparison between polymorphisms that have physiological impact and those that are neutral. We are exploiting this as we characterize genes responsible for Acriflavine resistance. Preliminary analysis showed that despite a wealth of polymorphisms among whole genome sequenced strains, the ORF NCU09975 encoding an abc3 transporter is not altered in the acriflavine resistant strain FGSC 1215. Additional analyses have pointed to the transcription factor gene NCU09974 and the polymorphism in this gene in the acriflavine resistant strain is unique both in comparison to the reference genome strain and among the growing number of strains subject to whole sequencing. Continuing work aims to test whether transfer of the NCU09974 allele from the acriflavine resistant strain to an otherwise sensitive strain will confer resistance. Additional studies will investigate whether the broad resistance seen in classical acriflavine resistant mutants can allow identification of a compound that can be used as a selectable agent in combination with the newly identified allele.

305. Genome based phylogeny of early diverging fungal lineages. A. P. Gryganskyi1, G. Bonito4, M. Rodriguez-Carres2, T. M. Porter2, Y. Chen4, S. Robb1, H.-L. Liao1, I. M. Anishchenko1, O. V. Savitskyl1, R. Ortega4, J. E. Stajich7, J. Heitman1, A. P. Litvintseva1, T. Y. James3, S. Sekimoto4, J. Spatafora5, R. Vilgalys1. 1) Biology, Duke University, Durham, NC; 2) Ecology and Evolutionary Biology, McMaster University, Hamilton, ON, Canada; 3) Duke University Medical School, Durham, NC; 4) Plant Pathology and Microbiology, University of California, Riverside, CA; 5) Institute of Botany, NASU, Kyiv, Ukraine; 6) Institute of Molecular Biology and Genetics, NASU, Kyiv, Ukraine; 7) Centers for Disease Control and Prevention, Atlanta, GA; 8) Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI; 9) Biological Sciences, The University of Alabama, Tuscaloosa, AL; 10) Botany and Plant Pathology, Oregon State University, Corvallis, OR.

The phylogeny of the early diverging fungal lineages remains controversial in spite of a growing database of morphological, ultrastructural, biochemical and molecular evidence. Here we present a comprehensive molecular phylogeny for the basal fungi using metagenomic data from 30 fungal taxa for which whole genomes or ESTs are available. Taxa include a dozen flagellated lineages, ten zygomycetous taxa, and key representative of the Dikarya with Entomophthoromycotina and Kickxellomycotina. Phylogenetic trees built from 434 orthologs (some missing data) and 29 orthologs (no missing data) are congruent and statistically well supported. Our results show a clear separation of most flagellated fungi from terrestrial taxa. An analysis of the presence of the genes associated with the flagellar apparatus supports the hypothesis that the flagellum was lost once concomitant with fungi transitioned to terrestrial habitats. Zygomycetous lineages occupy an intermediate position between flagellated fungi and the Dikarya with Entomophthoromycotina and Kickxellomycotina representatives as a basal clade.

306. Comparative analysis of 35 basidiomycete genomes reveals diversity and uniqueness of the phylum. Robert Riley1, Asaf Salamović2, Robert Otillar1, Kirsten Fagman, Bastien Boussau, Daren Brown, Bernard Henrisat, Anthony Levasseur, Benjamin Held, Laszlo Nagy, Dimitris Floudas, Emmanuelle Morin, Gerard Manning, Scott Baker, Robert Blanchette, Francis Martin, David Hibbett, Igor Grigoriev. 1) Joint Genome Institute, Lawrence Berkeley National Lab, Walnut Creek, CA; 2) Clark University, Worcester, MA; 3) UC Berkeley, Berkeley, CA; 4) USDA, Peoria, IL; 5) AFMB, Marseille, France; 6) UMN, St. Paul, MN; 7) INRA, France; 8) Salk Institute, La Jolla, CA; 9) Pacific Northwest National Lab, Richland, WA.

Fungi of the phylum Basidiomycota (basidiomycetes), make up some 37% of the described fungi, and are important in forestry, agriculture, medicine, and bioenergy. This diverse phylum includes symbionts, pathogen of the Glomeromycota, and bioenergy. This diverse phylum includes symbionts, pathogen of the Glomeromycota, and Basidiomycota. Phylogenetic analysis of pectin degradation, the third major plant cell wall polysaccharide. A combination of proteomics and transcriptomics was used to define the “toolbox” N.crassa uses to degrade this highly complex heteropolysaccharide, and to identify new components that seem to work both synergistically and antagonistically in this process. Moreover, in combination with the data from two earlier studies, describing the responses to cellulose and xylan, the acquired knowledge allowed for the first time to put the individual responses to each of these three main plant cell wall polysaccharides into perspective. Central to this analysis was the construction of a co-expression matrix covering the most relevant carbon source-related inducing conditions. The applicability of this matrix could be demonstrated by successfully guiding in the functional characterization of an unknown sugar transporter, which was identified to mediate L-arabinose uptake. Only if we understand the building blocks of the carbon-related response pathways we can attempt to put them together into the “bigger picture”. The comparative approach presented here therefore is an important step towards a more profound understanding of the fungal degradation process of complex biomass.
FULL POSTER SESSION ABSTRACTS


We wish to study the relationship of expanded genes and evolutionary adaptation in mushroom-forming fungi. We planned to (1) detect the enrichment of expanded genes specific for mushroom-forming fungi, (2) develop a pipeline to construct protein–protein interaction (PPI) networks and match them with the established pathways in REACTOME database for fungal proteins, and (3) characterize the functions and action stage of the expanded kinases in fruiting body development. The expanded genes in late evolved organisms contribute to adaptive functions and morphological characters. Mushroom-forming fungi can be differentiated from the simple fungi by the extra morphological status of fruiting bodies. We hypothesize that the expanded genes unique to mushroom-forming fungi are critical for fruiting body development. By comparing 70 species from basidiomycota and ascomycota, we found significant enrichment in some protein functional clusters in mushroom-forming fungi compared to simple fungi. Among these clusters, we chose to further analyze the group of Posttranslational modification related genes. We predicted and compared the PPM sites density. We found that the greater the genome size the lower the ubiquitylation site density. We developed a novel pipeline to search the literature for interacting proteins and construct PPI networks. With this pipeline, we proposed a light signal transduction phosphorylation cascade which involves some Funk1 kinases and components in PKA and MAPK pathways. To investigate the functional roles of these kinases in the putative cascade, we introduced the sirRNA of corresponding genes into Coprinopsis cinerea at five stages in the life cycle. We showed by transient knock down of expanded kinases that they play important roles in light signal transduction pathway and possess different functions in different developmental stages.


Many cellular processes are regulated by phosphorylation via protein kinases. To unravel the understanding of protein phosphorylation, normally the protein kinase complements (known as ‘kinomes’) are examined genome-wide in eukaryotic species. About 2% of eukaryotic genes are protein kinases. Bioinformatics and comparative genomics were used to determine kinomes from eukaryotes and to explore in evolutionary and functional context. Kinases are major regulators of cellular processes in fungi, in similar fashion as they regulate other eukaryotes. For example, 77 viable mutants for ser/thr kinase genes (of 86 in total) were identified in N. crassa and 57% illustrated at least one growth or developmental phenotype. Given that there is over 100 fungal genomes are known. Hence, there is a need of more comprehensive analysis of fungal kinomes. We have established kinomes of about 90 fungi with 5604 kinases, which also included pseudokinases (~1%). Fungi have expansion of ser/thr kinases in comparisons to other classes of kinases. We thus report how kinases in combination with ~80 other protein domains, evolved to perform their roles in different signalling cascades in these fungi. Furthermore, we annotated and analysed these kinomes for evolutionary mechanisms operating in fungal kinomes.


Asexual differentiation in Aspergillus nidulans involves complex control by a number of factors and is light-dependent. The VeLB/VeA/LaeA complex in A. nidulans coordinates light signal with development and secondary metabolism. We investigated the roles of velvet family genes, veA, veLB and veLc, and fluG (fluffy phenotype in A. nidulans) in an aflatoxicogenic flavus strain. Knockout strains of veA or veLB conidiated poorly in the dark but not in the light. Knockout strains of fluG also showed decreased conidiation but had increased sclerotial production. Deletion of fluG in the veA or veLB knockout resulted in a marked decrease in conidiation even in the light. Growth under stress (0.6 M potassium chloride) partially restored aforementioned defects in conidiation. The veA or veLB knockout mutant but not the veLC or fluG mutant was unable to produce aflatoxin. Overexpression of veA in the veLB mutant only restored conidiation while overexpression of veLB in the veA mutant failed to restore either conidiation or aflatoxin production. Yeast two-hybrid assays confirmed that VeA, VeLB and LaeA form a complex but suggested that FluG is also likely to be an interacting partner. Concerted interactions of A. flavus VeA and VeLB with LaeA are critical for conidiation in the dark and aflatoxin biosynthesis.

310. The Environmental Molecular Sciences Laboratory molecular analysis capabilities for fungal biology. S. E. Baker, Environmental Molecular Sciences Laboratory, Pacific Northwest Natl Lab, Richland, WA.

Tools for analysis of classical and reverse genetic mutants play an important role in fungal biology research. The Environmental Molecular Sciences Laboratory (EMSL) at the Pacific Northwest National Laboratory is a US Department of Energy national user facility. EMSL develops and utilizes cutting edge mass spectrometry, NMR, imaging and computational capabilities to accelerate research in a number of areas. We have used EMSL’s mass spectrometry capabilities to characterize glycosylation of secreted proteins of Aspergillus niger. In addition, we have explored the use of laser ablation and nano-DESI mass spectrometry for spatial localization of molecules associated with Trichoderma reesei mycelium. Finally, spores from wildtype and albino strains of Aspergillus carbonarius were characterized using helium ion microscopy. As a national user facility, the EMSL is open to the fungal biology community through a competitive, peer-reviewed proposal process.


We have sequenced the genome of Tolypocladium inflatum, the first sequenced representative of one of three major lineages of insect pathogens within the order Hypocreales. Comparisons of the gene space and transcriptome of T. inflatum with closely related plant pathogenic and endophytic fungi is providing insights into secondary metabolite arsenals specific to insect pathogens as well as shedding light on shifts in primary metabolism associated with a transition to an insect host. We address the role of secondary metabolites in insect pathogenesis using a combination of comparative genomics to track the evolution of secondary metabolite clusters across the Hypocreales and transcriptomics to characterize patterns of gene expression within metabolite clusters in media supplemented with insect cuticle (simulating insect infection) and hemolymph (simulating insect colonization). We also identify other gene families that are upregulated under these media conditions. GO enrichment analyses of upregulated genes showed that those involved in oxidation-reduction reactions, iron-binding, and transport of iron and inorganic ions are important during both the infection and colonization phases. Genes with serine peptidase and serine hydrolase activity were uniquely upregulated in cuticle media while a large proportion of genes upregulated in hemolymph were involved in transmembrane transport not only of iron, but also of sugars and other carbohydrates. We examine expansions and contractions of some of these gene families (e.g. proteases and P450s) that map to nodes in the phylogeny associated with shifts to insect hosts. We identify patterns that are shared across the three insect pathogenic lineages of Hypocreales versus those which have evolved independently in distinct lineages.
312. Integrated transcriptional profiling and analysis for identification of Cryptococcus neoformans genes regulated during human cryptococcal meningitis. Y. Chen\textsuperscript{1}, J. Tenor\textsuperscript{1}, D. Toffaletti\textsuperscript{1}, A. Litvintseva\textsuperscript{1}, T. Mitchell\textsuperscript{1}, J. Perfect\textsuperscript{1}. 1) Duke University School of Medicine, Durham, NC; 2) Centers for Disease Control and Prevention, Atlanta, GA.

Background: Cryptococcus neoformans is an opportunistic fungal pathogen that is the major cause of fungal meningitis in immunocompromised individuals worldwide. Accurate and comprehensive de novo transcriptome profiling of C. neoformans in the human host may allow a better understanding of how it survives and produces disease. Methods and Results: To identify genes, whose expression is differentially regulated under \textit{in vivo} and \textit{in vitro} conditions, we selected two strains of \textit{C. neoformans var. grubii} (serotype A), which were isolated from the cerebrospinal fluid (CSF) of two AIDS patients from Uganda and the United States. Multilocus sequence typing (MLST) showed that one strain was from VNI clade and one strain from VNII. Next-generation sequencing (RNA-Seq) was used to determine transcriptional profiles of these strains under three conditions: fungal cells were directly taken from CSF of the patients; fungal cells were grown in YPD at 37°C until the stationary phase; fungal cells that reached the stationary phase in YPD were exposed to sterile human CSF for 9 hours. The sequencing results showed that there was no major difference in sequencing quality and contaminations between \textit{in vivo} and \textit{in vitro} samples. Hierarchical clustering analysis revealed that the samples treated with same environment have more similarity in transcriptional profile. Comparative analysis of the expression pattern shows that 144 genes up-regulated in CSF when compared to YPD and 87 genes were up-regulated \textit{in vivo} compared to YPD and 39 genes overlapped between the CSF and \textit{in vivo} condition. Some of the overlapping genes in CSF and \textit{in vivo} have been reported to be related to the virulence composite of \textit{C. neoformans}, such as Rim101 and ENA P-type ATPase 1. Furthermore, we searched for the 100 most divergent expressed genes between the two strains. Gene Ontology (GO) term enrichment analysis showed an enrichment of GO terms in transporter activity between the strains. Conclusion: We provide the first transcriptome profiling of \textit{C. neoformans} taken directly from the CSF of two human patients. The comparisons between \textit{in vivo} and \textit{in vitro} samples helped us to identify a group of genes that may be important for surviving, adapting and proliferating of \textit{C. neoformans} in the CSF of the human host.


MicroRNAs are endogenous RNAs with a size of about 22 nt and post-transcriptionally regulate gene expression in metazoan and plants. MicroRNAs are derived from RNA hairpin precursors, which are usually transcribed by RNA polymerase II. Recent studies on small RNA binding components of the RNA-induced silencing complex (RISC) show the existence of microRNA-like RNAs (miRNAs) in \textit{Neurospora crassa}, which give a first hint of a post-transcriptional regulatory mechanism based on microRNAs in fungi [1]. So far only little is known about microRNA-like molecules in other fungi, especially about their role in fungal development and gene regulation. To investigate the occurrence of miRNAs and their involvement in the penicillin producing fungus \textit{Penicillium chrysogenum}, we performed predictions of putative microRNAs. Therefore small RNAs (19 - 50 nt) representing different growing conditions and developmental stages, were used for RNA next generation sequencing. The calculation of putative microRNA precursors was performed with the program mirDeep [2], and is based on the distribution of RNA sequence reads in afore predicted RNA hairpin molecules. By this approach, we were able to identify structures, which show the typical characteristics of microRNA precursors. To confirm the \textit{in silico} predictions, transcript analyses were performed. These analyses support the existence of small RNAs and their precursors and show various expression pattern of the putative miRNAs under different growing conditions. To investigate the regulatory role of the identified miRNAs, strains lacking or overexpressing miRNAs were generated. In addition, we have constructed artificial microRNAs to investigate their use as molecular genetic tools to mediate gene specific RNA interference (RNAi). The results of this study provide evidence for miRNAs in \textit{P. chrysogenum} and indicate a miRNA based silencing mechanism in this fungus.


314. Metatranscriptomic analysis of ectomycorrhizal root clusters in \textit{Pinus taeda}: new methodologies for assessing functional gene expression \textit{in situ}. H.-L. Liao\textsuperscript{1}, Y. Chen\textsuperscript{1}, T. D. Bruns\textsuperscript{3}, K. G. Peay\textsuperscript{2}, J. W. Taylor\textsuperscript{3}, S. Branco\textsuperscript{3}, J. M. Talbot\textsuperscript{3}, R. Vilgalys\textsuperscript{3}. 1) Department of Biology Duke University, Durham, NC; 2) School of Medicine, Duke University, Durham, NC; 3) Department of Plant and Microbial Biology, UC-Berkeley, Berkeley, CA; 4) Department of Biology, Stanford University, Stanford, CA.

A highly diverse community of ectomycorrhizal (ECM) fungi are known to associate with the needles of the genus \textit{Pinus}. Less is known about how diverse fungal communities affect functional diversity within ECM roots. Here we present an optimized method for metatranscriptomic analysis of the ECM-pine root interaction in a natural system. RNA was purified using a CTAB method from individual ECM root clusters collected at varying spatial scales across the distribution range of \textit{P. taeda}, and sequenced using Illumina HiSeq technology. About 35 million qualified reads were obtained. Sequences were initially assembled using reference based mapping (Bowtie) to sort the reads that represent rRNA from fungal and bacterial species. Reads from divergent regions (D1-D2) of fungal LSU rRNA were used to identify dominant ECM and other fungal community members. Subsequently, \textit{P. taeda} genes and functional genes of dominant fungal species were sorted using public cDNA databases. The Trinity package was used for \textit{de novo} assembly of un-mapped reads (mostly fungal genes). Blastx and Go packages were used for gene annotation. A typical ECM root cluster was found 45% \textit{P. taeda} genes, 3% fungal rRNA, 0.05% bacterial 16s rRNA, 30% fungal functional genes, 10% unknown sequences, and 12% unassembled reads. Analysis of D1-D2 LSU sequences confirmed that a single ECM fungal species usually dominates individual root clusters. \textit{De novo} assemblies of fungal genes yielded 120 thousand contigs from 10 million reads representing 90 thousand unique genes with highly similarity to known ECM fungi. Functional analysis revealed that most of the transcripts recovered were involved with translation, protein degradation, heat shock, superoxide metabolism, electron transfer, signaling, and C/N metabolism. Highly expressed transcripts recovered from \textit{P. taeda} and \textit{P. nigra}. Plasma membrane permeabilisation by chitosan depends on membrane fluidity, with FFA unsaturated membrane fungi (\textit{N. crassa}) being chitosan sensitive, the plasma membrane fluidity is an

315. Transcriptomic response of \textit{Neurospora crassa} germinating conidia to chitosan in sub-lethal dose. Federico Lopez-Moya\textsuperscript{1}, David Kowbel\textsuperscript{2}, N. Louise Glass\textsuperscript{2}, Luis Vicente Lopez-Lloerac\textsuperscript{2}. 1) Laboratory of Plant Pathology, Multidisciplinary Institute for Environment Studies (MIES) Ramón Margalef. University of Alicante, Alicante, SPAIN; 2) Department of Plant and Microbial Biology, University of California, Berkeley CA, 94720-3120 USA.

Chitosan is a natural polymer able to permeabilize \textit{Neurospora crassa} membranes, in an energy dependent manner. Plasma membrane permeabilisation by chitosan depends on membrane fluidity, with FFA unsaturated membrane fungi (\textit{N. crassa}) being chitosan sensitive, the plasma membrane fluidity is an
important factor in chitosan effect. Plasma membrane permeabilisation is also enhanced under starvation conditions, also causes intracellular ROS increases and is involved in cell division. Conidial germination is the most sensitive step to chitosan in filamentous fungi. We have used *Neurospora crassa* conidia germinating in Vogel medium with chitosan at a sub-lethal concentration for evaluating the transcriptomic response of the fungus. We determined chitosan IC50 for *N. crassa* (4, 8 and 16 hours after inoculation, hai) and analyzed the effect on gene expression over time. Using RNA-seq we have detected the genes involved in *N. crassa* response to chitosan, we analyzed with bioinformatic resources the expression involved on chitosan response in *N. crassa* conidia . We have also re-annotated the complete *Neurospora crassa* OR74A genome (Broad Institute) for allowing GO analyses (biological process, cell component and molecular function) of our RNA-seq data. Chitosan early induced (4-8 hai) some genes, involved in ROS protection (e.g. catalases, monooxygenase and SOD) and also in nitrogen compounds transporter and degradation. We also detected late expression of genes involved in biosynthetic processes, nitrogen compounds (including proteins) metabolism and transport. Our strategy has allowed us to gain an important insight on chitosan mode of action. This will open new possibilities for application this versatile natural compound.

316. Transcriptomic analysis of the interaction between *Trichoderma harzianum* and the phytopathogen *Sclerotinia sclerotiorum* using the RNA-seq approach. Andrei S. Steindorf1, Marcelo H.S. Ramada1, Robert Miller2, Georgios J. Pappas3, Cirano J. Ulhoa4, Eliane F. Noronha1. 1) Cell Biology Dept, Brasilia University, Brasilia, DF, Brazil; 2) Biochemistry Dept, Federal University of Goias, Goiania, Brazil. The plant pathogen *Sclerotinia sclerotiorum* is the causal agent of the common bean’s (Phaseolus vulgaris) root rot disease, white mold, and its occurrence is responsible for the great yield losses in irrigated areas of the Southeast and Midwest regions of Brazil. Species of the fungus genus *Trichoderma* have been used in the biological control of this pathogen as an alternative to chemical control. To gain new insights into the biocontrol mechanism used by *Trichoderma harzianum* against the phytopathogenic fungus, *Sclerotinia sclerotiorum*, our research group performed a transcriptomic analysis of this interaction using RNA-seq and quantitative real-time PCR (RT-qPCR) approaches. Six RNA-seq libraries from *T. harzianum* mycelium (isolate 303/02) grown on cell walls of *S. sclerotiorum* (CWSS) and glucose during 12, 24 and 36 h were constructed, sequenced by Illumina HiSeq 2000 x100pb and analyzed by TopHat/Cufflinks pipeline. The T. harzianum CBS 226.95 v1.0 genome (http://genome.jgi.doe.gov/Triha1/Triha1.home.html) was used as a reference for the bioinformatics analysis. Among the 13616 genes mapped, 1581 genes were found differentially expressed among the growth in the presence of glucose or plant pathogen cell wall. Moreover, the expression pattern was also time course dependent. Transporters, fungal cell wall hydrolases, peptidases, transcriptional factors and proteins presenting role in the environmental interaction were found high expressed by the *Trichoderma* isolate in the three different times of growth and in the presence of the pathogen. Some genes with no known functions were also found. The role of cell wall hydrolases, peptidases and other hydrolytic enzymes in the mycoparasitism by *Trichoderma* species was strongly recorded, therefore the description of the functions of those unknown functions genes in biocontrol will orientate future works. Indeed, the present work will contribute to an initial mapping of the transcripts quite related to the interaction among these two fungi and for its further analysis under in vivo interaction.

317. Genome evolution of the original Saccharomyces carlsbergensis lager yeast strain, Unterhefe No1, as revealed by whole genome sequencing. Andrea Walther, Ana Hesseltand, Jürgen Wendland. Carlsberg Laboratory, Copenhagen V, Denmark. The first lager beer yeast strain was purified by Hansen in 1883 who termed this original strain Unterhefe No1, also known as Saccharomyces carlsbergensis. It became evident that Unterhefe No1 is a hybrid between two closely related Saccharomyces species, particularly *S. cerevisiae* and a *S. bayanus*-like strain. We have compared key fermentation parameters including sugar utilization, ethanol and flavor production of Unterhefe No1 with currently used industrial lager yeast strains. We then sequenced the S. carlsbergensis genome using 454 next generation sequencing methods and determined its hybrid genome content. We found that the genome has evolved from a presumed ancestral tetraploid cell as a result of adaptation to brewing conditions resulting in its current allotetraploid state. In contrast to a hypothetical tetraploid genome with 24 Mb unique DNA contributed by its parents and distributed on 32 chromosomes, the Unterhefe No1 genome consists of only 20.7 Mb. It comprises chromosomes derived from *S. cerevisiae* and a non-*cerevisiae* parental strain as well as a *S. bayanus* like mitochondrial genome. In total, we found 29 different chromosomes including evolved chromosomes displaying several events of loss of heterozygosity and massive chromosomal rearrangements. Comparison of the genome of S. carlsbergensis with other yeast genomes provides insight into this brewing strain as a consequence of adaptation to lager beer fermentation conditions.

318. Retention of genes in a secondary metabolite gene cluster that has degenerated in multiple lineages of the Ascomycota. Daren W. Brown1, Hege H. Diven2, Erik Lyse3, Robert H. Proctor1. 1) Bacterial Foodborne Pathogens and Mycology Research, USDA/ARS, Peoria, IL; 2) Section of Mycology, Norwegian Veterinary Institute, PO Box 750, Sentrum, 0106 Oslo, Norway; 3) Department of Plant Health and Plant Protection, Bioforsk - Norwegian Institute of Agricultural and Environmental Research, 1432 Ås, Norway. Fungal secondary metabolite (SM) gene clusters encode proteins involved in SM biosynthesis, protection against SMs, and regulation of cluster gene transcription. RNA-Seq analysis of *Fusarium langsethiae* (class Sordariomycetes) revealed a cluster of six genes that were highly expressed during growth in oat-grain medium. All six genes share significant homology and synteny with genes in the *Alternaria brassicicola* (class Dothideomycetes) cluster responsible for production of the SM depudecin. HPLC analysis confirmed the presence of depudecin in oat-grain medium and absence from complete medium cultures. A survey of publically available genome sequences identified eight complete and 14 partial depudecin biosynthetic gene (DEP) cluster homologs in fungi across distantly related classes of Ascomycota. Most of the partial clusters included pseudogenes due to single nucleotide changes and/or multiple nucleotide deletions, indicating that the partial clusters are derived by degeneration of complete clusters. Most of the partial clusters also included apparently functional homologs of the major facilitator superfamily (MFS) transporter (DEP3) and transcription factor (DEP6) genes. Retention of these two genes may provide a defense mechanism against depudecin produced by other fungi. Alternatively, DEP3 and DEP6 in the partial clusters may have been repurposed to provide a selective advantage different from the advantage conferred by depudecin. The shared synteny of putative functional DEP3 and DEP6, as well as phylogenetic analysis of these genes, suggest that the DEP cluster has been transferred horizontally between fungi multiple times.

319. Functional characterization of unique non-ribosomal peptide synthetase genes in the cereal fungal pathogen *Cochliobolus sativus*. Yueqiang Leng, Shaobin Zhong. Department of Plant Pathology, North Dakota State University, Fargo, ND 58108. In filamentous fungi, nonribosomal peptide synthetases (NRPSs) are the major enzymes involved in biosynthesis of nonribosomal peptides (NRPs), some of which have been demonstrated to be involved in pathogenicity or virulence of fungal plant pathogens. However, the functions of many genes (NPS) encoding NRPSs are still not well understood. We identified 25 NPS genes from the genome sequence of the cereal fungal pathogen *Cochliobolus sativus*. Genome comparison among species in the genus of *Cochliobolus* identified 14 unique NPS genes in *C. sativus* with five (encoding protein ID: 130053, 140513, 104448, 115356 and 350779, respectively) being unique to the pathotype 2 isolate ND90PT of the fungus. Quantitative real time PCR revealed that
320. Phylogenomics unveils secondary metabolites specific to mycoparasitic lineages in Hypocreales. C. Alisha Owensby, Kathryn E. Bushley, Joseph W. Sapatara. Botany & Plant Pathology, Oregon State University, Corvallis, OR.

Hypocreales is an order characterized by a dynamic evolutionary history of interkingdom host jumping, with members that parasitize animals, plants, and other fungi. The monophyly of taxa attacking members of the same kingdom is not supported by molecular phylogenetics, however. For example, Trichoderma spp. and Elaphocordyceps spp. are both mycoparasitic, but are members of different families within Hypocreales, Hypocreaceae and Ophiocordycipitaceae, respectively. In fact, both genera are more closely related to insect pathogens, than they are to each other. Multiple species of Trichoderma have sequenced genomes, and recently genomes of several insect pathogens in Hypocreales have been completed (e.g. Metarhizium spp. and Tolyposcladium inflatum). The genus Elaphocordyceps represents a unique clade within Hypocreales, because whereas most species in the family Ophiocordycipitaceae are insect pathogens, most Elaphocordyceps parasitize truffles of the ectomycorrhizal genus Elaphomyces (Eurotiales, Ascomycota).

To compare genes of a truffle pathogen with hypocreaein insect pathogens and mycoparasites, we sequenced the genome of Elaphocordyceps ophioglissoides. Our draft assembly of the E. ophioglissoides genome is ~32 MB and has 10,779 gene models, 36 of which are predicted to produce secondary metabolites. We have identified three very large genes in E. ophioglissoides related to peptaiol producing nonribosomal peptide synthetase (NRPS) genes. Peptaiols, which disrupt osmoregulation by forming ion channels through lipid bilayers, have antibiotic and antifungal activity and are best described in Trichoderma spp. E. ophioglissoides and its beetl-pathogenic congener, T. inflatum, both possess three putative peptaiol synthetases which we identified through analysis of NRPS adenylation domains. Of the three peptaiol-specific domain clades, one is predicted to encode for the nonproteinogenic a-aminosubutryic acid residues. We also show that, despite being very closely related, E. ophioglissoides and T. inflatum each possess three different peptaiol-like genes, only two of which appear to be located in syntenic regions. The current distribution of fungi possessing peptaiol genes is restricted to mycoparasitic lineages of Hypocreales and is generating hypotheses about the role of secondary metabolites in mycoparasitism.

321. Genome and transcriptome sequence of the apomictic fungus Arnium arizonense (Podospora arizonensis). E. Coppin1,2, C. Drevet1, L. Peraza-Reyes1,2, D. Zickler1,2, E. Espagne1, J. Ait-Benkhali1,2, P. Sila1,2,4, A. E. Bell5, D. P. Mahoney5, R. Debuchy1,2. 1) Univ Paris-Sud, Institut de Génétique et Microbiologie, Orsay, France; 2) CNRS, Institut de Génétique et Microbiologie, Orsay, France; 3) Univ Paris-Sud, épitobioinformatics plateform, Orsay France; 4) UFR des Sciences du Vivant, Université Paris 7 Diderot, Paris, France; 5) Private Mycological Research, 45 Gurney Road, Lower Hutt, New Zealand.

The homothallic fungus Arnium arizonense is closely related to the heterothallic Podospora anserina but displays several unique features. It is apomorphic, i.e. dikaryotic croziers are formed inside the perithecia but neither karyogamy nor meiosis take place in the asci, although morphological changes in both chromosomes and spindle pole bodies are reminiscent of those associated with meiosis in heteromictic Pezizomycotina. Instead of meiosis, the two nuclei undergo two mitoses and the resulting eight nuclei are enclosed in uninucleate ascospores, among which four mature normally, and four abort. Arrangement of the two ascospore types in individual asci is random (Mainwaring and Wilson, 1968, Trans Br mycol Soc, 51, 663). A. arizonense has two chromosomes, while most fungi in this group have seven chromosomes. Analysis of the genome sequence revealed that A. arizonense contained linked counterparts of the P. anserina mating-type genes, a structure that is typical of homothallic life style. Deletion of the mating-type locus resulted in the loss of perithecum formation, thus confirming the role of the mating-type genes in the fruit-body development. Genome annotation identified 11,165 genes, of which 476 undergo alternative splicing. Comparison of A. arizonense proteins with their orthologs in P. anserina revealed that A. arizonense genome contains numerous pseudogenes. Direction for future work is to determine how apomixis takes place, as this process of asexual reproduction provides potential evolutionary applications in agriculture by allowing perpetuation of any important selected heterozygous genotype (reviewed by Oizas-Akins and van Dijk, 2007, Ann Rev Genet, 41, 509-537).

322. Role of MAP kinase pathways in the pathogenicity of the wheat pathogen Mycosphaerella graminicola. Elisabetta Marchegiani1, Julie Vallet1, Slain Deller1, Marc-Henri Lebrun1. 1) Biogeo, INRA, Thiverval-Grignon, France; 2) Syngenta Limited, European Regional Centre, Priestley Road, Surrey Research Park, Guildford, Surrey, GU2 7YH, United Kingdom.

Mitogen-activated protein kinases (MAPKs) are essential components of fungal signaling pathways involved in different developmental processes and are required for host plant infection. Mycosphaerella graminicola, the causal agent of Septoria tritici leaf blotch (STB) of wheat, has three MAPK pathways that are all required for infection (MgfUS3, MghG1, MgSLT2; Cousin et al., 2006; Mehrabi et al., 2006a, Mehrabi et al., 2006b). We showed that MgfUS3 null mutants are non-pathogenic on intact wheat leaves (paintbrush inoculation), but highly-reduced in pathogenicity when infiltrated into leaf tissues by syringe injection (reduced necrosis, low number of pycnidia). This suggests that MgfUS3 is involved in fungal penetration, host colonization and pycnidia formation. Mhag1 null mutants have pathogenicity defects similar to Mgfus3 null mutants. This result highlights that the role of HOG1 in pathogenicity on plants differs among fungi (Segmüller et al., 2007). MgSlt2 null mutants are fully non-pathogenic on inoculated wheat leaves either by paintbrush inoculation or injection. This phenotype is unusual among slt2 null mutants from other fungi. Therefore, Mycosphaerella graminicola MAPK pathways may have evolved to control regulatory networks differing from other fungal plant pathogens. To identify which genes are under the control of the MgSLT2 signaling pathway, we are developing different transcriptomics analyses. Expression profiling relies on the comparison of transcriptomes of MgSlt2 null mutants and wild type strains grown under conditions corresponding to either an active or an inactive SLT2 pathway. Additional transcriptomics analyses will be performed using an allele encoding a conditional activation MAPK expression under the control of an inducible/repressible promoter. Genes whose expression requires an active SLT2 MAPK will be further studied for their role in development and infection using reverse genetics. Cousin et al. (2006), Molecular Plant Pathology 7(4): 269-278. Mehrabi et al; (2006a), Molecular Plant-Microbe Interactions 19(4): 389-398; Mehrabi et al. (2006b), Molecular Plant-Microbe Interactions 19(11): 1262-1269; Segmüller et al. (2007), Eucaryotic Cell 6(2) 211-221.

323. WITHDRAWN

324. Ancient and abundant MITEs in epheloeae genomes. Damien Fleetwood1, Chris Scharf2, Carolyn Young3. 1) Forage Improvement Section, AgResearch, Auckland, New Zealand; 2) Dept of Plant Pathology, University of Kentucky, Lexington, KY; 3) Forage Improvement Division, Samuel Roberts Noble Foundation, Ardmore, OK.
The *Epichloë festucae* genome contains thirteen known degenerate miniature inverted repeat transposable element (MITE) families that make up almost 1% of the genome. Recent sequencing of a range of *Epichloë* and related Clavicipitaceae family genomes revealed that every *MITE* family was active early in the evolution of the *Epichloë* lineage although none are found in other closely related genera. Analysis of *MITE* integration sites showed that these elements have a target integration site preference for 5' genic regions of the *E. festucae* genome and are particularly enriched within alkaid gene clusters and within 10-kb of other NRPS and PKS genes. Very few individual insertion sites are apparently shared among different species although one ancestral insertion - three adjacent EFT-3m/Toru elements in the ergot alkaid synthesis cluster - has mediated recombination events that in one strain may have abolished synthesis of this bioprotective alkaloid. Overall these results suggest a potential role for *MITEs* in the evolution of the *Epichloë* and their symbiotic associations with plants.

325. Exploring the biomass modifying enzymes of new filamentous fungal isolates from Vietnam, using secretome and transcriptome analyses. George E Anastontzis1,2, Thanh Dang Tat1, Thuy Nguyen Thanh2, Hang Dinh Thi My1, Thanh Vu Nguyen1, Lisbeth Olsson1,2. 1) Industrial Biotechnology, Chalmers University of Technology, Gothenburg, Västra Götaland, Sweden; 2) Department of Microbiology, IFI - Food Industries Research Institute, Hanoi, Vietnam; 3) Wallenberg Wood Science Center, Chalmers, Gothenburg, Sweden.

In the bio-based economy concept, the current hydrocarbon fuels and non-biodegradable plastics will be replaced by new products which will derive from natural and renewable resources. The synthesis of such biofuels and biochemicals is still challenged by the difficulties to cost efficiently degrade lignocellulosic materials to fermentable sugars or to isolate the intact polymers. Biomass degrading and modifying enzymes play an integral role both in the separation of the polymers from the wood network, as well as in subsequent modifications, prior to further product development. The type of application usually defines the conditions where the reactions should take place. Thus, novel enzymes with variable combined properties, such as different thermostability, pH range of activity, substrate specificity and solvent tolerance, still need to be discovered and developed to achieve the highest possible efficiency in each occasion. We took advantage of the rapidly evolving and high biodiversity of the tropics and have been screening various isolates for their cellulases and hemicellulases activities. Promising strains were then cultivated in bioreactors with different carbon sources, such as wheat bran, spruce and avicel and their biomass degrading capacity was analysed through cross species protein identification of their secretome with iTRAQ. Information on the genes involved in the different stages of the fermentation and the carbon source are being acquired with next generation sequencing of the total transcriptome. Interesting transcripts will then be used to heterologously clone and express the respective genes and identify their role in the degradation process.

326. *Fusarium* Comparative Transcriptomics and Transcriptional Regulatory Network Reconstruction. L. Guo1, G. Zhao1, X. Zhao1, W. Jonkers2, L. Gao1, J. Xu1, C.H. Kistler2, L. Ma1. 1) Comparative Fungal Genomics Laboratory, University of Massachusetts Amherst, Amherst, MA; 2) Department of Electrical & Computer Engineering, University of Massachusetts Amherst, Amherst, MA; 3) Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN; 4) USDA-ARS, Cereal Disease Laboratory, St Paul, MN.

Genus *Fusarium* contains pathogens that infect hundreds of crop plants as well as humans and thus threatens global food safety and human health. As in other cellular organisms, diseases caused by this group of organisms are dynamically controlled through their transcriptional regulatory networks (TRNs). Reconstructing their TRNs will not only help us to comprehend the complexity of their cellular functions, but will also have broad implications for disease management and prevention. A robust searching algorithm using Bayesian networks model was developed based on nearly 200 gene expression datasets of *F. graminearum*. The algorithm infers the relationship between candidate regulators (transcription factors and signaling proteins) and the target genes regulated by them. Preliminary validation of the inferred network using prior biological knowledge proofs the effectiveness of the program.

Using comparative functional genomics approach, we have analyzed the microarray-based transcriptome data of *F. graminearum* (PH1), *F. verticillioides* (7600) and *F. oxysporum* f.sp. lycopersici (4287) in response to carbon (C) and nitrogen (N) starvation. In agreement with previous studies, under C and N starvation, fungal cells adjust to extreme environments via modulating expression of core orthologous genes to enhance cellulose degradation processes and identify their role in the degradation process.

327. The mycorrhizal genome initiative (MGI): Identification of symbiosis-regulated genes by using RNA-Seq. A. Kohler1, E. Tisserant1, E. Morin1, C. Veneault-Fourrey2, S. Abba3, F. Buscot3, J. Dore4, G. Gay5, M. Girlanda6, S. Herrmann1, T. Johansson1, U. Lahrmann1, E. Martino6, S. Perotto6, M. Tarrka2, A. Tunlid2, A. Zuccaro2, I. Grigoriev1, F. Martin1. 1) Lab of Excellence ARBRE, Tree-Microbes Department, INRA-Nancy, Champenoux, France; 2) Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Torino, Italy; 3) Department Soil Ecology, UFZ Centre for Environmental Research Leipzig-Halle Ltd., Halle, Germany; 4) Ecologie Microbienne UMR CNRS 5557, USC INRA 1193, Universite Claude Bernard Lyon 1, Villeurbanne, France; 5) Microbial Ecology, Lunds University, Lund, Sweden; 6) Max-Planck Institute for Terrestrial Microbiology, Marburg, Germany; 7) DOE Joint Genome Institute, Walnut Creek, California, USA.

Genome and transcriptome analyses of *Laccaria bicolor* and *Tuber melanosporum* (Martin et al., 2008, 2010) revealed that the ectomycorrhizal symbiosis probably developed several times during evolution by generating different ‘symbiosis molecular toolkits’. In *L. bicolor* a large set of small-secreted proteins acts as putative effectors but not in *T. melanosporum*, while the up-regulation of transporter-coding genes seems to be a common feature of both interactions. To better understand the evolutionary origin of mycorrhizal symbiosis and to elucidate the molecular mechanisms involved, a large sequencing project of species from different taxa, phylogenetic clades and symbiotic lifestyles (ectomycorrhizae, ericoid and orchid mycorrhizae) was started in 2011 by the Joint Genome Institute and the mycorrhizal genome initiative. To identify and to compare symbiosis-regulated genes large scale illumina transcriptome sequencing of mycelium and mycorrhiza roots from *Paxillus involutus, Piloderma croceum, Helbrooma cylindrosporum*, *Sebacina vermifera*, *Tulasnella calospora* and *Oidiodendron maius* was performed. Small-secreted proteins, transporters, CAZymes but also many lineage specific proteins were among the highly up-regulated transcripts.


328. Transcriptome, secreted enzymes and systematics of the white rot basidiomycete *Phlebia radiata*. Jaana Kuuskert1, Miia Mäkelä1, Kristiina Hildén1.
FULL POSTER SESSION ABSTRACTS

Pia Laine1, Lars Paulin1, Taina Lundell1, 1) Department of Food and Environmental Sciences, Division of Microbiology, Fungal Biotechnology Laboratory; 2) Institute of Biotechnology, DNA Sequencing and Genomics Laboratory, Viikki Campus, University of Helsinki, FINLAND.

The efficient wood-degrading white-rot basidiomycete Phlebia radiata Fr. is able to degrade all the main components of lignocellulose and secretes a repertoire of lignin-converting peroxidases and oxidasess as well as carbohydrate-acting enzymes. P. radiata is the second species of the genus Phlebia to be genome sequenced, thus giving more insight into to the phlebioid clade of the order Polyporales, class Agaricomycetes, and comparative data for functional analyses on white-rot fungal mechanisms of wood decay and decomposition of lignin. We carried out transcriptome sequencing and secretome analyses on the Finnish isolate 79. Molecular systematics of the genus Phlebia was inferred by performing a four-genome study on North-European Phlebia spp. isolates including 10 species. Ribosomal RNA-encoding (SSU 18S rDNA; ITS1-5.8S-ITS2; ITS2-28S) regions and two protein-coding genes (gapdh, rpb2) were partially PCR-amplified with fungal or basidiomycete-specific primer pairs. Phylogenetic sequence analyses resulted with no single taxonomic cluster of Phlebia. The genus is obviously polyphyletic, and the various species were scattered together with other genera of Polyporales, like Phanerochaete and Rhizoctocha, in the families Corticiaceae and Meruliaceae. P. radiata was the most related with Phlebia aerina and P. rufa along with P. tremellosa and P. brevispora whereas P. subserialis and Phlebiopsis gigantea are more distant. P. radiata transcriptome analysis resulted with 6,980 unique gene transcripts, and similarity searches with blastx (E-value cut-off 1e-6) matched 77% of the unique transcripts to known or predicted protein-coding gene sequences.

Functional annotation assigned Gene Ontology term for 54% of the gene transcripts. Most of the genes were annotated with term nucleotide binding whereas 7% were oxidoreductases. Among these were several lignin-modifying enzymes (class II heme-including peroxidases and laccases). From the dataset, 16% of the gene transcripts were unknown thus representing proteins potentially unique to P. radiata. Also, the mitochondrial genome of over 150 kb in size shows unique features and high degree of genetic flexibility. These results provide an insight into gene content of P. radiata and genome-level transcriptional information on fungal genetic machinery for growth on complex liquid media.

329. Characterization of molecular mechanisms underlying the multi-drug-resistant phenotypes of Mycosphaerella graminicola field isolates. Selim Omran1,2, Anne-Sophie Walker1, Hind Sghyer1, Catherine Lanen1, Lamia Aouni1, Gert Keema1, Sabine Filling1, 1) BIOGER, INRA, Thiverval-Grignon, France; 2) Plant Research International, Wageningen University, Wageningen, The Netherlands.

Multidrug resistance (MDR) is a common trait developed by many organisms to counteract chemicals and/or drugs used against them. The basic MDR mechanism is relying on an overexpressed efflux transport system that actively expels the toxic agent outside the cell. In fungi, MDR (or PDR) has been extensively studied in Saccharomyces cerevisiae and Candida albicans, but also plant pathogenic fungi, e.g., Botrytis cinerea, Oculimacula yallundae and Mycosphaerella graminicola are concerned by this phenomenon. In agriculture, it is currently under investigation if MDR strains may threaten the efficacy of current fungicide treatments. MDR strains were detected in seiptoria leaf blotch (M. graminicola) field populations since 2008. These strains are slightly more resistant to DMl (inhibitor of the sterol 14 a-demethylase) fungicides than comparable cyp51 genotypes and cross-resistant to fungicides with different modes of action. The identification of the molecular mechanism explaining the MDR phenotype in two isolated strains (MDR6 and MDR7) was the main goal of this study. By the use of C14-prochiorzor, a DMl, we demonstrated increased fungicide efflux in both MDR strains in comparison to sensitive strains. RNA-sequencing led to the identification of several overexpressed transporter genes, out of which one MFS (major facilitator family) transporter had particularly abundant mRNA in both MDR strains. Crosses between both MDR strains showed that mdr6 and mdr7 loci are closely linked. We applied bulk-progeny sequencing to progeny of the crosses MDR6 x sensitive and MDR7 x sensitive in order to map the genomic regions co-segregating with the MDR phenotypes. SNP frequency analysis in sensitive and resistant bulks showed a clear co-segregation between phenotypes and the left arm of chromosome 7. This region harbors a gene cluster including the MFS transporter gene mentioned above. After sequencing the mfs promoter, we identified a 514 bp insertion in both MDR strains. Further studies are needed to validate the role of this insertion leading putatively to mfs overexpression and to clarify its relation to the MDR phenotype in the two studied strains. Financial support: Avrisal Institut du Vegetal, BASF Agro SAS, Bayer SAS, DuPont de Nemours SAS, Syngenta Crop Protection AG.

330. Candidate pathogenesis gene identification via Ustilago maydis ‘first gen’ genomic analyses. M.E. Donaldson1, S. Meng2, B.J. Saville1,2, 1) Environmental & Life Sciences, Trent University, Peterborough, Ontario, Canada; 2) Lineberger Comprehensive Cancer Center, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, N.C., USA; 3) Forensic Science Program, Trent University, Peterborough, ON, Canada.

Three different approaches were used to identify candidate pathogenesis genes for the model plant pathogen Ustilago maydis (DC) Corda: 1) suppressive subtractive hybridization (SSH) cDNA library analysis, 2) a bioinformatics approach, selecting genes unique among pathogenic basidiomycete fungi, and 3) microarray hybridization analyses. The SSH cDNA library was constructed to capture subtractive hybridization (SSH) cDNA library analysis, 2) a bioinformatics approach, selecting genes unique among pathogenic basidiomycete fungi, and 3) microarray hybridization analyses. The SSH cDNA library was constructed to capture U. maydis genes expressed in planta, as well as Zea mays genes up-regulated during the infection process. The resulting ESTs represented 23 U. maydis, and 159 Zea mays transcripts, respectively. Analysis of the U. maydis transcripts revealed 14 genes which have been previously characterized, 8 genes of unknown function, and 1 putative non-coding RNA. The fact that not all of the transcripts code secreted proteins is consistent with the observation by others that not all U. maydis effectors have recognizable secretion signals. RT-PCR results supported in planta transcript levels for a subset these genes. To evaluate the three strategies, one gene from each series of experiments was chosen for selective gene deletion experiments. Deletion strains were created for: 1) a hypothetical gene (um03046) that had high representation in the SSH cDNA library, 2) a conserved hypothetical gene (um02632) unique among basidiomycetes, and 3) the calcineurin B regulatory subunit (cncb, um01226), identified through microarray hybridization two-fold more highly expressed in the dikaryotic filamentous growth form compared to the diploid filamentous form. All U. maydis deletion strains were capable of mating on DCM medium containing charcoal. Mutant U. maydis clones disrupted for um03046 and um01632 did not show aberrant growth phenotypes; and the morphology of these cells was indistinguishable from wild-type strains. In contrast, the cncb mutants did not appear to separate after budding. In pathogenesis assays, the um03046 and um01632 mutants were slightly more, or less pathogenic than wild-type infections, respectively. Results for the cncb mutants were more striking, with a marked 77% decrease in the disease index, compared to wild-type infections. Together, these results support SSH cDNA library and microarray hybridization analyses as useful tools in identifying genes expressed during specific stages of in planta development and those genes involved in pathogenesis.

331. A biocontrol agent among wild-types: How Pseudozyma flocculosa genome relates to singular lifestyle. F. Lefebvre1, D.L. Joly2, G. Bakkeren2, F. Belisle3, R.R. Belanger1, 1) Centre de recherche en horticulture, Département de phytologie, Université Laval, Québec, QC, Canada; 2) Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, BC, Canada; 3) Institut de biologie intégrative et des systèmes, Département de phytologie, Université Laval, Québec, QC, Canada.

Most fungal species belonging to the Ustilaginales are well known for their pathogenic activity towards a variety of plant species. One interesting exception is Pseudozyma flocculosa that rather acts as a biocontrol agent against powdery mildews. In order to better understand the factors underlying these opposed lifestyles among closely related organisms, the genome of P. flocculosa was first sequenced and annotated on the basis of homology to
known protein sequences, RNA-Seq data and ab initio predictors. Then, based on comparative genomics with the pathogenic species *Ustilago maydis*, *Ustilago hordei* and *Sporisorium reilianum*, we identified key features that could explain both the avirulent nature of *P. flocculosa* toward plants and the virulent features of the pathogenic Ustilaginales.

First, the genome structural annotation highlighted similarities in total gene number, gene density and average gene length. But these similarities hide major differences with regards to average intron per gene and GC content. In fact, *P. flocculosa* has about 4 times more introns than *U. maydis* and has a very high GC content of 65.1%. Moreover, *P. flocculosa* genome shows a lower level of synteny than pathogenic species compared together.

Second, comparison of gene content revealed unexpected results. On one hand, the genome of *P. flocculosa* harbor many traits usually associated with pathogenic species like plant cell-wall degrading enzymes and genes involved in the synthesis of secondary metabolites that are highly conserved compared to pathogenic species. On the other hand, genes coding for candidate secreted effector proteins (CSEPs) show a much lower level of conservation, that seem to explain, in part, the differences in lifestyle.

Finally, this work led to the identification of certain of the most interesting genetic features in the study of pathogens and biocontrol agents.

**332. A tale of two poplar pathogens - Moving from sequence to function.** B. Dhillon1, N. Feau1, P. Tanguay1, M. Sakalidis1, S. Beausiegle1, R. Ohm1, A. Aerts1, I. Grigoriev1, G. H. J. Kema1, S. B. Goodwin1, R. Hamelin1. 1) Forest Sciences, University of British Columbia, Vancouver, BC, Canada; 2) CFS Laurentian Forestry Centre, Succ. Sainte-Foy, Québec, Canada; 3) DOE Joint Genome Institute, Walnut Creek, California, USA, DOE, USA; 4) Plant Research International B.V., Wageningen, The Netherlands; 5) USDA-Agricultural Research Service, Purdue University, West Lafayette, Indiana, USA.

Two closely related, morphologically indistinct fungal pathogens of poplars, *Mycosphaerella populorum* and *M. populicola* are prevalent in North America. In natural stands, these two fungal species closely follow the distribution of their host, with *M. populicola* being found on *Aigeiros* botanical section and *M. populicola* on the *Tacamahaca* section of poplars. Epidemiologically, *M. populicola* is considered to be more aggressive, as in addition to leaf-spots, it has the ability to infect woody tissue and cause cankers, that cankers *M. populicola* lacks. Moreover, introduction of hybrid plants has added to *M. populicola* host range. Availability of genomes will allow us a window into understanding the genetic basis for these observed differences in epidemiology and host-specificity for these two pathogens. Historical observation of host-specificity was confirmed by comparative sequence analysis the estimated the divergence time between the two poplar pathogens to be ~6.4 Mya, which agrees with the divergence time estimates for the poplar botanical sections (6.8 - 7.8 Mya). Despite the remarkable macro-synteny exhibited between these two recently diverged pathogens, several genes specific to each pathogen were identified in genomic regions where synteny broke down. In addition to being candidates for the different physiological and epidemiological attributes, these species-specific genes could be utilized for diagnostic and monitoring assays. A consistent expansion of several pathogenicity-related gene families was observed in *M. populicola*, suggesting a role for gene-dosage in determining its ability to cause cankers. Preliminary enzyme assays showed significant differences in beta-glucosidase and xylanase activities between these two fungi.

**333. Defining Open Chromatin Regions in Coprinopsis cinerea Oidia by FAIRE.** Virginia K. Hench1,2, Patricia J. Pukkila1,2. 1) Department of Biology, University of North Carolina at Chapel Hill, NC 27599; 2) Office for Undergraduate Research, University of North Carolina at Chapel Hill, NC 27599.

Changes in chromatin organization are principal regulatory mechanisms controlling multiple cellular processes including gene expression and meiotic crossover formation. Here we present FAIRE (formaldehyde assisted isolation of regulatory elements) data that reveals regions of open chromatin in *Coprinopsis cinerea* oidia, the asexual spore stage of the *C. cinerea* life cycle. A standard FAIRE protocol was developed and optimized for oidia and used to enrich for nucleosome-free stretches of chromatin. FAIRE peaks were identified from single-end read whole genome sequence data using ZINBA (Zero-Inflated Negative Binomial Algorithm), which identified 7,276 peaks covering 6.3% of the genome. FAIRE peaks are predominantly intergenic with 78% of FAIRE domains overlapping noncoding sequence. The peak widths range from 98-1390 bps, with an average width of 310 bps. Nearly half or 47% of annotated genes (Broad version 3) contain a FAIRE peak in the proximal promoter region (defined as 500 bps immediately upstream of the gene start). Differential transcription has been characterized throughout the synchronous meiotic process in *C. cinerea* (Burns, C. et al., PLOS Genetics, vol 6, issue 9, 2010), but the extent to which nucleosome occupancy might contribute to gene regulation in this multicellular fungus was not known. We found that a minority of meiotic specific (MS; genes expressed in meiosis and not in vegetative tissue) genes had promoter FAIRE peaks (of 819 genes 37% had promoter FAIRE peaks). In contrast, 61% of genes significantly changing during meiosis (SCDM, 2,455 genes) had promoter FAIRE peaks. Out of 295 genes that were MS and SCDM, 38% had promoter FAIRE peaks in oidia. Genes with known meiotic function including spo11, dmc1, and rec8 were amongst the MS/SCDM genes that did not have promoter FAIRE peaks in oidia. In summary, all examined meiotic gene sets included genes associated with and without FAIRE peaks in their promoter regions, indicating that complex gene regulation mechanisms contribute to differential, tissue-specific gene expression in *C. cinerea*. Supported by the U.S. Department of Energy Joint Genome Institute Community Sequencing Program. The work conducted by the U.S. DOE JGI is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

**334. Ensembl Fungi - genome-scale data portal from fungal species.** Uma Maheswari, Heldér Pedro, Mark McDowall, Daniel M. Staines, Paul Kersey. European Bioinformatics Institute (EMBL-EBI), Cambridge, United kingdom.

Ensembl Fungi (http://fungi.ensembl.org) is a portal offering access to genome-scale data from fungal species, using the Ensembl genome analysis system, through a common set of interfaces shared with non-fungal species also represented in the Ensembl system. These include a web-based genome browser, Perl and RESTful APIs, a public MySQL server and a query-oriented data warehouse (BioMart). The current release (January 2013) provides access to 36 fungal genomes across 12 different taxonomic orders, including the model species *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, (for which data are imported from the Saccharomyces Genome Database and PomBase respectively) but focuses mainly on plant pathogen species: genomic data from these is being integrated with information about infectious phenotypes (derived from PHI-base (http://www.phi-base.org) on a per-gene basis, thro ugh a new targeted resource PhytoPath (http://www.phytopathdb.org).

Core data provided for all species includes genome sequence, sequence patterns, annotation of protein and non-coding genes and functional annotation imported from direct curation, UniProt and InterPro. Information about gene regulation, sequence variation, evolution and conservation is also integrated in the system. Protein alignments are used to reconstruct evolutionary trees and infer homology relationships, while pairwise alignments between DNA sequences are performed between closely related species. Genomic polymorphisms are presented in the context of the reference genome sequences of *Saccharomyces cerevisiae* and the phytopathogens *Gibberella zeae*, *Puccinia graminis* and *Fusarium oxysporum*.

Ensembl Fungi will continue to expand with the increase in genomic data, we seek to work with the communities actively generating and using data, and are participants in a growing range of collaborations involved in the annotation and analysis of genomes.
335. A draft genome of the ectomycorrhizal fungus Rhizopogon vesiculosus: Characterization of mating system and heterozygosity within the dikaryon. Alia Muic, Joseph Spatafora. Botany and Plant Pathology, Oregon State University, Corvallis, OR.

Species of Rhizopogon are EM symbionts of trees in family Pinaceae and produce basidiospores within hypogeous false truffles that are dispersed by mycophagous mammals. All known members of R. subgenus Villoullsi form obligate EM relationships with Pseudotsuga spp. (Douglas Fir) and are the only members of the genus known to possess this host association. R. vesiculosus, along with its cryptic sister species R. vinicolor, possess a sympatric distribution where sampled within the range of their host tree, P. menziesii. While the sporocarp and EM morphology of these fungi may be highly similar; they possess striking life history differences with R. vesiculosus producing larger vegetative genets and displaying greater population structure at both local and landscape scales. We have sequenced the genome of R. vesiculosus using dikaryotic tissue and a whole genome shotgun sequencing approach on the Illumina HiSeq platform. De novo assembly of the genome was performed using VELVET 1.19 and gene predictions were made using AUGUSTUS with Laccaria bicolor as a training model. The draft genome assembled to a total length of 46 Mb in 6700 contigs with an N50 of 26,783, a maximum contig size of 446,818 bp, and 12,604 predicted genes. Here we characterize the mating system of R. vesiculosus, which possesses both an A-locus encoding a heterodimer transcription factor, as well as a B-locus encoding transmembrane pheromone receptors and pheromone precursor genes. We present comparisons of the mating system of R. vinicolor and its similarities to other members of Boletales (e.g., Serpula) and differences with Agaricales (e.g., Laccaria). Due to the dikaryotic nature of the genome sequence produced for R. vesiculosus, single nucleotide polymorphisms (SNPs) can be observed and used to characterize allelic variation. SNPs observed in protein coding regions of both MAT loci indicate that R. vesiculosus is likely heterothallic. We have also characterized heterozygosity across the whole genome in order to identify hypervariable regions. This genome will allow for comparative analysis of gene content, mating type system with other Basidiomycota and, ultimately, for population/species-level genomic studies within Rhizopogon.

336. Diverse Lifestyles and Strategies of Plant Pathogenesis Encoded in the Genomes of Eighteen Dothideomycetes Fungi. Robin A Ohm1, Nicolas Feau2, Bernard Henrissat3, Conrad L Schoch3, Benjamin A Howitz4, Rosie E Brashaw4, Lynda Ciuffetti4, Richard C Hamelin5,6, Gert Hj Kema7, Christopher Lawrence2, James A Scott3, Joseph W Spatafora3, B. Gillian Turecke7, Pierre JGM de Wit1, Shaobin Zhong8, Stephen B Goodwin9, Igor V Grigoriev1,2, Other members of the Dothideomycetes community. 1) United States Department of Energy (DOE) Joint Genome Institute (JGI), Walnut Creek, CA, United States of America; 2) Faculty of Forestry, Forest Sciences Centre, University of British Columbia, Vancouver, BC, Canada; 3) Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille Université, CNRS, Marseille, France; 4) NIH/NLM/NCBI, Bethesda, MD, United States of America; 5) Department of Biology, Technion - IIT, Haifa, Israel; 6) Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand; 7) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, United States of America; 8) Natural Resources Canada, Ste-Foy, QC, Canada; 9) Plant Research International, Wageningen, The Netherlands; 10) Virginia Bioinformatics Institute & Department of Biological Sciences, Blacksburg, VA, United States of America; 11) Division of Occupational & Environmental Health, Dalla Lana School of Public Health, University of Toronto, Toronto, Canada; 12) Department of Plant Pathology & Plant Microbiology, Cornell University, Ithaca, NY, United States of America; 13) Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands; 14) Department of Plant Pathology, North Dakota State University, Fargo, ND, United States of America; 15) United States Department of Agriculture, Agricultural Research Service, Purdue University, West Lafayette, Indiana, United States of America.

The class Dothideomycetes is one of the largest groups of fungi with a high level of ecological diversity including many plant pathogens infecting a broad range of hosts. Here, we compare genome features of 18 members of this class, including 6 necrotrophs, 9 (hemi)biotrophs and 3 saprotrophs, to analyze genome structure, evolution, and the diverse strategies of pathogenesis. The Dothideomycetes most likely evolved from a common ancestor more than 280 million years ago. The 18 genome sequences differ dramatically in size due to variation in repetitive content, but show much less variation in number of (core) genes. Gene order appears to have been rearranged mostly within chromosomal boundaries by multiple inversions, in extant genomes frequently demarcated by adjacent simple repeats. Several Dothideomycetes contain one or more gene-poor, transposable element (TE)-rich putatively dispensable chromosomes of unknown function. The 18 Dothideomycetes offer an extensive catalogue of genes involved in cellulose degradation, proteolysis, secondary metabolism, and cysteine-rich small secreted proteins. Ancestors of the two major orders of plant pathogens in the Dothideomycetes, the Capnodiales and Pleosporales, may have had different modes of pathogenesis, with the former having fewer of these genes than the latter. Many of these genes are enriched in proximity to transposable elements, suggesting faster evolution because of the effects of repeat induced point (RIP) mutations. A syntenic block of genes, including oxidoreductases, is conserved in most Dothideomycetes and upregulated during infection in L. maculans, suggesting a possible function in response to oxidative stress.

337. Domains of meiotic DNA recombination and gene conversion in Coprinopsis cinerea (Coprinus cinereus). Patricia J. Pukkila1, Wendy Schackwitz1. 1) Dept Biol, Univ North Carolina, Chapel Hill, NC, USA; 2) US DOE Joint Genome Institute, Walnut Creek, CA, USA.

We have previously shown that rates of meiotic recombination are highly non-uniform along the assembled chromosomes of C. cinerea (Stajich et al. PNAS 107: 11889-11894, 2010). That study revealed an over-representation of paralogous multicopy genes in regions with elevated levels of meiotic exchange. In addition, retrotransposon-related sequences were not found in large segments of the genome with low levels of meiotic exchange. However, the study was limited by the available markers, and only 31 Mb of the 36 Mb genome could be mapped. More recently, we have resequenced 45 meiotic segregants and 4 complete tetradets. We developed a simple script to detect crossover and gene conversion events involving over 75,000 SNPs spanning 35 Mb. The data were analyzed using MSTmap (Wu et al. PLoS Genetics 4: e1000212, 2008). The new dataset revealed sub-telomeric recombination hotspots at every chromosome end, and 36% of the crossovers were associated with uninterrupted tracts of gene conversion. The conversion tracts (2-8 SNPs) were quite short (8-219 nt), and the median distance between the flanking SNP markers was also small (500 nt). Since these subtelomeric hotspots correspond to sites of synaptic initiation in C. cinerea (Holm et al. Carlsberg Res. Commun. 46: 305-346, 1981) and other genomes, these data may contribute to our understanding of how homologous chromosome pairing and synopsis are coordinated with meiotic recombination. Supported by the U.S. Department of Energy Joint Genome Institute Community Sequencing Program. The work conducted by the U.S. DOE JGI is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.
339. **Letters from the front:** The *Microbotryum violaceum* genome and transcriptome project. *Su San Toh*¹, *Jared Andrews*¹, *Sebastien Duplessis*², *David Treves*¹, *Christina Cuoio*³, *David Schultz*², *Michael Perlin*¹. ¹University of Louisville, Louisville, KY, USA; ²Centre INRA de Nancy, Champenoux, France; ³Indiana University Southeast, New Albany, Indiana, USA; ⁴Broad Institute, Cambridge, Massachusetts, USA. *Microbotryum violaceum* is a fungal species complex that includes related smut species primarily infecting members of the Caryophyllaceae (pinks). Individual species of this group are limited to successional infection and reproduction on a specific host species. We have produced a draft sequence at 18x coverage for a haploid strain derived from meiosis of teliospores isolated from the host Silene latifolia. The draft sequence is currently in the process of annotation and is publicly available through a website at the Broad Institute. Using Illumina Next Gen sequencing, we are generating deep transcriptome information about a variety of stages in the lifecycle of the fungus, with particular emphasis on the late stages of infection, where teliosporegenesis occurs. Through the analysis we have performed so far, we were able to identify a suite of secreted proteins (SPs) that are potentially involved in host-pathogen interactions. Some of these include plant cell degradation enzymes like pectinesterase, laccase, subtilase and glycoside hydrolase. Moreover, some of these SPs are small, unique and cysteine-rich proteins, that might be involved in pathogenicity. Finally, since no reliable transformation system has been adapted for this fungus and, as a consequence, no targeted gene disruption has been demonstrated, we are developing constructs that rely on the newly completed genome to devise new strategies to allow such functional analyses in the future.

340. **The Aspergillus and Candida Genome Databases: Recent Developments and Future Plans.** *Martha B. Arnaud*¹, *Gustavo C. Cerqueira*², *Diane O. Ingles*³, *Marek S. Skrzypek*⁴, *Jonathan Binkley*¹, *Clinton Howarth*¹, *Prachi Shah*¹, *Farrell Wymore*¹, *Gail Binkley*¹, *Stuart R. Miyasato*¹, *Matt Simison*¹, *Gavin Sherlock*¹, *Jennifer Russo Woodward*¹. ¹Dep. of Genetics, Stanford University School of Medicine, Stanford, CA; ²Broad Institute, Cambridge, MA. *The Aspergillus and Candida Genome Databases* (AspGD, http://www.aspgd.org and CGD, http://www.candidagenome.org/) are freely available, web-based resources for researchers studying the molecular biology of these fungi. The interfaces of both web sites and databases now provide streamlined, ortholog-based navigation of the genomic and functional annotation for multiple species concurrently. We have completed manual curation of the published literature about multiple *Candida* and *Aspergillus* species. As part of our community-oriented mission, we also provide resources to foster interaction and dissemination of community information, tools, and data, including collecting, archiving, and providing large-scale datasets for download. AspGD also offers a full-featured genomics viewer to facilitate comparative genomics analysis. We have added new servers to improve web site performance and page loading speeds. Areas of future expansion include incorporation and curation of additional species, as well as improvements to the reference genome sequences and gene sets, utilizing high-throughput sequence to correct errors in sequence and gene structure, and display of additional regulatory elements and gene products, including alternate splice forms. We also plan to develop and incorporate improved tools for query, display and analysis of data, especially large-scale and comparative data such as gene synteny and the evolution of genes and gene substructure (e.g., intron gain and loss). We welcome, encourage, and appreciate your questions, feedback or suggestions. AspGD and CGD curators can be reached at aspergillus-curator@lists.stanford.edu and candida-curator@lists.stanford.edu, respectively. *AspGD* is funded by grant R01 AI077599 from the National Institute of Allergy and Infectious Diseases, and *CGD* is funded by R01 DE015873 from the National Institute of Dental and Craniofacial Research at the US National Institutes of Health.

341. **The Trichoderma reesei polyketide synthase gene pks1 is necessary for yellow-green pigmentation of conidia and is involved in the establishment of environmental fitness.** *Lea Atanasova*¹, *Benjamin P. Knox*², *Christian P. Kubicek*¹, *Scott E. Baker*², *Irina S. Druzhinina*¹. ¹Microbiology Group, Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, 1060 Vienna, Austria; ²Chemical and Biological Process Development Group, Pacific Northwest National Laboratory, Richland, WA, USA. The economically important genus *Trichoderma* (Hypocreales, Ascomycota, Dikarya) is well known for its mycotrophic lifestyle and for the broad range of biotrophic interactions with plants and animals. Moreover it contains several cosmopolitan species characterized by their outstanding environmental opportunism. These properties have given rise to the use of several species in agriculture as biopesticides and biofertilizers while *T. reesei* is applied for production of bioenergy-related enzymes. The molecular basis of the opportunistic success of *Trichoderma* is not yet well understood. While there is some evidence for a role of secreted enzymes and proteins, less is known about a possible role of secondary metabolites. Recently it was predicted that the PKS encoding gene pks1 from *T. reesei* and its orthologues are most likely responsible for the characteristic yellow-green pigmentation of conidia. To reveal the full function of the gene we deleted it from the wild-type strain QM 6a what resulted in complete loss of the green coloration of conidia. The ecophysiological profiling of Dpks1 showed that the gene is also involved in multiple functions at different stages of the *T. reesei* life cycle. Testing the antagonistic antifungal potential of the *T. reesei Dpks1* mutant against several host/prey fungi suggested that the loss of pks1 reduced the ability to combat them by means of both mechanisms: the pre-contact inhibition and direct overgrowth. However the overall analysis of mycoparasitic interactions suggests that the gene is most likely involved in protection against other fungi rather than in attacking them. Interestingly, we noticed the increased production of volatile compounds by the Dpks2 strains. The phenotype microarrays showed that PKS1 encoding gene restricts *T. reesei* from conidiation on a number of the best utilized carbon sources but does not influence the sexual development except the alternation of stroma pigmentation. The data for transcriptional response of genes putatively involved in above mentioned processes will be presented.
342. Functional Analysis of Genes in Regions of Introgression in Coccidioides. Bridget M. Barker. Immunology & Infectious Diseases, Montana State Univ, Bozeman, MT.

Coccidioides immitis and C. posadasii are dimorphic fungi endemic to the Americas. Genomic analysis of sequenced strains of C. posadasii and C. immitis reveals insights into the population biology of these organisms. There is strong evidence for hybridization and introgression, such that for many of the C. immitis strains, there are several regions that have a closer match to C. posadasii, but few regions within C. posadasii matching C. immitis. Multiple hybridization regions were located in several genomes analyzed, and at least one region containing ten genes exhibits a pattern consistent with introgression in C. immitis. This conserved region was further evaluated in a larger collection of isolates. Approximately half of the C. immitis isolates contain the C. posadasii fragment, and the majority of those are from the southern California and Mexico populations. The region of introgression represents a unique opportunity to functionally assess genes that are likely to be relevant for species-specific virulence and adaptation to mammalian hosts or the environment. This region has a shared recombination point flanking a metalloproteinase, Mep4, genes that are highly expressed in the parasitic phase; and genes of unknown function. Importantly, evolutionary selection has preserved this region in multiple strains of C. immitis further emphasizing the possible role in virulence of these genes. Variation among strains for virulence in murine models of coccidioidomycosis has been observed, but has not been tested in the context of the newly discovered species or with a targeted underlying genetic mechanism hypothesis to test. Gene deletion mutants are being generated for three genes in the conserved introgression region to determine effects on in vitro growth and morphological change under host relevant conditions.


Functional prediction of carbohydrate-active enzymes is difficult due to low sequence identity hampering recognition of the functional relationship. However, similar enzymes often share a few short motifs, e.g., around the active site even when the overall sequences are very different. To exploit this notion for functional prediction of carbohydrate-active enzymes we developed a simple algorithm, Peptide Pattern Recognition (PPR) that can divide proteins into groups of sequences that share a set of short conserved sequences. When this method was used on 118 functionally characterized GH5 proteins with 9% average pairwise identity and representing four enzymatic functions, 97% of the GH5 proteins were sorted into groups correlating with their enzymatic activity. Furthermore, we analyzed 8138 GH13 proteins including 204 experimentally characterized enzymes with 28 different functions. There was a 91% correlation between group and enzyme activity. These results indicate that the function of carbohydrate-active enzymes can be predicted with high precision by finding short conserved motifs in their sequences. The GH61 family is important for fungal biomass conversion but only few GH61s have been functionally characterized. Interestingly, PPR divided 743 GH61 proteins into 16 subfamilies useful for targeted investigation of the function of these proteins, and pinpointed three conserved motifs with putative importance for enzyme activity. The conserved sequences were useful for discovery and cloning of new, subfamily-specific GH61 proteins from 14 different fungi. In conclusion, identification of conserved sequence motifs is a new approach to sequence analysis that can predict carbohydrate-active enzyme functions with high precision. Furthermore, these motifs can be used to mine genomes and more complex data such as metagenomes and -transcriptomes for genes encoding proteins with specific, enzymatic activity.

344. The mechanism of intron-like element multiplication in fungi. Ate van der Burg1, Edouard Severing2, Valeria Ochoa Tufiño1, Pierre de Wit1, Jérôme Collemare1. 1) Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands; 2) Laboratory of Bioinformatics, Wageningen University, 6708PB Wageningen, The Netherlands.

The recent discovery of intron-like elements (ILEs) in six fungal species shed new light on the origin of regular spliceosomal introns (RSIs). ILEs are novel spliceosomal introns that are found in hundreds of near-identical copies in unrelated genes. They account for the vast majority of intron gains in these species and are not associated with intron losses. Remarkably, ILEs are longer than RSIs and harbor predicted stable secondary structures. However, they are prone to quickly degenerate in sequence and length to become undistinguishable from RSIs, suggesting that ILEs are predecessors of most RSIs. Further analyses are being performed in order to understand the multiplication mechanism of ILEs, which is hypothesized to resemble the retro-homing mechanism of self-splicing group II introns. The dynamics of ILE’s secondary structures could be predicted and two conserved motifs were identified in almost all fungal ILEs, which might play an important role in direct insertion into DNA. We also have developed a genetic screen in yeast in order to capture and characterize ILE insertion events. These ongoing studies should provide hints about the mechanism of ILE multiplication, i.e. how new spliceosomal introns are gained in fungi.

345. Fungi use prion forms for signal transduction processes involving STAND proteins. Asen Daskalov, Khalid Salamat, Sven J. Saupe. CNRS, IBGC UMR5095, BORDEAUX, AQUITAINE, France.

Prions are proteins embedding genetic information into their structural state. Generally, those proteins exist in a soluble state and sporadically as infectious amyloid aggregates. Podospora anserina’s [Het-s] is one of the best characterized fungal prions with a remarkably high prevalence in wild populations. [Het-s] functions in vegetative incompatibility - a biological process occurring during anastomosis between two genetically incompatible strains. The [Het-s] protein exists in a soluble state - [Het-s]+ - or can switch to an aggregated amyloid state - [Het-s] - the prion form. When an [Het-s] prion infected strain fuses with a strain expressing the alternative allelic variant of the [Het-s] locus - [Het-S] - a cell death reaction of the heterokaryon occurs. Recent studies shed light on the mechanism of [Het-s]/[Het-S] incompatibility reaction. Differing by 13 amino acids both proteins shares a two domain architecture; a globular N-terminal domain called HeLo and a C-terminal Prion Forming Domain (PFD). The latter is able to adopt a b-sheet rich conformation with a specific b-solenoid fold. It has been demonstrated that in presence of [Het-s] amyloid fibers [Het-S] turns into a pore-forming toxin: transconformation of the [Het-S] PFD by [Het-s] fibers triggers the refolding of the [Het-S] HeLo domain, inducing the cell death reaction. In an attempt to better characterize the conserved features of the [Het-s] b-solenoid fold and identify new distant homologues of [Het-S], we have generated a minimal consensus sequence motif of it. Surprisingly, the second best hit in a BLASTp search is in the N-terminal region (3-23) of the product encoded by NW2D2, the immediately adjacent gene to [Het-s]. NW2D is a STAND protein. STAND proteins form signal transducing hubs through oligomerization upon ligand recognition. That in mind and several bioinformatics observations led us to propose that [Het-S] and NW2D are functional partners in various filamentous fungal species using the amyloid fold in a signal transducing pathway. We will present experimental evidence that NW2D is able to trigger [Het-S] toxicity in much the same way as [Het-s] does. Further in silico analysis identify a number of these STAND/prion-like gene pairs and suggest that signal transduction through an amyloidial prion-like fold is a general widespread mechanism in fungi.

346. RNA silencing in poplar anthracnose fungus Colletotrichum gloeosporioides. Simeng Li, Yonglin Wang, Chengming Tian. The Academy of Forestry, Beijing Forestry University, Beijing, China.

Poplar anthracnose is one of the most destructive diseases on Populus sp, whose causal agent is Colletotrichum gloeosporioides. Although the fungus is a
347. Comparative Genomics of L and S Morphotypes of Aspergillus flavus. Mana Ohkura, Peter Cotty, Marc Orbach. Division of Plant Pathology, University of AZ, Tucson, AZ.

Aspergillus flavus is a widely distributed facultative pathogen of plants and animals and the most common causal agent of crop contamination with aflatoxins. Isolates of A. flavus vary widely in aflatoxin producing ability, ranging from atoxigenic to being capable of producing many mg/g. Variability in aflatoxin production makes specific attribution of etiology very complex. Aspergillus flavus exists in two morphotypes the large (L) and small (S) sclerotial producing strains. The S strains have consistent high aflatoxin-producing ability while the L strains vary greatly in toxin production with atoxigenic strains commonly found. Some atoxigenic strains are active ingredients in biocontrol products used commercially to prevent contamination. We are applying comparative genomics to L and S strains in an attempt to reveal clues to potential differential adaptations associated with the variation in aflatoxin-production between these morphotypes. In addition to aflatoxin producing potential, several characteristics diverge between the morphotypes including sclerotial morphology as well as spore and hydrolyase production and prevalence. We hypothesize there are genomic differences between the L and S morphotypes that reflect their divergent evolution leading to differential adaptation. To evaluate this, we have sequenced the genomes of three L morphotype and three S morphotype isolates from agricultural fields in Arizona belonging to 6 different genetically compatible groups. L and S strain isolates from across Arizona were selected. Strains were sequenced to ~40-45 X coverage on the Illumina HiSeq 2000 platform. The genomes were assembled de novo using VelvetOptimiser and gaps were filled using GapFiller. Preliminary assessment of the assemblies indicate there is ~90% syntenic coverage with the published genome of A. oryzae RIB40, a close relative of A. flavus. The genomes were annotated transversely with RATT using the genome of A. oryzae RIB40 as a reference. Comparisons of genome statistics, secondary metabolite clusters, and morphotype specific genes will be presented.


The genome of Coprinopsis cinereus contains both Class I and Class II repetitive elements, making up about 2.5% of the total genomic DNA. While bioinformatics techniques were used to identify and classify these elements based on sequence similarity, it is uncertain whether any of the elements are functional. Although the nature of repetitive elements is to expand their numbers within the genome, the genome acts to suppress activity through mutation and methylation. Detailed analysis to survey functionality and expression was conducted on two families of repetitive elements, the Gypsy-like retrotransposons (Class I) and the hAT Transposons (Class II). The potential for functionality was determined based on size, structure and flanking repeat sequence integrity. Evidence for expression was determined based on reviewing EST, SAGE and methylation data for these elements. Of the 31 largest gypsy elements analyzed, all appear to be inactive. However, one of the nine hAT elements appears to be structurally intact and shows evidence of expression. The dimerization domain of this element is being studied as a potential region to assay for activity.

349. Genome-wide analysis of small RNA machineries in fungal kingdom. Jiayao Wu1, Jaeyoung Choi2, Fred O. Asiegbu1, Jari P.T. Valkonen3, Yong-Hwan Lee4. 1) Forest Science, Helsinki University, Helsinki, Finland; 2) Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea.

RNA interference (RNAi) is a phenomenon widely conserved in eukaryotes to regulate gene expression through diverse pathways at transcriptional (TGS) or post-transcriptional level (PTGS). In fungi, the RNAi pathways are found with three major functions: genomic defence, heterochromatin formation, and gene regulation. The mechanisms of RNAi in fungi seem to be unique and highly differentiated from plant and animal kingdoms, although the core mechanisms are relatively similar. We identified 3 key genes such as Argonaute, Dicer and RNA-dependent RNA Polymerase (RdRP) in the pathway from 143 fungal and 66 other genomes. They were found in most genomes with very different gene numbers, while some of fungal genomes appear to be lack of all the components indicating the absent of the whole pathways. In general, fungi have the same domains in Argonautes with plant and animal, but longer in length in the number. Compared to plant and animal, fungi have more Dicers, but they do not contain PAZ domain, which is essential for RNAi in plant and animal. Phylogenetic analysis indicates that most fungal Argonautes belong to AGO-like subfamily. However, fungal Dicers could be divided into two subfamilies; one is closely related to plant and animal Dicers and the other only exists within fungal kingdom. Further analysis using codonW shows RNAi proteins are evolved into different subfamilies under natural selection not due to random mutation. Taken together fungi RNAi pathway is likely to be much complex than we expected with multiple functions in diverse regulatory pathways. All information on proteins analyzed is archived in Fungal Small RNA Machinery Database (http://funrna.icebergshot.snu.ac.kr/).

350. Comparative Analysis of Malassezia Mating Loci. Jun Xu1, Wenjun Li2, Anastasia Giotia3, Björn Nystedt4, Anna Averetec4, Charles Saunders4, Thomas Dawson5, Joseph Heitman6, Annika Schwenke7, 1) Procter & Gamble Co., Mason Business Center, USA; 2) Department of Molecular Genetics and Microbiology, Duke University Medical Center, USA; 3) Science for Life Laboratory, Translational Immunology Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden; 4) Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden; 5) Translational Immunology Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden.

Malassezia fungi are naturally found on the skin surfaces of many animals and are associated with skin disorders such as dandruff and atopic dermatitis/eczema. Whole genome sequence analysis showed that M. globosa has a bipolar mating type with two MAT alleles encoding the homeodomain (HD) and pheromone/receptor (P/R) loci separated by 167 kb of intervening sequence. We compared the M. globosa mating locus with the newly sequenced M. sympodialis genome. Our analysis showed that the M. sympodialis MAT region has extensive well-conserved synteny with M. globosa and the HD and P/R mating subloci are physically linked with a 141 kb separating the two. Interestingly, MAT sequences derived from a population of M. sympodialis isolates suggests that M. sympodialis does not fit a traditional bipolar or tetrapolar system. Instead, it is more similar to a pseudo-bipolar model previously reported for Sporidiobolus salmonicolor in which the HD and P/R genes are physically linked similar to bipolar mating type configurations.

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**Education and Professional Development**


This study was conducted to ascertain the effectiveness of the internet gaining students enrolled at college of education the scientific facts, concepts and disagreement about Biofuel issue according to the Responsibility Spiral model. The study used a quasi-experimental design. The study sample consisted of 76 students divided into two groups. The experimental group consisted of 37 students, whereas the control group consisted of 39 students. The study used materials and activities according to the Responsibility Spiral model. Each stages of the waks (1992) model consisted of objectives, content, teaching and learning activities and evaluation tools. The results showed statistically significant differences between the mean score of the experimental group which gained the scientific facts and concepts about Biofuel issue according to the Responsibility Spiral model and the mean score of the control group. The result showed that there is an opposite relationship between the model stages and t-test that if the model expands the value of t-test decreased.

Based on the results, the researcher recommends that the internet is not enough tools for advanced stages of the Responsibility Spiral model.

352. ComGen Authentic Research Experiences (C-ARE): Fungal genetic analysis. Gita Bangera1, Andrea Gargas2. 1) Bellevue College, Bellevue, WA, USA; 2) Symbiology LLC, Middleton, WI, USA.

ComGen (Community College Genomics Research Initiative) teaches students the skills of self-directed learning, critical thinking, and analysis. Community college students in this program receive a mini-graduate school experience, following a single requisite course in cell biology. Students work on original research projects, learn to troubleshoot their experiments, organize lab meetings and student journal clubs, and network within the scientific community. In one research track students work with DNA from described fungal collections, learning DNA-based techniques including PCR amplification, DNA sequencing and sequence analysis. Student-gathered sequence information is used to advance identification and phylogenetic results for these collections. With NSF Award DUE #1225857 ComGen (C-ARE): Dissemination, Enrichment and Expansion Project the project will be expanding to community college partners throughout the Seattle/Tacoma region of Washington State.

353. Facilitating an Interdisciplinary Learning Community Amongst Undergraduate Research Fellows By Emphasizing Scientific Inquiry as the Unifying Thread. Virginia K. Hench1,2, Patricia J. Pukkila1,2. 1) Department of Biology, University of North Carolina at Chapel Hill, NC 27599; 2) Office for Undergraduate Research, University of North Carolina at Chapel Hill, NC 27599.

The HHMI-Future Scientists and Clinicians (HHMI-FSC) fellowship is 1 of 3 components of the HHMI Science Learning Communities program at UNC Chapel Hill. The HHMI-FSC program was designed to foster an intellectual community that empowers high-ability students from low-income backgrounds to engage in biomedical research for 2 summers. Each year, 12 new fellows are matched with mentors in labs spanning a range of biomedical areas. They work fulltime in labs on their own research project and meet weekly as a group to engage in interactive programming that targets skills critical for success in science beyond the bench. One area of emphasis has been the process of inquiry itself. The goal is for students to transition from being a pair of hands executing protocols to active learners invested in their own projects and able to speak with authority about why experiments are performed in particular ways and what conclusions can be drawn from data generated. This starts with coaching students to state the questions that they are trying to answer and think through whether an experimental setup is consistent with what they say they are trying to find out. Assignments and feedback are designed to reinforce this principle. One of the most satisfying aspects of doing science is getting to follow one’s own instinctive curiosities and develop the methodologies needed to navigate new terrains. Undergraduates are usually still trying to define their own specific curiosities. Pushing students to describe what they are curious and passionate about is one feasible strategy that can help students identify pursuits that fit their interests and talents. Another successful strategy has been to require returning second year fellows to share science learning experiences via 15-30 minute long talks for their peers. Some took the opportunity to become more immersed in their lab’s focus, while others branched into questions like what motivates scientists to work in foreign countries and what has genonomic anthropology told us about human evolution. Project aims were developed through conversations between the fellow and instructor. The one constraint was for fellows to organize their presentations around questions. Feedback indicated that presenters benefited from having to give presentations and others enjoyed learning about a broader array of topics.

**Gene Regulation**

354. YAB-An Agrobacterium-based vector system for direct cloning of eukaryotic gene constructs via yeast recombination. M Alejandra Mandel, Marc J Orbach. School of Plant Sciences and The Bio Institute, University of Arizona, Tucson, AZ.

*Agrobacterium tumefaciens*-mediated transformation (ATMT) has become the preferred method to introduce modified genes in many fungal systems. Therefore, there is a need for a simple and efficient method to create gene constructs in an ATMT vector. Generation of ATMT constructs usually requires PCR-amplification of DNA fragments and multiple cloning steps into binary *Agrobacterium* vectors. Advances have been made with attB/attP-based systems (e.g. pTRoy, Gene-blast, DelsGate) that shorten the number of steps required for generating plasmid constructs, however these constructs require a final sub-cloning step into an *Agrobacterium* vector. One of these systems, OSCAR, combines PCR and attB/attP-based technology in an *Agrobacterium* platform. All of these methods require the use of commercial enzymes and usually involve one or more PCR amplification steps. There are also plasmid vectors that use yeast transformation-associated recombination (TAR) which do not require the use of enzymes for cloning, but can only be used to transform fungi via electroporation, protoplast transformation or biolistics. We present here YAB (for Yeast-*Agrobacterium*-Bacteria), a vector that combines the homologous recombination properties of yeast in an *Agrobacterium* vector backbone that can be used to create any kind of gene construct (e.g. gene deletion mutants, fluorescence-tagged genes, overexpression, RNAi) to directly transform fungi in one step. DNA fragments are cloned into YAB via recombination between 22-nucleotide compatible ends generated by PCR, or by using short oligomers that “bridge” the DNA fragment with the vector, thus avoiding the need for amplification steps. YAB-Hph has a 1.4-kb Hygromycin phosphotransferase cassette flanked by the left and right borders of the Ti plasmid. This vector was used to generate whole-gene deletion mutants by TAR in one step, that were directly introduced into fungi.

355. Removal of C4-methyl Sterol Accumulation in a SREBP-null Mutant of Aspergillus fumigatus Restores Hypoxia Growth. Sara J. Blosser1, Brittney Whitmire, Khoa K. Tran, Scott K. Alasn. 1) Department of Plant Science, New Mexico State University, Las Cruces, NM 88003.

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356. VeA Regulates Conidiation, Glutolixin Production and Protease Activity in the Opportunistic Human Pathogen Aspergillus fumigatus. Sourabh Dhingra1, David Andes2, Ana M. Calvo1. 1) Biological Sciences, Northern Illinois University, Dekalb, IL; 2) Medical Mycology and immunology, University of Wisconsin-Madison, Madison, WI.

Aspergillus fumigatus is the causative organism of invasive aspergillosis. Our study shows that normal levels of veA expression are necessary for wild-type morphological differentiation in this medically important fungus. Specifically, deletion or overexpression of veA reduce conidiation. Parallel, briA expression was also affected by alterations in veA transcription levels. In addition, our studies revealed that veA regulates glutolixin production in A. fumigatus. Glutolixin production was decreased in the deletion veA and over-expression veA strains, where gli2 and gli5 expression was altered. Interestingly, veA also controls hydrolytic enzyme activity in this human pathogen. Deletion of veA resulted in a reduction of protease activity; this is the first report of a veA homolog with a role in controlling fungal hydrolytic activity. Although veA affects several cellular processes in A. fumigatus, pathogenicity studies in a neutropenic mouse infection model indicated that veA is dispensable for virulence. More research will be conducted to study the effect of veA in non-neutropenic mice.

357. HapX C-terminal truncation impairs Aspergillus fumigatus iron homeostasis. Fabio Gsaller1, Veronika Klammer1, Beatrix E. Lechner1, Peter Hortschansky1, Axel A. Brakhage2, Ernst R. Werner1, Hubertus Haas1. 1) Division of Molecular Biology, Medical University of Innsbruck, Austria; 2) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany; 3) Division of Biological Chemistry, Medical University of Innsbruck, Austria.

The maintenance of iron homeostasis is indispensable as iron is essential for various cellular processes but can be toxic at iron excess. In A. fumigatus the bZIP-like transcription factor HapX is important for adaption to iron starvation and consequently virulence due to its role in repression of iron consuming pathways (i.e. heme biosynthesis, TCA cycle, respiration) and activation of iron uptake (i.e. siderophore biosynthesis and uptake, reductive iron assimilation). In this study we demonstrate that conditional hapX overexpression using the xylose-inducible xyIP promoter leads to repression of genes involved in iron consumption (i.e. heme biosynthetic hemA and leucine-biosynthetic leuA) and activation of iron acquisition-related genes (i.e. siderophore-biosynthetic siderC and siderophore transporter-encoding mirB) within one hour of induction. In agreement, elevated hapX expression decreased the cellular accumulation of protoporphyrin IX, the iron-free precursor of heme, and increased production of the extracellular siderophore TAF. HapX-truncation studies revealed that the C-terminal 93 amino acid residues are essential for its activating as well as repressing functions. HapX N-terminally tagged with Venus green fluorescent protein localized to the nucleus during iron starvation but was undetectable after an one hour-shift to iron sufficient. These data demonstrate tight regulation of hapX expression at the protein level as previously shown at the transcript level. Consistently, HapX-deficiency is detrimental only during iron limitation. Two hapX copies and in particular xyIP promoter-mediated overexpression of hapX caused growth defects independent of the iron availability, which underscores the importance of a precisely regulated HapX level. This work was supported by the Austrian Science Foundation grant FWF P21643-B11 to HH.

358. The CCAAT-Binding-Complex mediates Iron Regulation in Aspergillus fumigatus. Hubertus Haas1, Christoph Joechl1, Thorsten Heinekamp1, Ilse D. Jacobsen1, Markus Schrettl2, Axel A. Brakhage2, Lukas Schafferer1. 1) Division of Molecular Biology, Biocenter, Innsbruck Medical University, Austria; 2) Department for Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany.

Iron is essential for a wide range of cellular processes but its excess is toxic. Therefore, microorganisms evolved fine-tuned mechanisms for uptake and storage of iron. In the opportunistic fungal pathogen Aspergillus fumigatus, the bZIP-type transcription factor HapX mediates adaptation to iron starvation by activating siderophore biosynthesis and repressing iron-dependent pathways. HapX-deficiency attenuates the virulence of A. fumigatus underlining the importance of adaptation to iron starvation in pathogenicity. The HapX N-terminal amino acid sequence predicts interaction with the DNA-binding, heterotrimeric CCAAT-binding complex (CBC), which is conserved in all eukaryotes and believed to co-regulate up to 30% of all genes. Here, we characterized the role of the CBC in iron regulation of A. fumigatus by analysis of the phenotypic consequences of genetic inactivation of the CBC subunit HapC. HapC-deficiency was deleterious during both iron starvation as well as iron sufficient, demonstrating iron-dependent regulatory functions of the CBC. In contrast, HapX is important during iron starvation only. As shown previously for HapX-deficiency, HapC-deficiency derepressed genes involved in iron-consuming pathways during iron starvation but decreased siderophore metabolism at transcriptional and metabolic levels. Inhibition of reductive iron assimilation by ferrous iron chelation blocked colony formation of both HapC-deficient and HapX-deficient conidia. Moreover, inactivation of HapC was epistatic to HapX-deficiency. Taken together, these data indicate that the CBC mediates both the activating and the repressing functions of the iron-regulatory transcription factor HapX. The central role of the CBC in environmental adaptation is underlined by HapC-deficiency rendering A. fumigatus avirulent in a murine model of aspergillosis. This work was supported by the Austrian Science Foundation grant FWF 1282-809 to HH.

359. Protein kinase A signaling in Aspergillus fumigatus: Identification of downstream targets. Juliane Macheleit1,2, Wolfgang Schmidt-Heck2, Ilse D.
FULL POSTER SESSION ABSTRACTS

Jacobsen¹, Thorsten Heinkeamp¹, Axel A. Brakhage¹, Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Molecular and Applied Microbiology, Jena, Germany; 2) Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Systems Biology / Bioinformatics, Jena, Germany; 3) Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Microbial Pathogenicity Mechanisms, Jena, Germany; 4) Institute of Microbiology, Friedrich Schiller University, Jena, Germany.

Aspergillus fumigatus is a saprophytic mould normally inhabiting the soil. The fungus also represents a medically important pathogen causing severe systemic infections in immunocompromised patients. To survive in these entirely different habitats, A. fumigatus needs mechanisms to sense environmental signals and transduce them intracellularly. One of these signal transduction pathways is the cAMP dependent protein kinase A (PKA) pathway. For A. fumigatus, components of this signaling cascade have been characterized in detail and its significance for virulence is evident.

To identify target genes of PKA, we performed microarray analyses using a mutant strain overproducing the PKA catalytic subunit. Following this approach, 23 transcription factors potentially regulated by PKA were identified. From these, 15 were deleted and the mutant phenotypes were characterized. A gene encoding a C6 finger domain protein that showed highest upregulation of all identified transcription factors is located in a potential secondary metabolite gene cluster. Deletion of the transcription factor gene resulted in reduced growth and sporulation of the mutant strain. This phenotype was observed even more drastically for a strain lacking the nonribosomal peptide synthetase of the same cluster. Because genes of this cluster were shown to be transcribed in infected mouse lungs, a virulence study was performed using an embryonated egg infection model. However, the transcription factor deletion mutant showed no altered virulence compared to the corresponding wild type.

To get deeper insights into the function of the secondary metabolite gene cluster, the gene encoding the C6 finger domain protein was overexpressed using an inducible promoter. Overproduction of the transcription factor resulted in induced transcription of all cluster genes and furthermore in the formation of a brown substance which is currently under investigation.


The cell wall is essential for fungal survival in natural environments. Many fungal wall carbohydrates are absent from humans, so they are a promising source of antifungal drug targets. Galactofuranose (Gal-f) is a sugar that decorates certain carbohydrates and lipids. It comprises about 5% of the Aspergillus fumigatus cell wall, and may play a role in systemic aspergillosis. We are studying Aspergillus wall formation in the tractable model system, A. nidulans. Previously we showed single-gene deletions of three sequential A. nidulans Gal-f biosynthesis proteins each caused similar hyphal morphogenesis defects and 500-fold reduced colony growth and sporulation. Here, we controlled A. nidulans ugeA, ugmA or ugtA using the alcA(p) or niaI(p) promoter. For repression and expression, alcA(p)-regulated strains were grown on complete medium with glucose or threonine, whereas niaI(p)-regulated strains were grown on minimal medium with ammonium or nitrate. Expression was assessed by qPCR and colony phenotype. The alcA(p) and niaI(p) strains produced similar effects: colonies resembling wild type for gene expression, and resembling deletion strains for gene repression. Gal-f immunolocalization using the L10 monoclonal antibody showed that ugmA deletion and repression phenotypes correlated with loss of hyphal wall Gal-f. None of the gene manipulations affected itraconazole sensitivity, as expected. Deletion of any of ugmA, ugeA, ugtA, their repression by alcA(p) or niaI(p), OR, ugmA overexpression by alcA(p), increased sensitivity to Caspofungin. Strains with alcA(p)-mediated overexpression of ugeA and ugtA had lower caspofungin sensitivity. Gal-f appears to play an important role in A. nidulans growth and vigor. We are extending these studies to A. fumigatus UgmA and UgtA to determine which amino acids are critical for function and Gal-f generation. Constructs with mutated A. fumigatus sequences failed to rescue the AnugmD phenotype.

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Mutations in the cysB, sconB and sconC genes affect sulfur metabolism in Aspergillus nidulans in different ways. The cysB mutation blocks synthesis of cysteine and leads to a shortage of this amino acid which is toxic for the fungus. The sconB and sconC genes encode transcription factors that regulate expression of genes encoding enzymes that synthesize cysteine. We have identified 109 genes that are regulated by SconB and SconC and have used them to construct a gene expression map of the Aspergillus sulfur metabolism. The map shows that these genes are regulated in a coordinated manner, and that they are activated by two different mechanisms: an oxidative stress response and a transcriptional regulator that is activated by the cysB gene.


A large body of work has elucidated the mechanisms associated with mRNA degradation and translational repression - which processes are fundamental to biological systems. For the majority of eukaryotic transcripts, deadenylation is known to lead to transcript degradation and translational repression. However, the surveillance mechanism which determines the point at which functional transcripts are effectively switched off is poorly defined. This transition is generally associated with the mRNA being tagged at the 3' end with a short run of pyrimidine nucleotides. For example, cell cycle-regulated decapping and degradation of mammalian histone mRNA is triggered by uridylation. In fission yeast and the filamentous fungus, Aspergillus nidulans, polyadenylated transcripts are decapped and degraded in response to 3' tagging. In A. nidulans and Arabidopsis tagging involves the addition of a C/U rich element when the transcript's poly(A) tail is shortened to ~15 nucleotides. Disruption of the Aspergillus nucleotidyltransferases, CUTa and CUTb, results in loss of tagging, lower rates of degradation and an accumulation of transcripts with short poly(A) tails. Recently we have shown that 3' tagging is also
induced by nonsense mediated decay (NMD) and is required for efficient dissociation of the resulting termination complex. Upf1, a major NMD component, is implicated not only in this but, surprisingly, in all mRNA tagging to date. This led us to propose that deadenylation of mRNA results in an "NMD-like" event, where dissociation of poly(A) binding protein results in recruitment of Upf1 to the mRNA thereby triggering translational repression, clearance of the termination complex and efficient RNA degradation. We will describe our recent findings which relate to the mechanisms that trigger mRNA tagging and the downstream events which ultimately repress transcript expression.


Secondary metabolism in the model fungus Aspergillus nidulans is controlled by the global conserved regulator VeA, which also governs morphological differentiation. Among the secondary metabolites regulated by VeA is the mycotoxin sterigmatocystin (ST), where the presence of VeA is necessary for the biosynthesis of this carcinogenic compound. We identified revertant mutants (RM) capable of synthesizing ST in the absence of VeA. The point mutation in the RM7 mutant occurred at the coding region of a gene encoding a novel putative C2H2 zinc finger domain type transcription factor that we denominated MtfA. As expected, the A. nidulans mtfA gene product localizes at nuclei. Deletion of the mtfA gene restored mycotoxin biosynthesis in the absence of VeA, but drastically reduced mycotoxin production when mtfA gene expression was altered, by either deletion or overexpression, in the Aspergillus nidulans wild type strain. Our study revealed that mtfA regulates ST production by controlling the expression of the specific ST gene cluster activator cfrA. Importantly, mtfA also controls sexual and asexual development in A. nidulans. Deletion of mtfA results in a reduction of conidiation and sexual development.

365. Histidine 704 of the Aspergillus nidulans GATA factor AreA is required for nuclear export. Damien Downes1, Brandon Pfannenstiel1, Cameron Hunter1, Kendra Siebert1, David Clarke2, Meryl Davis3, Richard Todd1. 1) Department of Plant Pathology, Kansas State University, Manhattan, USA; 2) Department of Genetics, University of Melbourne, Australia.

In A. nidulans, the GATA transcriptional activator AreA controls the preferential utilization of nitrogen nutrients as well as the response to nitrogen starvation. During nitrogen starvation AreA accumulates in the nucleus, and a strong increase in target gene expression is observed. Addition of nitrogen nutrients to nitrogen starved cells results in rapid translocation of AreA to the cytoplasm and arrest of elevated AreA-dependent gene expression, indicating that regulated nuclear export is the control mechanism for AreA nuclear accumulation. AreA contains a single conserved CRM-A-dependent Nuclear Export Sequence (NES). We propose that regulated AreA nuclear export is controlled by post-translational modification of residues within the NES. We show that deletion of the AreA NES confers nuclear accumulation. Substitution of individual amino acids within the AreA NES identified a single histidine residue, which when mutated to a non-modifiable alanine residue leads to constitutive nuclear accumulation. This suggests that histidine modification may promote AreA nuclear export. We show that fusion of the AreA NES to the constitutively nuclear protein PrnA confers nucleocytoplasmic distribution and a proline utilization loss of function phenotype. We have used this phenotype to select mutants affecting AreA-dependent nuclear export.

366. Redundant Nuclear Localization Signals Mediate Nuclear Import of the Aspergillus nidulans Transcription Activator of Nitrogen Metabolic Genes. AreA. Cameron C. Hunter1, Kendra S. Siebert1, Damien J. Downes1, Koon Ho Wong2, Sara Lewis3, James A. Fraser3, David F Clarke2, Michael J. Hynes1, Meryl A. Davis3, Richard B. Todd1. 1) Department of Plant Pathology, Kansas State University, Manhattan, KS; 2) Department of Genetics, The University of Melbourne, Parkville VIC 3010, Australia.

The Aspergillus nidulans GATA transcription factor AreA activates transcription of nitrogen metabolic genes. AreA accumulates in the nucleus during nitrogen starvation but not in the presence of nitrogen sources. AreA contains five putative classical nuclear localization sequences (NLSs) and one putative non-canonical bipartite NLS. We used two approaches to identify the functional NLSs. First, we constructed epitope-tagged gene replacement areA mutants affected in individual NLSs or combinations of NLSs to identify sequences required for nuclear localization. Deletion of all five classical NLSs did not affect utilization of nitrogen sources and did not prevent AreA nuclear localization. Mutation of the bipartite NLS conferred inability to utilize alternative nitrogen sources but did not prevent AreA nuclear localization. Combinations of mutations of the six NLSs indicate redundancy among the AreA NLSs. Second, we constructed Green Fluorescent Protein (GFP)-AreA NLS fusion genes and introduced them into A. nidulans. The bipartite NLS strongly directs GFP to the nucleus, one of the classical NLSs weakly directs GFP to the nucleus and the other four classical NLSs collaborate to direct GFP to the nucleus.

367. Conditional expression of the phospho-transmitter gene ypdA and the signaling interaction of YpdA with response regulators; SskA and SrrA in Aspergillus nidulans. Mayumi Nakayama1,2, Yura Midorikawa1,2, Akira Yoshimi2, Daiuske Hagiwara3, Keietsu Abe1,2,3. 1) Tokoh University, Senda, Miyagi, Japan; 2) ABE-project New Industry Hatchery Center (NIChe) Tokoh University, Senda, Japan; 3) Present address: MMRC, Univ. of Chiba, Chiba Japan.

The histidine-to-aspartate (His-Asp) phosphorelay signaling transduction system has been conserved widely in both prokaryotes and eukaryotes. The systems typically consist of three types of common signal transducers: His-kinase (HK), a response regulator (RR), and a histidine-containing phosphotransfer intermediate (HPT). Generally, HPT acts as an intermediate between HK and RR and is indispensable for inducing appropriate responses to environmental stresses through His-Asp phosphotransfer signaling. In Aspergillus nidulans, we revealed His-Asp phosphorelay signal transducers: HK (NikA), RR (SsA and SrrA), and HPT (YpdA) were essential for the response of high-osmotic and oxidative stresses. Nevertheless the ypdA is the essential gene, the molecular mechanism underlying the importance of YpdA remains unclear. To identify the function of the YpdA, we constructed A. nidulans mutant in which expression of the ypdA gene is conditionally regulated under the control of the A. nidulans aClA promoter (CypdA strain) and analyzed their phenotype. We constructed mutant strain from CypdA by deleting the response regulator gene ssrA (CypdA/DssrA) and sskA (CypdA/DsskA). When ypdA was downregulated, CypdA showed remarkable growth retardation and formed abnormal hyphae, and CypdA/DsskA unexpectedly showed more severe growth retardation than the parent CypdA, in contrast, the growth retardation of CypdA/DsskA partly recovered. It is suggested that the growth retardation of CypdA was only partly suppressed by switching off the HogA pathway. We further constructed a mutant (CypdA/DssrA/DsskA) from CypdA by deleting the two RR genes. Here, we discussed two-component signaling under the inhibitory conditions of signaling between YpdA and response regulators.

368. WITHDRAWN

369. FigA, a putative member of low-affinity calcium system, is involved in both asexual and sexual differentiation in Aspergillus nidulans. Shizhu Zhang, Hailin Zheng, Nanbiao Long, Sha Wang, Ling Lu. College of life Sciences, Nanjing Normal University, Nanjing, Jiangsu, China.

Calcium-mediated signaling pathways are widely employed in eukaryotes and are implicated in the regulation of diverse biological processes. In baker’s yeast Saccharomyces cerevisiae, at least two different carrier systems have been identified—a high-affinity calcium influx system (HACS) and a low-affinity calcium system.
Peptide mass fingerprinting revealed that FIa (clbRox) increased 6 gene XlnR carboxymethylcellulase 1 (Sumitani, T. Kawaguchi. Life & Environmental Sci, Osaka Prefecture 373. The effect of the productivity under the designed culture conditions using were divided into two groups depending on the type of nutrition. Combining some culture con pathways and found some positive and negative correlations between primary and secondary ones. We also found that secondary metabolic pathways and assignment of transcriptome data, and applications. Since types and amounts of the secondary metabolites greatly change according to strains and culture conditions, controlling the environmental growth conditions is mainly because of its ability to secrete large amounts of protein into the growth medium, and carry out the eukaryotic post-translational modifications of glycosylation, proteolytic cleavage and disulfide bond formation. Although A. niger can express some native and foreign proteins at high levels, many native proteins and most foreign proteins are expressed at very low levels. Compartmentalization of native proteins for rate limiting steps in the secretory pathway, transcription, mRNAs processing and translation are bottlenecks that can limit levels of secreted protein production. Furthermore, proteins expressed using A. niger often require extensive purification, due to the presence of high levels of many native secreted proteins that can interfere with the downstream characterization or restrict protein stability. To address these issues we have been developing recombinant promoters that support high transcription rates, engineering strains that produce reduced levels of native secreted protein, and identifying secertory pathway bottlenecks that limit secreted protein yields. We have created a set of novel expression cassettes that are capable of supporting significantly higher transcription rates than are obtained with the widely used glucoamylase gene (glaA) promoter. We have also, engineered new “clean” expression strains that combine dramatically reduced levels of “contaminating” native extracellular protein production with increased levels of target protein expression. RNA-seq analysis and conditional gene expression are being used to identify additional bottlenecks that limit the production of foreign proteins.

Aspergillus niger is widely used commercially and for basic research as a host for native and foreign protein production. This is mainly because of its ability to secrete large amounts of protein into the growth medium, and carry out the eukaryotic post-translational modifications of glycosylation, proteolytic cleavage and disulfide bond formation. Although A. niger can express some native and foreign proteins at high levels, many native proteins and most foreign proteins are expressed at very low levels. Compartmentalization of native proteins for rate limiting steps in the secretory pathway, transcription, miRNA processing and translation are bottlenecks that can limit levels of secreted protein production. Furthermore, proteins expressed using A. niger often require extensive purification, due to the presence of high levels of many native secreted proteins that can interfere with the downstream characterization or restrict protein stability. To address these issues we have been developing recombinant promoters that support high transcription rates, engineering strains that produce reduced levels of native secreted protein, and identifying secretory pathway bottlenecks that limit secreted protein yields. We have created a set of novel expression cassettes that are capable of supporting significantly higher transcription rates than are obtained with the widely used glucoamylase gene (glaA) promoter. We have also, engineered new “clean” expression strains that combine dramatically reduced levels of “contaminating” native extracellular protein production with increased levels of target protein expression. RNA-seq analysis and conditional gene expression are being used to identify additional bottlenecks that limit the production of foreign proteins.

Aspergillus niger is an important cell factory in biotechnology. Productivity of this fungus is strongly influenced by the environmental growth conditions and the subsequent morphological adaptation. In eukaryotic organism, mitogen activated protein kinase cascades are important signaling pathways mediating cellular responses to environmental cues. In order to gain a deeper understanding of the molecular mechanisms of cellular adaptation in A. niger, we characterized the Saccharomycetes cerevisiae FUS3 MAP kinase homologue MAKB by using a combination of molecular genetics, biochemical analysis and light and fluorescent microscopy. We found that the absence of makb results in specific developmental defects, which strongly depend on environmental conditions. For example, the mutant secretes a dark pigment in a nutrient dependent manner in constant darkness, but not while growing in a dark-light-rhythm. In addition, MAKB is also involved in asexual spore formation, indicated by its accumulation in developing conidiophores and a reduced sporulation of the makb deletion mutant. Detailed comparison of sporulation in the wild-type and the mutant under different growth conditions let us to the hypothesis that two independent trigger induce conidiation: nutrient starvation and cell age or density. Interestingly, MAKB appears to be only involved in the latter one, indicated by suppressed sporulation of the mutant under nutrient sufficient growth conditions. Taken together, these observations indicate that in A. niger, MAKB is intimately involved in the adaptation of secondary metabolism and cell differentiation in response to environmental influences.

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decreased. Northern blot analysis revealed that the expression of cbhIb decreased in the cbBox strain under cellulose inducing condition. However, the cbBox overexpression increased xynA expression under wheat bran inducing condition, but not under the avicel inducing condition. The effect of the cbBox overexpression was not equal to the enzyme production and the gene expression even though they were under the control of CbfB. These results suggest that CbfB coordinates their expression with another factors.

374. Regulation of the a-tubulin B-encoding gene during early development of the phytopathogenic fungus Botrytis cinerea. Y. Faiivre Talmey, I. R. Gonçalves, A. Simon, M. Viald, C. Bruel. 1) CNRS, University Claude Bernard, LYON, France; 2) INRA, BIOGER, Grignon, France.

The fungal plant pathogen Botrytis cinerea is able to infect more than 250 different hosts and is responsible for major economic losses worldwide. The success of the fungus life cycle relies on distinct developmental stages and specific cellular structures like appressoria, infection cushions, conidiophores, apothecia and sclerotia. Based on the important role that cytoskeleton plays in cellular organization and shape in all eukaryotes, attention was given to tubulins and their putative specific role in the virulence of B. cinerea. One b-tubulin and two a-tubulin encoding genes are found in B. cinerea's genome. Alpha-tubulin A and B share 60% identity at the protein level and phylogenetic analyses revealed that orthologs to the a-tubulin A gene exist in all ascomyceta species whereas the a-tubulin B gene seems to be present in some ascomyceta only. Expression studies showed that the a-tubulin B gene is more expressed than the a-tubulin A gene. Besides, a peak of expression is observed for the a-tubulin B encoding gene early during conidia-derived fungal development. In order to understand the regulation of the a-tubulin B encoding gene, combinations of promoter deletions and transcriptional fusions were used. Putative regulatory regions were identified and the one hybrid yeast system was used to search for putative transcription factors that would interact with these regions and play a role in the regulation of this gene.

375. The regulation of D-galacturonic acid utilization in Botrytis cinerea. Lisha Zhang, Joost Stassen, Sayantani Chatterjee, Maxim Cornelissen, Jan van Kan. Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands.

The plant cell wall is the first barrier to pathogen invasion. The fungal plant pathogen Botrytis cinerea produces a spectrum of cell wall degrading enzymes for the decomposition of host cell wall polysaccharides and the consumption of the monosaccharides that are released. Especially pectin is an important cell wall component, and the decomposition of pectin by B. cinerea is a well studied phenomenon. D-galacturonic acid is the most abundant component of pectin and effective utilization of D-galacturonic acid is important for virulence of B. cinerea. The D-galacturonic acid catabolic pathway comprises three enzymatic steps, involving D-galacturonic reductase (encoded by Bcgar1 and Bcgar2), L-galactonate dehydratase (encoded by Bclg1), and 2-keto-3-deoxy-L-galactonate aldolase (encoded by Bclg1). Therefore, an effective concerted action of the appropriate pectin depolymerising enzymes, monosaccharide transporters and catabolic enzymes is important for complete pectin utilization by B. cinerea. In this study, RNA sequencing was performed to compare genome wide transcriptional profiles in B. cinerea grown in media containing glucose and pectate as sole carbon sources. We identified 31 genes that are significantly upregulated in pectate containing culture, including Bcgar2, Bclg1a, and a putative monosaccharide transporter. In addition, conserved cis-regulatory elements were predicted in the promoters of genes involved in pectate decomposition and D-galacturonic acid utilization. Functional analysis was carried out of the bidirectional promoter of the Bcgar2-Bclg1a gene cluster to study which of the cis-regulatory elements is required for induction by D-galacturonic acid. Furthermore, potential regulatory protein(s) were isolated by DNA-protein pull down assays using one important cis-regulatory element.


Candida albicans grows within a wide range of fluctuating host niches, and the ability to rapidly adapt enhances its success as a commensal and as a pathogen. The recently expanded telomere-associated (TLO) gene family consists of fourteen expressed members in C. albicans. Each TLO gene encodes a paralog of a single Mediator complex component. Thirteen expressed TLOs are located at the chromosome ends as the most telomere-proximal open reading frame. Individual TLO expression at both the transcript and protein level was extremely noisy. Noise originated from single cell variability in TLO expression due to intrinsic factors. Deletion of chromatin modifying enzymes that function in subtelomeric silencing abolished TLO noise, as did ectopically expressing a TLO from an internal locus. Conversely, transcriptional variation of a low noise gene increased significantly when ectopically expressed in the subtelomere. Interestingly, deletion of the Mediator component MED3, which inhibits Tlo from incorporating into Mediator, also drastically reduced TLO noise and supports an autoregulatory mechanism for TLO noise. These data suggest subtelomeric chromatin structure regulates TLO gene noise through the action of chromatin modifiers and Mediator. We propose that TLO noise is beneficial to C. albicans by producing heterogeneous cell populations that incorporate different Tlo proteins in Mediator, producing a range of transcriptional profiles in the population that allows some cells to survive in altered environmental conditions.


Candida albicans is currently the most common human fungal pathogen, and its propensity for causing infection has been closely linked with its ability to form biofilms. Central to understanding its behavior is the white-opaque phenotypic switch, in which cells can undergo an epigenetic transition between the white state and the opaque state. The phenotypic switch regulates multiple properties including biofilm formation, virulence and sexual mating. In particular, opaque cells are the mating competent form whereas white cells do not mate but generate biofilms in response to pheromone. In this work, we identify the master transcriptional regulator involved in pheromone-induced biofilms in white cells as well as sexual mating in opaque cells as C. albicans Cph1, ortholog of Saccharomyces cerevisiae Ste12. In contrast, Cph1 is not required for the formation of conventional biofilms (biofilms that can form without pheromone treatment). Transcriptional profiling analysis under biofilm conditions during pheromone treatment revealed a number of potential downstream targets of Cph1 and Tec1. In total, we observed 23 genes that exhibited decreased induction (>2-fold) by pheromone in both Dcph1 and Dtec1 mutants. Of these genes, six candidates were chosen for further analysis due to their dependence on Cph1 and Tec1 for pheromone-induced expression, and because they were also not induced in pheromone-treated opaque cells. Interestingly, one novel gene product shown to influence biofilm formation is Hgc1. Similar to the Dtec1, deletion of HGC1 resulted in decreased biofilm formation in white cells responding to pheromone, and also abolished formation of conventional biofilms. It is therefore apparent that Hgc1 is important for cell adhesion and biofilm development in the two distinct models of biofilm formation. Most importantly, these results suggest that both shared and unique components operate in different models of biofilm formation in this important human pathogen.

The ability of Candida albicans to colonize or invade multiple host environments requires that it rapidly adapts to different conditions. Our group has been exploiting CHIP-chip and transcription profiling technologies, together with computer modeling, to provide a better understanding of select transcription factor (TF) networks. We used DNA microarrays to measure the changes in transcriptional profiles that occur immediately following the infection of C. albicans to hypoxic growth conditions. The impressive speed of this response is not compatible with current models of fungal adaptation to hypoxia that depend on the inhibition of sterol and heme biosynthesis. Functional interpretation of these profiles was achieved using Gene Set Enrichment Analysis, a method that determines whether defined groups of genes exhibit a statistically significant bias in their distribution within a ranked gene list. The 5It4p phosphatase, Ccr4p mRNA deacetylase and Sko1p TF were identified as novel regulators of the early hypoxic response. While cells mutated in these regulators exhibit a delay in their transcriptional responses to hypoxia their ability to grow in the absence of oxygen is not impaired. Promoter occupancy data on 26 TFs was combined with the profiles of 375 significantly-modulated target genes in a Network Component Analysis (NCA) to produce a model of the dynamic and highly interconnected TF network that controls this process. The NCA also allowed us to observe correlations between temporal changes in TF activities and the expression of their respective genes, thus allowing us to identify which TFs are potentially subjected to post-transcriptional modifications. The TF network is centered on Tye7p and Upc2p which are associated with many of the genes that exhibit the fastest and strongest up regulations. While Upc2p only associates with downstream promoters, Tye7p is acting as a hub, its own promoter being bound by itself and 7 additional TFs. Rap1 and Ahr1 appear to function as master regulators since they bind to a greater proportion of TF gene promoters, including those of Upc2p and Tye7p. Finally, Cbf1p, Mrp1p and Rap1p show the greatest numbers of unique target genes. The high connectivity of these models illustrates the challenges that lie in determining the individual contributions of specific TFs.

379. Gene expression and function during invasive Candida infection. Wenjie Xu1, Norma Solís2, Carol Woolford2, Scott Filler3, Aaron Mitchell4. 1) Biological Sciences, Carnegie Mellon University, Pittsburgh, PA; 2) Departments of Medicine and Pathology, Harbor-UCLA, Los Angeles, CA. What genes does a pathogen express during infection? What regulatory pathways contribute to expression of those genes in vivo? Which pathogen gene functions evoke specific host responses? These questions are beginning to be addressed for many plant pathogens, in which a lesion that is enriched for infected tissue can be isolated readily. There have also been pioneering studies with human and animal pathogens, but they have been limited by the ability to isolate infected tissue and by background problems from microarray technology. We implemented a recently developed technology, nanoString profiling, to investigate these long-standing questions with the fungal pathogen C. albicans in a murine model of hematogenously disseminated candidiasis. We used whole kidneys, a major target organ, for profiling the time course of both pathogen and host gene expression. NanoString technology is not genome-wide, so we have selected high-priority fungal and host genes for investigation. On the pathogen side, we find that hyphal genes and neutral/alkaline pH response genes are induced early in infection, while oxidative and cell wall stress genes are induced later. These results are consistent with the idea that the influx of neutrophils causes cell wall and oxidative stress. Among the 222 C. albicans genes that specify transcription factors, those highly expressed or highly up-regulated during infection are enriched for previously demonstrated virulence regulators, and also include many genes not previously known to govern virulence. We have profiled attenuated mutants despite their limited growth during infection, and we have found that transcription factor target genes in vivo differ considerably from the target genes identified in vitro. Finally, we find that the host displays progressive induction of cytokines, pattern recognition receptors, and innate immune signaling pathways, with expression changes detectable at 12 hours post-infection, when fungal burden is extremely low. An attenuated rim101 mutant causes a muted host response, but also alters the kinetic profile to yield precocious induction of late host response genes. Our data allow the most detailed sketch to date of the dynamics and functions at the host-pathogen interface during disseminated candidiasis.

380. Effects of histone H3 point mutations on centromere maintenance. Steven Friedman1, Eric Selker2, Michael Freitag3. 1) Biochemistry & Biophysics, Oregon State University, Corvallis, OR; 2) Institute of Molecular Biology, University of Oregon, Eugene, OR. Post-translational modifications (PTM) of histone amino acid residues are known to play important roles in chromatin structure and function. In Neurospora crassa, trimethylation of histone H3 lysine 9 (H3K9me3) is essential for cytosine DNA methylation [1]. There is also evidence for a role of H3K9me3 in the maintenance of centromeres [2]. An in-depth study of a larger set of histone H3 point mutations revealed additional recessive and dominant mutations involved in DNA methylation, including some mutations that proved lethal [3]. In this study, hH3 alleles were integrated ectopically or at the his-3 locus in the presence of a mutant hH3 allele at the endogenous hH3 locus. Here we describe a gene replacement system that allows mutant hH3 alleles to be integrated at the endogenous loci by homologous recombination, yielding hH3 replacement strains. The general approach is versatile and applicable to studying the role of specific point mutations in other genes. We will present results on how hH3 mutations affect the deposition of centromere-protecting proteins (e.g., CenH3, CEN-T and CEN-C).


381. Analysis of the transcriptional regulation of genes involved in the synthesis and organization of the cell wall of Ustilago maydis during infection of an alternative host. Angélica Mariana Robledo Briones, José Ruiz Herrera. Centro de Investigaciones y de Estudios Avanzados del IPN, Km 9.6 Libramiento Norte Carretera Irapuato-León.

The cell wall is the most external structure of the cell. Its function is to protect it against the difference in osmotic pressure with the environment and provide the morphology. The cell wall of fungi is made of mannan and glucans) immersed in a matrix of glycoproteins. Ustilago maydis is a dimorphic basidiomycota, pathogen of maize, but under axenic conditions it may infect other plants including Arabidopsis thaliana. Considering the role of the cell wall in the pathogenic process, we made a transcriptomic analysis of the genes involved in its structure and synthesis, and encoding secreted proteins during the infection of A. thaliana. For this study, one channel chips with high density oligonucleotide were used. Plantlets of Arabidopsis were infected with a haploid or a diploid strain, and at intervals RNA was isolated, complementarity CDA was synthesized and used to hybridize the microarrays. Data of microarrays allowed to identify genes involved in cell wall synthesis and organization, and encoding proteins from the secretome of U.maydis whose expression was regulated during the transition from saprophytic to pathogenic stages. These accounted to about 60 per cent; of the total of 639 genes existing in U. maydis, a proportion being slightly higher in the haploid strain. Some differences were observed in the regulation of these genes in the haploid and diploid strains. We observed that genes involved in N- and O- glycosylation of proteins were up-regulated during infection. In addition, some CHS and CDA genes, and some genes involved in the synthesis of b-1,6-glucans and GPI proteins, were differentially regulated. A great number of genes encoding secreted proteins with a degradative function were up-regulated (more in the haploid), this increased transcription may be related with degradation of the plant cell wall necessary to establish the infection. All these results demonstrate the usefulness of the Ustilago maydis-Arabidopsis thaliana pathosystem for identification of the pathogenic mechanisms of U. maydis, and in the case of this
study the role of genes encoding proteins of the wall and secretome, and the differences existing in the behavior of the diploid and haploid, taking into consideration that only the first one is pathogenic to the natural host.

382. Expression of the *Trichophyton rubrum* ace2 and pacC genes during degradation of keratinized substrates. Larissa Silva, Nalu Peres, Gabriela Persinoti, Elza Lang, Vanderlei Oliveira, Antonio Rossi, Nilce Martinez-Rossi. University of Sao Paulo, Ribeirão Preto/SP/Brazil, Sao Paulo, Brazil.

*Trichophyton rubrum* is a pathogenic, cosmopolitan and anthropophilic fungus that infect keratinized tissues, mainly skin and nails. The genomes of several dermatophytes, including *T. rubrum*, were sequenced by the Broad Institute/NIH, enabling studies on the regulation of the expression of genes related to several cellular processes. The transcription factor (TF) Ace2 participates of a network of genes called RAM (Regulation of Ace2 activity and cellular morphogenesis), involved in the regulation of morphogenesis, cell division, and development of conidiophores. The TF PacC regulates the transcription of genes in response to extracellular pH and also genes related to the biosynthesis of cell wall, suggesting a crosstalk between these two pathways. Therefore, the aim of this study was to analyze the expression profile of the pacC and ace2 genes in different nutritional sources (nail and skin ex vivo infections) to understand the regulation of these TF during pathogenesis and development. In silico analyses of the putative promoter regions of the pacC and ace2 genes revealed the presence of the DNA binding motifs of both TFs, suggesting a possible cross- and co-regulation of these TFs expression in *T. rubrum*. Gene expression analyses during growth in keratinized tissues suggested an opposite expression profile in nail interaction assays, the ace2 gene was up-regulated and pacC was down-regulated. Moreover, in *ex vivo* skin infective both genes presented a similar expression level. These results suggest a different gene expression modulation of ace2 and pacC according to the nutrient source and possibly the infection site. Moreover, this evaluation provides a better comprehension of the involvement of both pathways in regulating a variety of cellular processes that enable cell viability during infection of keratinized tissues.

383. Control and Function of Two Fatty Acid Regulators in *Neurospora crassa*. Erin L. Bredeweg1, Fei Yang2, Kristina Smith3, Rizgiz Dekkang4, Jillian Emerson1, Jay Dunlap5, Deborah Bell-Pedersen6, Matthew Sachs3, Michael Freitag2. 1) Program for Molecular and Cellular Biology, Department of Biochemistry and Biophysics, and Center for Genome Research and Biocomputing (CGRB), Oregon State University, Corvallis, OR 97331; 2) Department of Biology and Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX; 3) Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH.

The filamentous saprobe *Neurospora crassa* is an excellent model for describing the behavior of transcriptional regulators. We describe the genome-wide behavior of two Fatty Acid Regulators (FAR) proteins, transcription factors that modulate the response of *N. crassa* to the presence of fatty acids. We used ChiP-seq to find the localization of FAR-1 and FAR-2 under nutrient conditions targeting short and long chain fatty acid carbon sources, with sucrose as a control. Bioinformatic analyses describe variant binding sites for FAR-1 and FAR-2, with overlap in about a third of all target regions. Functions under the control of ChiP-seq targets were further examined by phenotypic assays for siderophore production, oxidative stress, and linear growth. We found reduced siderophore production, and increased vulnerability to oxidative stress in far-1 mutants, but not far-2 mutants. Linear growth showed a carbon-specific reduced growth rates for far-2, as well as TWEEN-20 sensitivity and conidiation defects for far-1. RNA-seq identified numerous differentially regulated transcripts under different growth conditions and in the single or double mutants. Many of these transcripts are part of the gene set identified by ChiP-seq, and many were affected by the absence of one or both FARs. Our analyses identified groups of co-regulated proteins not previously identified as affected by FAR transcription factors, in addition to those involved in the control of the core cellular machinery for energy production by beta-oxidation.

384. Characterization of genomic targets for the *Neurospora crassa* hypothetic transcription factor NCU04390 by ChiP-sequencing. R. Gonçalves1, E. Bredeweg2, M. Freitag2, M. C. Bertolini2. 1) Instituto de Química, UNESP, Araquara, São Paulo, Brazil; 2) Department of Biochemistry and Biophysics, OSU, Corvallis, OR, USA.

The mechanisms by which glycogen content is controlled in microorganisms are intricate, involving co-regulation of many proteins. In *Neurospora crassa*, glycogen reaches maximal levels at the end of the exponential growth phase, however under heat shock, glycogen content and transcription of the glycogen synthase gene (gsn) rapidly decrease. In a previous analysis, the NCU04390 gene showed a drastic increase in glycogen levels and up-regulation of the gsn gene after heat shock when compared to the wild-type strain, suggesting that the NCU04390 gene product is involved in the regulation of glycogen metabolism. Because the product of this ORF is annotated as a hypothetical transcription factor (TF) with an N-terminal zinc-finger and a central fungal-specific TF domain, chromatin immunoprecipitation followed by high throughput DNA sequencing (ChIP-seq) was expected to reveal genes that are directly regulated by the NCU04390 gene product. First, GFP tag was fused to the 3’-end of the ORF NCU04390 by gene replacement. ChiP was performed with NCU04390-GFP at 30ºC and 45ºC with antibodies against the GFP tag. ChiP-libraries were sequenced on a HiSeq2000 (Illumina/Solexa) genome analyzer and data from 45ºC experiment revealed that most of the genes regulated by the transcription factor encode hypothetical proteins. However genes encoding proteins with known functions, such as proteins involved in carbon metabolism and transporters were also identified. Among these genes, it is important to mention the glycogen debranching enzyme coding gene (ORF NCU00743), which participates in the glycogen degradation. The His::4390 recombinant protein was produced in *E. coli*, partially purified by IMAC and used in EMSA experiments to validate the result found in the ChiP-Seq assay. The results showed specific binding of the recombinant His::4390 in the NCU00743 promoter, suggesting that the transcription factor might regulate glycogen metabolism under heat stress through the gene encoding the debranching enzyme. Data from EMSA validation analysis for more peaks found in ChiP-seq will be presented. Supported by FAPESP, CNPq, CAPES and US NIH.

385. The KMT6 Histone H3 K27 Methyltransferase Regulates Expression of Secondary Metabolites and Development in *Fusarium graminearum*. Kristina M. Smith, Lanelle R. Connolly, Michael Freitag. Department of Biochemistry and Biophysics, Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331.

The cereal pathogen *Fusarium graminearum* produces secondary metabolites toxic to humans and animals, yet coordinated transcriptional regulation of secondary metabolite gene clusters remains largely a mystery. By ChiP-sequencing we found that regions of the *F. graminearum* genome with secondary metabolite clusters are enriched for a histone modification, trimethylated histone H3 lysine 27 (H3K27me3), associated with gene silencing. This modification was found predominantly in regions that lack synteny with other *Fusarium* species, generally subtelomeric regions. H3K27me3 and di- or trimethylated H3K4 (H3K4me2/3), modifications associated with gene activity, are found in mutually exclusive regions of the genome. To better understand the role of H3K27me3, we deleted the gene for the putative H3K27 methyltransferase, KMT6, a homolog of *Drosophila* Enhancer of zeste, E(z). The kmt6 mutant lacks H3K27me3, as shown by western blot and ChiP-sequencing, displays growth defects, is sterile, and produces mycotoxins under conditions where they are not generated in wildtype (WT) strains. RNA-sequencing showed that genes modified by H3K27me3 are most often silent, as about 75% of the 4,449 silent genes are enriched for H3K27me3. Surprisingly, we found 22% of the 8,855 expressed genes enriched for H3K27me3. A subset of genes that were enriched for H3K27me3 in WT gained H3K4me2/3 in kmt6 (1,780 genes), and an overlapping set of genes showed increased
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expression. Almost 95% of the remaining 2,720 annotated silent genes showed no enrichment for either H3K27me3 or H3K4me2/3. In these cases absence of H3K27me3 is insufficient for expression, which suggests a requirement for additional factors for gene expression. Taken together, we show that absence of H3K27me3 allows expression of 14% of all annotated genes, resulting in derepression of predominantly secondary metabolite pathways and other species-specific functions, including potentially secreted pathogenicity factors. This study provides the framework for novel targeted strategies to control the "cryptic genome" and specifically secondary metabolite expression.

386. Circadian clock-gated cell division cycles in Neurospora crassa. C. Hong1, J. Zamborszky1, M. Baek2, K. Ju3, H. Lee1, L. Larrondo1, A. Goity2, A. Csikasz-Nagy1. 1) Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH; 2) Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile; 3) Randall Division of Cell and Molecular Biophysics, and Institute for Mathematical and Molecular Biomedicine, King’s College London, London, SE1 UL, UK.

Asynchronous nuclear divisions are readily observed in filamentous fungi such as Ashygo gossypii and Neurospora crassa. Our computational simulations, however, predict synchronous circadian clock-gated mitotic divisions if the division cycles of such multinucleated organisms are coupled with circadian rhythms. Based on this hypothesis, we investigate the coupling between the cell cycle and the circadian clock in Neurospora crassa. First, we show WC-1-dependent light-induced expression of stk-29 mRNA (homolog of wee1), which suggests that there exists a conserved coupling between the clock and the cell cycle via STK-29 in Neurospora as in mammals. Second, we demonstrate that G1 and G2 cyclins, CLN-1 and CLB-1, respectively, show circadian oscillations with luciferase bioluminescence reporters. Moreover, clb-1 and stk-29 gene expressions show circadian clock-dependent light-induced phase shifts, which may alter the timing of divisions. Third, we show circadian clock-dependent synchronized nuclear divisions by tracking nuclear morphology with histone hH1-GFP reporter. Synchronized divisions occur late in the evening, and they are abolished in the absence of circadian rhythms (frq(ff)). Our findings demonstrate the importance of circadian rhythms for synchronized mitotic cycles and establish Neurospora crassa as an ideal model system to investigate mechanisms that couple the cell cycle and the circadian clock.

387. Protein Binding Microarrays and high-throughput real-time reporters studies: Building a four-dimensional understanding of transcriptional networks in Neurospora crassa. A. Montenegro-Montero1, A. Goity2, C. Olivares-Yañez1, A. Stevens-Lagos2, M. Weirauch3, A. Yang4, T. Hughes1, L.F. Larrondo1. 1) Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Santiago, Chile; 2) CAGE, Cincinnati Children’s Hospital Medical Center, University of Cincinnati, U.S.A; 3) Banting and Best Department of Medical Research, University of Toronto, Canada.

It has been suggested that ~20% of the Neurospora-transcriptome may be under circadian control. Nevertheless, there is scarce information regarding the regulators that are involved in the rhythmic expression of clock-controlled genes (ccgs). We are using a high-throughput platform, based on various codon-optimized luciferase transcriptional- and translational-reporters, to monitor time-of-day-specific gene expression and to identify key elements mediating circadian transcriptional control. Thus, we have identified transcription factors -such as SUB-1- that affect the expression of known and novel ccgs, among which there are transcriptional regulators that give access to a group of third-tier ccgs. In addition, we are characterizing several rhythmic bZIP-coding genes as potential nodes of circadian regulation. In order to characterize regulatory networks in which these and all Neurospora transcription factors participate, we are using double-stranded DNA microarrays containing all possible 10-base pair sequences to examine their binding specificities and in that way, predict possible targets on a genome-wide manner. Currently, these Protein Binding Microarray studies have provided DNA-binding specificities for over 120 Neurospora transcription factors granting an unprecedented and powerful tool for transcriptional network studies. Finally, we have generated graphic tools to explore the spatial differences observed in the temporal control of gene expression. Funding: Conicyt/Fondecy/regular 1090513.

388. Glycogen metabolism is regulated by the circadian clock in Neurospora crassa. S. Virgilio1, T. Candido, M. C. Bertolini. Instituto de Química, UNESP, Araraquara, São Paulo, Brazil.

The fungus Neurospora crassa has been widely used in studies of circadian rhythms and photobiology. Our research group has been using this model organism to study the molecular mechanisms involved in glycogen metabolism regulation and recent findings have revealed that circadian rhythms control a variety of physiological and metabolic functions in different organisms. In a screen of a mutant strains set we identified a number of transcription factors/cofactors likely acting as regulators of glycogen metabolism. Among them, several transcription factors were previously described as controlled by regulators of the circadian clock in N. crassa. The result led us to start to investigate whether glycogen metabolism is under control of circadian clock in N. crassa. Experiments were performed to verify whether glycogen was rhythmically accumulated in a wild-type strain. In circadian clock experiments, the glycogen content varied according to the circadian rhythm, with cyclical periods ranging from 22 to 24 h. The glycogen synthase activity (GSN) was quantified in the presence and absence of the allosteric activator glucose-6-phosphate (G6P). The +/- G6P ratio is considered as an index of phosphorylation, higher levels correlating with lower phosphorylation. The GSN phosphorylation was influenced by the biological clock, showing changes in the GSN phosphorylation status along the experiment. The expression of the gsn and gpn (encoding glycogen phosphorylase) genes was evaluated in the same experiments and in light-induced experiments as well. In circadian clock analysis, the gsn and gpn transcripts showed rhythmic expression although not as pronounced as the levels of the cgp-1 transcript (positive control). In light-induced experiments, the levels of glycogen were kept constant during different times after exposure to light, however the expression of the gsn and gpn genes showed to be delayed light-induced. The results suggested a connection between the energy derived from the glycogen metabolism and the circadian clock in N. crassa. Supported by FAPESP and CNPq.

389. Genetic and Molecular Dissection of the Neurospora Circadian Oscillatory System. Qijun Xiang1, Bin Wang1, Chandru Mallappa2, Jennifer Hurley2, Arko Dasgupta1, Jennifer Loro3, Jay Dunlap2. 1) Department of Genetics, Dartmouth Medical School, Hanover, NH03755; 2) Departement of Biochemistry, Dartmouth Medical School, Hanover, NH03755.

Transcription/ translation feedback loops are central to all eukaryotic circadian clocks. In the circadian oscillator, the negative feedback loop drives periodic expression of proteins that feed back to reduce their own expression. A heterodimer of proteins, WC-1 and WC-2, acts as a transcription factor to drive expression of the frq gene. Its product FRQ dimerizes and forms a complex with another protein FRH. This complex inhibits the activity of the WC heterodimer creating the negative feedback loop. While canonical clock proteins such as FRQ work exclusively in timing, all systems utilize additional proteins performing other functions in the cell. Among these in Neurospora is the essential putative RNA helicase, FRH. A novel, unbiased genetic screen for circadian negative feedback mutants uncovered a point mutation that completely complements the essential functions of FRH yet is totally arrhythmic, thus genetically separating essential functions from clock-associated roles. In other experiments we used mass spectrometry to look for interactors of FRH, FRQ, and to follow posttranslational modifications of these proteins over the day. Although few modifications are found on FRH, FRQ is extensively modified with nearly 100 phosphorylations. By examining the phenotypes of strains bearing mutants that have lost these sites individually and in groups, we begin to see how temporally regulated phosphorylation has opposing effects directly on overt circadian rhythms and FRQ stability. For over 60% of the
confirmed phosphorylation sites, loss of the individual or neighboring sites have no apparent effect on the free running period length, suggesting that sites may work in groups as dynamically regulated charged domains. Some domains promote FRQ stability and lengthen period and other promote turnover and shorten period. Modifications are dynamic such that at near all times of day “FRQ” describes a heterogeneous mix of proteins with the same amino acid sequence but variable and distinguishable structure and surface chemistry. We have also used luciferase as a reporter to follow the FRQ-WC core oscillator under conditions where growth rhythms are manifest. Under these conditions the FRQ/WC oscillator cycles with a normal compensated circadian period length even when the overt rhythm of growth moves out of the circadian range.

390. Non-optimal codon usage determines the expression level, structure and function of the circadian clock protein FREQUENCY. Mian Zhou1, Jinhu Guo2, Joonseok Cha3, Michael Chae4, She Chen5, Jose Barral6, Matthew Sachs7, Yi Liu8. 1) Department of Physiology, UT Southwestern Medical Center, Dallas, TX; 2) National Institute of Biological Sciences, Beijing, China; 3) Departments of Neuroscience and Cell Biology and Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, TX; 4) Departments of Biology, Texas A&M University, College Station, TX; 5) School of Life Sciences, Sun Yat-sen University, Guangzhou, China.

Codon usage bias has been observed in the genomes of almost all organisms and is thought to result from selection for efficient and accurate translation of highly expressed genes 1-3. In addition, codon usage is also implicated in the control of transcription, splicing and RNA structure 4-6. Many genes, however, exhibit little codon usage bias. The lack of codon bias for a gene is thought to be due to lack of selection for mRNA translation. Alternatively, however, non-optimal codon usage may also have biological significance. The rhythmic expression and the proper function of the Neurospora FREQUENCY (FRQ) protein are essential for circadian clock function. Here, we show that, unlike most genes in Neurospora, frq exhibits non-optimal codon usage across its entire open reading frame. Optimization of frq codon usage results in the abolition of both overt and molecular circadian rhythms. Codon optimization not only increases FRQ expression level but surprisingly, also results in conformational changes in FRQ protein, impaired FRQ phosphorylation, and impaired functions in the circadian feedback loops. These results indicate that non-optimal codon usage of frq is essential for its circadian clock function. Our study provides an example of how non-optimal codon usage is used to regulate protein expression levels and to achieve optimal protein structure and function.

391. Two putative long non-coding RNAs upstream of transcription factor Znf2 may regulate morphogenesis (or dimorphic transition) in Cryptococcus neoformans. Nadia Chacko, Linqi Wang, Xiaorong Lin. Biology, Texas A&M University, College Station, TX.

Cryptococcus neoformans is an opportunistic human pathogen and the causative agent of fungal meningitis, one of the leading causes of death in immunocompromised patients. The virulence of this dimorphic fungus is closely tied to its morphology as the yeast form is pathogenic while the filamentous form is non-pathogenic. The morphological switch from yeast to filament occurs typically during asexual and bisexual mating but can occur under mating limiting conditions too. Recently we found that transcription factor Znf2 directs morphological transition from yeast-to-filament and its activity is reversely correlated with fungal virulence. In a search to identify upstream regulators of ZNF2, we screened 60,000 insertional mutants that mimic znf2D phenotype. Insertions in two of the selected mutants were found to be in a potential long non-coding RNA located in the intergenic region of ZNF2. This IncRNA was named ZRE2. The zre1D mutant phenotype resembles the znf2D mutant phenotype, supporting our hypothesis that ZRE1 functions upstream of ZNF2. Interestingly, the expression of ZRE1 is increased under host relevant conditions but not under mating-inducing conditions, suggesting that ZRE1 could be involved in the adaptation to the host during infection. Surprisingly the expression of ZNF2 is only modestly reduced in the ZRE1 insertional mutants, indicating the existence of other potential regulators or non-transcriptional regulation of ZNF2 by ZRE1. Further analysis of the intergenic region of ZNF2 revealed the presence of another IncRNA, which was named ZRE2. Long ncRNAs are known to regulate genes by transcriptional activation, repression and epigenetic control. The investigation of extent of regulatory role of ZRE1 and ZRE2 on ZNF2, the conditions under which they exert regulation, and the mode of action (transcriptional, translational, or epigenetic control) of these ncRNAs will further clarify the role of ZNF2 in morphogenesis and virulence in C. neoformans.


Cryptococcus neoformans is an encapsulated basidiomycetous yeast responsible for deadly meningocencephalitis in immunocompromised patients. The analysis of its genome sequence revealed that nearly all the genes contain introns. These introns are short (67 bp) and each gene contains 5 introns on average. RNAseq data showed that alternative splicing is also very common. Moreover, for most of the genes tested introns appeared to be essential for gene expression. We have studied the pathways by which these introns regulate gene expression in C. neoformans. We indentified a polyA binding protein as a major key factor in this regulation controlling the degradation of the mRNA transcribed from intronless alleles.

393. Unravelling of sexual differentiation mediated by Ire1 via Hx1-independent manners in Cryptococcus neoformans. Kwang-Woo Jung, Yong-Sun Bahn. Department of Biotechnology, College of Life science and Biotechnology, Yonsei University, Seoul, South Korea.

Sexual differentiation is a key biological process for generating genetically diverse offspring, which contributes to the increased fitness of certain species in its environmental niches. A human fungal pathogen, Cryptococcus neoformans, undergoes both bisexual and unisexual differentiations. Our previous study revealed that UPR (unfolded protein response) components include an evolutionarily conserved ER stress sensor Ire1 and a unique transcription factor Hxl1 that modulate ER stress, cell wall integrity, antifungal drug resistance, and virulence in C. neoformans. In this study, we for the first time provide several evidences showing that the UPR pathway governs both bisexual and unisexual differentiation of C. neoformans. In the serotype A strain backgrounds (H99 and KN99a), the ire1D mutants exhibit severe defects in bilateral cross whereas Hx1 appears to be dispensable for mating in both unilateral and bilateral crosses. Cell fusion efficiency of unilateral and bilateral crossing with ire1D mutants is significantly reduced when compared to WT cross, indicating that Ire1 promotes cell-to-cell fusion during mating. Moreover, deletion of the Ire1 gene blocked induction of pheromone-mediated conjugation tubes in crg1D mutants, which lack a RGS protein that negatively regulates pheromone responsive G-protein signaling. Unexpectedly, however, expression of the mating pheromone gene (MFA1) was strongly induced by the mutation of Ire1 gene in serotype A strain, suggesting that Ire1 has both positive and negative roles in mating of serotype A. C. neoformans. The ire1D mutants constructed in serotype D (JE221a and JEC20a) strains also exhibit mating defects similar serotype A ire1D mutant strains, whereas the hx1D mutants are dispensable for mating. It indicates that the role of Ire1 in sexual differentiation is evolutionary conserved in both serotype A and D strains. The unisexual mating, also known as monokaryotic fruiting, is an alternative differentiation process in C. neoformans. The deletion of Ire1 in the XL280 strain, which is used as a tester strain for same-sex mating, causes significant defects in filamentation whereas the hx1D mutants exhibit levels of filamentation indistinguishable from those of XL280. In conclusion, the Ire1 regulates both bisexual and unisexual mating of C. neoformans in Hx1-independent manners.

The Puf (Pumilio and FBF) family of RNA binding proteins is known to bind to 3′UTRs of mRNAs and is associated with regulatory functions including translation, stabilization and localization of transcripts. Investigation of the C. neoformans genome has revealed that it encodes four PUF proteins. PUF proteins are typically characterized by the presence of 8 consecutive Puf repeats, however variations do occur. Sequence analysis and evolutionary studies of Puf proteins in fungi has predicted Puf1, Puf2 and Puf3 of C. neoformans to contain 5, 3 and 8 PUM-HD repeats respectively. We hypothesize that Puf1, Puf2 and Puf3 act as RBPs and regulate gene expression. The PUF proteins characterized to date have been reported to bind to 3′ UTR sequence encompassing a UGUR tetranucleotide in their target RNA. Previous studies have reported that the core motif of UGUA followed by a variable region that Puf3 binds to is conserved from yeast to humans. Scanning of 3′UTRs of all the annotated genes of C. neoformans revealed Puf3 binding consensus sequence in the transcripts involved in phenomere signaling cascade. To test this hypothesis we performed bilateral mating assays for wild type and puf3D mutants. When equal numbers of opposite mating cells of puf3D mutants were cocultured on V8 agar, we observed that they were defective in filamentation as compared to the wild type cross. To determine the ability of puf3D mutants to produce pheromone, northern blot was done. The RNA obtained from puf3D mutants bilateral cross was probed for mating pheromone MFa in comparison to the wild type cross (H99a x K99a) and the induction of MFa was found to be normal in puf3D mutants mating. Also, a fusant colony formation assay revealed that filamentation defect of puf3D is not due to impaired cell fusion. Using fluorescent microscopy we have shown that mCherry tagged Puf3 localizes to areas of hyphal growth. Our results suggest that mate recognition and fusion do occur when puf3D mutants are crossed. We predict that the defect is in Puf3 mediated post fusion hyphal extension. Future studies will determine the mechanism of Puf3 regulation on potential target transcripts. Also we will identify the target/s of Puf1 and Puf2 and their mechanism of regulation which would enable us to establish a link between the physiology and the Puf regulon of C. neoformans.

395. Determining the direct targets of two master regulators of sexual development in Cryptococcus neoformans. Matthew E Mead², Emilia K Kruzel¹, Christina M Hull¹. 1) Department of Biomolecular Chemistry, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA, 53706; 2) Department of Medical Microbiology and Immunology, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA, 53706.

Cryptococcus neoformans is a major global fungal pathogen that causes disease primarily in immunocompromised individuals. Proposed infectious particles include spores, which are produced as a result of sexual development. In crosses between a and a cells this process is in part controlled by the homeodomain transcription factors Sxi1a and Sxi2a. To understand the molecular events governing development, we set out to identify direct transcriptional targets of the Sxi1a/Sxi2a heterodimer.

First, we created a haploid strain in which galactose-inducible promoters control the expression of Sxi1a and Sxi2a. This Sxi1 inducible strain allowed us to assess global transcript levels in the presence and absence of Sxi1 expression. At the same time, we compared changes in transcript levels in crosses between wild type (a x a) and sxi deletion (sxi1Δa x sxi2Δa) strains. We discovered that 185 genes exhibited a “Sxi-induced” regulation pattern in both experiments.

Upstream regions of these highly regulated genes were then analyzed using motif-finding algorithms, and a subset of the Sxi-induced genes was found to contain a sequence similar to one bound by Sxi1a/Sxi2a in vitro. Individual occurrences of the motif were tested in a Yeast 1-Hybrid system and shown to be bound by Sxi1a/Sxi2a in a sequence-specific and heterodimer-specific manner. An in vivo reporter assay was then used to show that these binding sites confer Sxi-dependent regulation to their downstream targets that is also sequence specific.

The list of direct targets studied so far includes numerous uncharacterized genes and putative transcriptional regulators likely important for controlling subsequent developmental transitions. Future studies will focus on building a complete, Sxi-dependent transcriptional network of development. This work will help us better understand a process that results in the production of a likely infectious particle in mammalian disease.

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396. Post-transcriptional gene regulation contributes to host temperature adaptation and virulence in Cryptococcus neoformans. Amanda L. Misener Bloom¹,², Kurtis Downey¹, Nathan K. Wool¹, John C. Panepinto¹. 1) Microbiology/Immunology, SUNY University at Buffalo, Buffalo, NY; 2) Witebsky Center for Microbial Pathogenesis and Immunology, SUNY University at Buffalo, Buffalo, NY.

In response to the hostile host environment, pathogens must undergo rapid reprogramming of gene expression to adapt to the stresses they encounter. Upon exposure to host temperature, Ribosomal protein (RP) transcripts are rapidly repressed in C. neoformans. We are interested in investigating specific mechanisms involved in this repression as there may be a critical process in host temperature adaptation. Using a mutant null of the major heat shock protein, CcpA, we discovered that repression of RP transcripts is in part due to enhanced degradation of RP transcripts. CcpA lacks a nucleic acid binding domain and therefore must be recruited to mRNA targets via RNA binding proteins. Using MEME analysis and chromaticographic techniques, we have identified a shared cis element in the 3′UTR of RP transcripts that is recognized by the zinc knuckle protein, Gis2. We are currently investigating the importance of this protein-RNA interaction in the expression of RP genes.

Host temperature-induced enhanced degradation of RP transcripts is also dependent on the dissociable RNA polymerase II subunit, RpB4. Specifically, we demonstrated that in an rpBΔ4 mutant, RP transcript deadenylation is impaired, suggesting that RpB4 may be required for Ccr4-targeted degradation. In addition, we observed that upon a shift to 37°C, RpB4 travels from the nucleus to the cytoplasm, supporting a role for RpB4 in coupling transcription and degradation. Interestingly, this coupling is not restricted to the RP transcripts, as RpB4 is also involved in enhanced decay of ER stress transcripts following their peak induction, one hour after a shift to host temperature. We have demonstrated that signaling through PKH enhances the degradation of the RP-transcripts in response to host temperature, but not the ER stress transcripts, highlighting the complexity of this system. We report that when transcription and degradation are uncoupled by the loss of RpB4, growth at host temperature is impaired and virulence in a mouse model of disseminated cryptococcosis is attenuated. Our data suggests that coupling of transcription and degradation via RpB4 allows the cell to co-ordinate different responses at specific times following exposure to host temperature, contributing to the ability of C. neoformans to adapt to this stress.


Cryptococcus neoformans is an environmental fungus that opportunistically infects immune compromised individuals, and is widely studied as a model basidiomyctete. The ability to adapt to host temperature is an essential pathogenic trait of C. neoformans, and the degradation of mRNA initiated by the major deadenylase Ccr4 appears to play an integral role in the temperature stress response of C. neoformans. Microarray analysis revealed that the largest functionally related group of mRNA stabilized in the ccr4D mutant encode ribosomal protein (RP) transcripts. An RNA-binding protein, identified as Gis2, has been shown to interact specifically with a cis element in the 3′UTR of RP transcripts. Gis2 is a small protein (19kDa), predominantly comprised of a
A series of zinc knuckles and a single glycine-arginine rich (GAR) region, which may serve as a target for protein arginine methyltransferases (RMTs). Primary sequence analysis revealed that, Gis2 from *C. neoformans* (CnGis2) shares significant primary sequence identity with Gis2 from *S. cerevisiae* (ScGis2) and the human CNBP/ZNF9 protein. However, ScGis2 does not contain a GAR motif; in contrast, the human ortholog contains a GAR element, and has been shown to undergo methylation by RMTs. An in vitro methylation assay demonstrated that recombinant CnGis2 can be methylated by one (or more) Cryptococcal RMTs. Investigation of the *C. neoformans* genome revealed there are 5 putative RMT genes. A *C. neoformans rmt5D* deletion mutant exhibited a severe growth defect and aberrant cell morphology at 39°C, suggesting that Rmt5 activity impacts temperature adaptation in *C. neoformans*. Further analysis of the rmt5D mutant and the remaining 4 *C. neoformans* RMTs will determine to contribution of protein arginine methylation to the virulence and stress tolerance of *C. neoformans*. 

**FULL POSTER SESSION ABSTRACTS**
398. UVE1 is a Photo-regulated Gene Required for the Protection of Mitochondrial DNA in Cryptococcus neoformans from UV Induced DNA Damage. Surbhi Verma, Alexander Idrumr. School Of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO 64110.

The UVE1 gene is an apurinic/apyrimidinic endonuclease, identified in a T-DNA insertion mutagenesis screen in the pathogenic fungus Cryptococcus neoformans. UVE1 mutation or deletion leads to a UV hypersensitive phenotype. The homologous gene in fission yeast Schizosaccharomyces pombe encodes apurinic/apyrimidinic endonuclease acting in the UVDE-dependent excision repair (UVER) pathway. C. neoformans UVE1 complements a S. pombe udve knockout strain, hence functionally similar. In Cryptococcus, the Bwc1-Bwc2 photoreceptor complex regulates mating, virulence and ultraviolet radiation (UV) stress tolerance. How the Bwc1-Bwc2 complex regulates these functions is not clear. We discovered that UVE1 is photoregulated in Bwc1-Bwc2 dependent manner in Cryptococcus, and in Neurospora crassa and Phycymyces blakesleeanus that represent two other major lineages in the fungi. Overexpression of UVE1 in bwc1 mutants rescues their UV sensitivity phenotype and gel mobility shift experiments show binding of Bwc2 to the UVE1 promoter. These experiments indicate that UVE1 is a direct downstream target for the Bwc1-Bwc2 complex, required for UV stress tolerance. Uve1-GFP fusions localize to the mitochondria in C. neoformans. Hence in Cryptococcus, UVE1 is a key photo-regulated gene responsible for tolerance to UV stress for protection of the mitochondrial genome.

399. Uve1 endonuclease protects Cryptococcus neoformans from UV damage through regulation by the White collar complex. Surbhi Verma, Alexander Idrumr. School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO.

In Cryptococcus neoformans the Bwc1-Bwc2 photoreceptor complex regulates mating, virulence and ultraviolet radiation (UV) stress tolerance. We identify and characterize a gene, UVE1, whose mutation leads to a UV hypersensitive phenotype. The homologous gene in Schizosaccharomyces pombe encodes an apurinic/apyrimidinic endonuclease acting in the UVDE-dependent excision repair (UVER) pathway. C. neoformans UVE1 complements a S. pombe Udve knockout strain. UVE1 is photoregulated, in a Bwc1-dependent manner in Cryptococcus, as well as in Neurospora crassa and Phycymyces blakesleeanus. Overexpression of UVE1 in bwc1 mutants rescues their UV sensitivity phenotype and gel mobility shift experiments show binding of Bwc2 to the UVE1 promoter, indicating that UVE1 is a direct downstream target for the Bwc1-Bwc2 complex. Uve1-GFP fusions localize to the mitochondria. In C. neoformans UVE1 is a key gene regulated in response to light that is responsible for tolerance to UV stress for protection of the mitochondrial genome.


The saprophytic white rot fungus S. commune, is involved in the degradation of complex organic molecules including lignin as well as refractory organic matter from black slate with the help of different exoenzymes. Thus, the genome sequence of S. commune was used to gain an insight into the functional analysis of laccases and laccase-like enzymes. Laccases are multi-enzyme oxidases that catalyze oxidation of a wide spectrum of organic and inorganic substances. In most fungi, laccases are found to be multigene families producing isoenzymes with multiple functions. Two laccases and four laccase-like genes have been identified from the genome of S. commune. Differential regulation of individual genes was analysed at the transcript level by quantitative real-time PCR. Individual laccase genes showed distinct expression profiles during fungal development, morphogenesis and during substrate utilization. Genes icc1 and icc4 seem to play a role during the primordial formation for fruiting bodies phase and icc2 and icc6 were found to be related to the dikaryotic phase and vegetative growth. Gene icc5 is regulated during fruitbody formation. Various stress responsive elements (XRE, STRE) could be detected in all respective promoters indicating an influence of aromatic compounds and stress molecules in transcriptional regulation. Characterization of laccase mutants with respect to organic matter and black slate degradation is linking these data to different functions of single laccase and laccase-like genes.

401. Regulation of DNA repair genes expression by UV stress in Neurospora crassa. Tsukasa Takahashi, Makoto Fujimura, Akihiko Ichishi. Faculty of Life Science, Toyo University, Ora-gun, Gunma, Japan.

In all organisms, DNA is constantly damaged by endogenous and exogenous factors such as environments and chemicals. In these genotoxicins, ultraviolet (UV) irradiation induces DNA damage such as cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs). N. crassa has three mechanisms to repair UV-damaged DNA; nucleotide excision repair (NER), UV dependent repair (UVDR), photoreactivation (PR). Because UV-induced DNA lesions are efficiently removed by these DNA repair systems, N. crassa shows high resistance to UV as compared to other species. In human and S. cerevisiae, it has been reported that some of DNA repair genes involved in removal of UV-damaged DNA were induced by UV irradiation. Furthermore, some MAP kinase pathways were activated in response to UV irradiation in human. In N. crassa, characteristics of DNA repair gene mutants have been performed in detail, but relationship between expression of these genes and UV stress are not clear yet. Thus, we investigated whether UV stress is involved in regulation of expression of DNA repair genes, and whether UV stress activates MAP kinase pathway like a human. We show that some DNA repair genes such as mus-40, mus-43 were up-regulated by UV irradiation. The OS-2 MAP kinase, involved in response to osmotic stress in N. crassa, was activated by UV irradiation, and then expression of mus-40, mus-43 were not induced after UV irradiation in os-2 mutant. In addition, os-2 mutant was more sensitive to UV irradiation than the wild-type. These data suggest that UV stress upregulates some DNA repair genes and UV signal was partially transmitted by OS MAP kinase cascade, in N. crassa.

402. Diverse classes of small RNAs originating from genomic hotspots, trNA and the mitochondrial genome in Phytophthora infestans. Sultana N. Jahan1, Anna K. M. Åsman1, Ramesh R. Vetukuri1, Anna D. Avrova2, Stephen C. Whisson2, Christina Dixiels3. 1) Department of Plant Biology and Forest Genetics, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, PO-Box 7080, SE-75007, Uppsala, Sweden; 2) Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee DD2 5DA, United Kingdom.

Phytophthora infestans is the oomycete pathogen responsible for the devastating late blight disease on potato and tomato. P. infestans is notorious for its ability to evolve to overcome resistant potato varieties. The genome of this pathogen has been sequenced and revealed vast numbers of transposon sequences, and hundreds of disease-promoting effector proteins. We are aiming at understanding gene-silencing mechanisms in P. infestans including deciphering roles of small non-coding RNAs. In our previous work we have shown that P. infestans has an active RNA silencing pathway (Vetukuri et al. 2011). We also performed deep sequencing of sRNAs from P. infestans and knocked down the genes encoding the RNA silencing components Argonaute and Dicer in order to investigate their roles in sRNA biogenesis (Vetukuri et al. 2012). Here, we describe the distribution of genomic sites from which sRNAs originate. Genome-wide analysis of sRNAs revealed diverse classes mapping to genomic sources such as tRNAs, rRNAs, genomic RNA ‘hotspots’, and the mitochondrial genome. Most tRNA-derived RNA fragments (tRFs) mapped to the sense strand of the 5’-halves of mature tRNAs and peaked at 27 and 30 nt lengths. In accordance with reports from other organisms (Franzén et al. 2011), the tRFs mapped to different tRNA isoacceptors with unequal frequencies, the lile_tRNA_Cluster_0 showing the highest proportion of mapping sRNAs. We are presently using our Dicer knockdown transformants to investigate the tRF biogenesis mechanism. Another interesting group of sRNAs are those that map to transposons that have close-by neighboring RXLR-effector genes.
One such example is PiAV2, which is located just 231 bp from a class II transposon. The presence of sRNAs mapping to both PiAV2 and the nearby transposon indicate that RNA silencing may play a role in regulation of this important effector gene. Over a hundred additional predicted genes were found to be sRNA hotspots in our data: Cinkler effector genes, arrays of duplicated genes, potentially antisense overlapping transcripts, and genes containing transposon insertions. Present paper task is to unveil the role that sRNAs might play in their regulation.


Plant pathogenic microbes secrete an arsenal of small secreted proteins (SSPs) acting as effectors that modulate host immunity to facilitate infection. In Eukaryotic phytopathogens, SSP-encoding genes are often located in particular genomic environments and show waves of concerted expression at diverse stages of plant infection. To date, little is known about the regulation of their expression. *Leptosphaeria maculans* is an ascomycete fungus responsible for the most devastating disease of oilseed rape (*Brassica napus*). The sequencing of its genome revealed a bipartite structure alternating gene rich GC-isochores and gene poor AT-isochoress made up of mosaics of transposable elements. The AT-isochoress encompass one third of the genome and are enriched in putative effector genes that present the same expression pattern (no or a low expression level during in vitro growth and a strong over-expression during primary infection). Here, we investigated the involvement of an epigenetic control in the regulation of effector gene expression. For this purpose, we silenced expression of two key players of heterochromatin remodeling, i.e. HP1 and DIM5, by RNAi and used HP1::GFP as a heterochromatin marker. Whole genome oligoarrays were done in silenced-HP1 and silenced-DIM5 isolates to analyze the involvement of HP1 and DIM5 on gene expression according to their function and location. We evaluated the effect of a change of genomic context from AT-isochoress to GC-isochoress on the expression of effector genes. Silencing of DIM5 resulted in lack of chromatin condensation. The silencing of HP1 and DIM5 resulted in an over-expression of pathogenicity-related genes during in vitro growth, with a favored influence on SSP-encoding genes in AT-isochoress. The “moving” of effector genes corroboration transcriptomic analysis as it led to a strong overexpression of effector genes during in vitro growth. These data strongly suggest that an epigenetic control represses the expression of effector genes located in AT-isochoress during in vitro growth, which is, to our knowledge, the first description of an epigenetic control, relying on HP1 and DIM5, exerted on effector-encoding genes expression. Switch toward pathogenesis lifts this repression based on chromatin-structure, rendering promoters of effector genes accessible to specific transcription factors.


Recently we were successful in establishing a genetic network for sexual development and ascospore germination in the homothallic filamentous fungus *Sordaria macrospora* [1, 2]. Central components of this network are three G-protein alpha subunits (GSA), an adenyl cyclase SAC1, and the transcription factor STE12. The three G-proteins (GSA1, GSA2 and GSA3) have different roles in developmental processes. GSA1 and GSA2 are important for sexual propagation and the generation of perithecia, while GSA3 is essential for proper ascospore germination. Interestingly, the phenotypes of mutants lacking fungal NAD(P)H oxidases (NOX) resemble the known Dgsa phenotypes: DnoxA shows an arrest of sexual development and ascospores from a DnoxB mutant fail to germinate. These similarities raised the question, whether the GSA proteins and NOX enzymes are part of identical signaling pathways. To verify this hypothesis, we generated knockout mutants of both NOX A and B isoforms and their regulator NOXR in S. macrospora. Our hypothesis was further supported by the comparison of these mutants with gsa deletion mutants by measuring hyphal fusion events, quantification of reactive oxygen species and ascospore germination. The generation of double mutants and complementation studies with constitutive gsa1 derivatives enabled us to propose an interactive NOX-GSA network for sexual development and ascospore germination. References: 1. Kameredwed, J., M. Jansson, M. Nowrousian, S. Pöggeler, and U. Kück, *Sordaria macrospora*, a model organism to study fungal cellular development. European journal of cell biology. 2010. 89(12): p. 864-72.

405. The bZIP transcription factor Atf1 acts as a global regulator for secondary metabolite production in *Fusarium fujikuroi*. Sabine E. Albermann, Bettina Tuczynski. IBBP, WWU Muenster, Schlossplatz 8, 48143 Muenster, Germany.

The activating transcription factor 1 (Atf1) belongs to the bZIP transcription factor family and is known to have a great impact on stress responses mediated by the mitogen activated protein kinase (MAPK) cascade in fission yeast. In this pathway, activation of the transcription factor is achieved by phosphorylation via the kinase Sty1. Furthermore, the transcription factor plays a role in sexual and asexual development which was observed for several filamentous fungi e.g. in *Aspergillus* species where it affects conidiospore germination. Atf1 can also act as a virulence factor which was described for its homologue in the rye pathogen *Claviceps purpurea*. However, involvement of Atf1 in secondary metabolism was first observed in the grey mould Botrytis cinerea. As Atf1 seems to play a crucial role in different processes, this transcription factor was also investigated in the rice pathogen *Fusarium fujikuroi*. For this purpose, deletion mutants of atf1 and the Sty1 homologue sak1, the putative kinase for Atf1, were cultivated under varying conditions. HPLC analysis of the secondary metabolite spectrum revealed a drastic change in the production level of several metabolites. Gibberellic acids, for instance, are down-regulated up to 50 % in DAtf1 compared to the wild-type, whereas the amount of gibberellins in the Dsak mutant is about twice as much in the wild-type. Furthermore, applied salt stress dramatically enhances mycotoxin production in the DAtf1 mutants, while the deletion mutant Dsak1 is not able to grow at all. Plate assays applying different stressors to the strains revealed involvement of both proteins in the osmotic stress response. However, reactive oxygen species and cell wall damaging agents do not seem to have an impact on their growth. In contrast, reduced protoplast formation was observed for DAtf1 mutants and even more significantly in Dsak. Therefore, it is very likely, that the cell wall composition and integrity is changed in these mutants. Summarizing, Atf1 and Sak1 are involved in various processes such as secondary metabolite production, cell wall integrity as well as in stress responses. The obtained information leads to the conclusion that Sak1 might be the kinase responsible for Atf1 phosphorylation. But there certainly have to be more factors to be involved in activation of this transcription factor.


*Fusarium fujikuroi* is well known for its ability to produce gibberellins, growth-promoting plant hormones with agricultural applications. Recently, this fungus has become a model system in the research of other metabolic pathways, including carotenoid biosynthesis. This fungus produces an acidic apocarotenoid, neurosporaxanthin, through the activity of the enzymes encoded by five structural genes, whose expression is induced by light. We are interested in the molecular basis of this regulation. As usually found in fungi, the *F. fujikuroi* genome contains genes for WC-1 and WC-2 orthologs. In contrast to other species with light-induced carotenogenesis, e.g., *Neurospora crassa* or *Phycomyces blakesleeanus*, this photoresponse is not impaired in null mutants of the only wc-1-like gene of *F. fujikuroi*, *wcaA*. Therefore, we are analyzing the role of other blue-light photoreceptors. Here we described...
the identification, regulation and targeted mutation of the gene vvd4, ortholog of the N. crassa vivid (vvd) gene. The predicted F. fujikuroi VdA protein is similar to VVD in size (198 aa compared to 186 aa) and sequence (87 identical positions). Deletion of vvdA in F. fujikuroi results in a significant reduction in pigmentation and carotenoid production, a regulatory effect opposite to the enhanced carotenoid accumulation characteristic of the vvd mutants of N. crassa. Additionally, vvdA mutant colonies exhibit a different aspect in the light, apparently due to more compact development or aerial mycelia. As found for vvd in N. crassa, expression of vvdA in F. fujikuroi cultures is strongly stimulated by light, an activation which is severely reduced in the wcOA mutants. Accordingly, the alterations exhibited by the vvdA mutants are only apparent under illumination. Our results suggest that VdA participates in the photoreceptor machinery responsible for carotenoid photoinduction in F. fujikuroi.

407. Gene expression of secondary metabolism gene clusters by different Fusarium species during in planta infection. J. Espino, M. Muensterkoetter, U. Guldener, B. Tudyński. 1) Institut of Plant Biology and Biotechnology, Westf. Wilhelms University, Münster Germany; 2) 2Institut of Bioinformatics and Systems Biology, Helmholtz Zentrum Muenchen (GmbH), Ingolstaedter Landstrasse 1. 85764 Neuerberg, Germany.

The filamentous fungus Fusarium fujikuroi, complex (GFC) comprises about 50 Fusarium species with similar characteristics, which are responsible for an array of plant diseases, causing devastating losses in agriculture. The majority of their members is able to produce different toxins which can contaminate food and feed worldwide. Despite of their similarities, they differ in their spectrum and amount of secondary metabolites (SM) production, probably due to different natural hosts. For example, Fusarium verticilloides is considered as a fumonisins producer and attacks mainly maize, whereas Fusarium fujikuroi causes the bakanane disease in rice, secreting the phytotoxone gibberellin beside several other products. Another fungus, Fusarium mangiferae, causes the mango malformation and is neither able to produce fumonisins nor gibberellins. And Fusarium proliferatum produces a very broad spectrum of mycotoxins and infects mainly maize. In the present study we compared the in planta expression profiles for different secondary metabolism gene clusters in these four species of the GFC, and also the one of Fusarium oxysporum as an outgroup not belonging to the GFC. So far, gene expression studies have been done for these fungi mainly in vitro, showing differential regulation mechanisms, e.g. in response to nitrogen availability. But not much is known about the gene expression during plant infection. We have performed an infection assay in maize and rice and quantified the fungal biomass in the roots by quantitative PCR using genomic DNA to determine the ratio between plant and fungal biomass. The results of this study show differences between the species regarding colonization of the host and expression of SM. Surprisingly the high expression of some gene clusters, which were never expressed before in vitro, suggests a specific induction by plant signals.

408. A cis-acting factor modulating the transcription of FUM1 in Fusarium verticilloides. Valeria Montis, Matias Pasquale, Ivan Visentin, Petr Karlovsky, Francesca Cardinale. 1) Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, 10095 Grugliasco (TO), Italy; 2) Environment and Agrobiotechnology Dept, CRP GABRIEL LIPPMANN, Belvaux, Luxembourg; 3) Department of Crop Sciences, Molecular Phytopathology and Mycotoxin Research, University of Göttingen, D-37077 Göttingen, Germany.

Fumonisins-biosynthetic FUM genes are clustered and co-expressed in toxicon producing. By overexpression analyses, we identified a motif in promoters of clustered FUM genes in both fumonisins producers F. verticilloides and A. niger. The same motifs was not found in various FUM gene homologues of fungi that do not produce fumonisins. Deletion of the main 6-mer in FvFUM1 promoter compromises its gene expression both in planta and in vitro. We hypothesize that such motif may be important for clustered FUM genes coordinated transcription, being the core of a transcriptional factor-binding site for a putative Zn-finger protein.


The rice pathogen Fusarium fujikuroi produces economically important secondary metabolites like gibberellic acids and carotenoids as well as mycotoxins like biakverin and fusarin C. Their production is activated in response to environmental stimuli such as light, pH or nutrient availability. In this study, we evaluate the effects of light and different putative light receptors on growth and differentiation as well as secondary metabolism. Bimolecular fluorescence complementation proved that homologs of the Neurospora crassa White Collar proteins in F. fujikuroi (WcOA and WcOB) form a nuclear localized complex (WCC) that is needed for full functionality. Deletion and complementation of both genes revealed that the WCC represses biakverin gene expression in constant light conditions and induces immediate light-dependent carotenoid gene expression as shown by northern blot analyses. Additionally the WCC represses conidiogenesis in response to light. The effects observed regarding biakverin and carotenoid gene expressions as well as conidiogenesis are antagonistically to the ones observed in the velvet mutant, making a connection between the WCC and the velvet complex feasible, similarly to the situation in Aspergillus nidulans. Since carotenoid production was maintained in both wcOA and wcOB single as well as in wcOA/Δ double mutants in constant light conditions, we focused on characterization of additional putative light receptors in F. fujikuroi. Deletion of the phytochrome-like-encoding gene phl1 did not show any significant phenotype. Deletion of phl1 coding for a cryptocrome/photolyase demonstrated impaired carotenoid biosynthesis gene expression upon exposure to light. Additionally, gene expression and HPLC analyses of these mutants demonstrated loss of fusarin C gene expression and concomitant production formation as compared to the wild type, suggesting a distinct transcriptional activity for this rarely characterized class of enzymes. Finally UV mutagenesis experiments and qRT-PCR demonstrate that WcOA, WcOB and Phl1 are involved in UV-damage repair most likely by transcriptionally activating phl1, encoding a CDP-photolyase. The data presented here allow us to draw a first model of how light receptors function in a signaling network in the rice pathogen F. fujikuroi.


The filamentous fungus Fusarium graminearum infects cereals and corn. It is one of the main causal agent of “Fusarium Head Blight” and “Maize Ear Rot”. During infection, it produces mycotoxins belonging to the trichothecenes family that accumulate in the grains. Although the biosynthetic pathway involving specific Tri genes has been elucidated, the global regulation of toxin biosynthesis remains enigmatic. It is now established that oxidative stress modulates the production of toxins by F. graminearum. H₂O₂ added in liquid cultures of this fungus enhances trichothecenes accumulation and increases Tri genes expression. Our working hypothesis is that a transcription factor regulates redox homeostasis, and is involved in Tri genes regulation. In the yeast Saccharomyces cerevisiae, the transcription factor Yap1p mediates response to oxidative stress via nuclear re-localization and activation of genes coding for detoxification enzymes. In this study, we investigate the role of Yap1p homolog in F. graminearum, Fgap1, in response to oxidative stress and its eventual role in the regulation of trichothecene production. A deleted mutant and a strain expressing a constitutively activated form of the Fgap1 factor in F. graminearum were constructed. We cultured these mutants in GYEPE liquid medium supplemented with H₂O₂ to evaluate their sensitivity to oxidative stress and analyse their toxin production. The nuclear localization of constitutively activated Fgap1p as well as wild-type Fgap1p under oxidative stress by
H₂O₂ was analyzed. Expression profiles of genes encoding oxidative stress response enzymes potentially controlled by Fgap1p and of genes involved in the biosynthesis of type B trichothecenes were analyzed by Q-RT-PCR. Trichothecene accumulation is strongly enhanced in the deleted strain, with an increase in Tri genes expression. On the other hand, Tri genes expression and toxin accumulation are drastically repressed in the mutant in which Fgap1p is constitutively activated. Moreover, the level of expression of two genes encoding catalases is modulated in both mutants. The involvement of Fgap1 in other types of stress has also been investigated. In particular, cadmium and osmotic stress affect growth in the deleted strain.


Fusarium graminearum (telomorph: Gibberella zeae) is the casual agent of the head blight of cereal crops and produces mycotoxins such as trichothecenes and zearalenone in infected plants. The expression of genes involved in biosyntheses of these mycotoxins are controlled at the different levels ranging from by a pathway-specific transcription regulator (encoded by TR16 or ZEB2) to by a global regulator involved in chromatin remodeling. Here we focused on the function of FgLaeA in F. graminearum, which is an ortholog of the Aspergillus nidulans LaeA, encoding the global regulator for both secondary metabolism and sexual development. For functional analysis of FgLaeA in mycotoxin production, we used a transgenic F. graminearum strain expressing a firefly luciferase gene under control of TR16 or ZEB2 promoter as a reporter system. Targeted deletion of FgLaeA led to a dramatic reduction of luminescence in the reporter strain, indicating that FgLaeA controls the expression of both TR16 and ZEB2 in F. graminearum; the reduced toxin accumulation was further confirmed by HPLC analysis. In addition, the FgLaeA deletion strains exhibited not only albino phenotype on CM medium but also earlier formation of sexual fruiting bodies (perithecia) on carrot agar than its wild-type progenitor, the latter indicating that FgLaeA seems to negatively control the perithelial induction. Quantitative real-time PCR revealed that FgLaeA was expressed constitutively under both mycotoxin production and sexual development. Overexpression of a GFP-FgLaeA fusion construct in a FgLaeA-deletion strain recovered all the phenotypic changes to the wild-type levels, and led to constitutive expression of GFP in the entire cells at different developmental stages. A split luciferase assay for in vivo protein-protein interaction demonstrated that FgLaeA could not interact with FgveA, an ortholog of A. nidulans veA. Taken together, it is likely that FgLaeA controls both secondary metabolism and sexual development in F. graminearum, but the regulation pattern operated by FgLaeA is somewhat different from that by LaeA in A. nidulans.


The cellulolytic system of the fungus Stachybotrys microspora is characterized by the existence of several b-glucosidases. From a compilation of fungal b-glucosidases belonging to family GH1, we designed primers to isolate b-glucosidases by PCR. Using different primers combination, three different fragments genes were firstly obtained. Two of them are overlapping and constitute a novel gene named Smgb1A while the third one is a part of a second gene named Smgb1B. RT-PCR analysis showed the first gene is induced by cellulase and repressed by glucose while Smgb1B is equally expressed on both conditions. The identification of putative catalytic residues as well as the conserved glycone and aglycone binding sites was performed on Smgb1B deduced aminoacid sequence. The predicted secondary structure of Smgb1 confirmed its appurtenance to GH1 family: the presence of a classical (b/a)8 barrel and all the characteristic of subite -1 (glycone site).

413. The transcriptional factors XYR1 and CRE1 regulate the expression of Cellulolytic and Xylanolytic genes at carbon source dependent-manner in Hypocreac jecorina (Trichoderma reesei). Amanda C.C. Antônio, Lilian S. Castro, Wellington R. Pedersoli, Roberto N. Silva. Department of Biochemistry and Immunology, School, University of São Paulo, Ribeirão Preto-SP, São Paulo, Brazil.

The ascomycete Hypocrea jecorina (anamorph of Trichoderma reesei) is one of the most well studied cellulolytic fungus and widely used in the biotechnology industry, such as in the production of second generation ethanol, because it is a strong producer of hydrolytic enzymes such as cellulases and xylanases. The objective of this study was evaluate the gene expression and enzymatic activities of cellulases and xylanases in the Dxyr1 and Dcr1 mutants and compare with the parental T. reesei (QM9414), in three different carbon sources. The strains were grown in Mandels-Andreatto medium, supplemented with cellulose, sorphorose or glucose. The expression of 22 set cellulases and xylanases genes were evaluated by real-time PCR (qRT-PCR) and cellulolytic and xylanolytic activities were observed using different substrates. The cel6a, cel3a, cel7b, cel12, cel3e, xyn2 and sgo genes showed a significantly higher expression in the mutant Dcr1 when compared with the parental QM9414 and low expression of the cel1a, cel3d and cel61b genes was observed when compared the mutant Dxyr1 with the QM9414 on cellulose, sorphorose and glucose. Overall, all of cellulase and xylanase showed higher expression in mutant Dcr1 and low expression in mutant Dxyr1 in all studied conditions, when compared to QM9414. Concerning to enzymatic profiles, the activity of CMCase, b-glucosidase and Xylanases ranged also for the presence of specific carbon source. These results suggest that the deletion of the genes xyr1 and cre1 affects the formation of cellulases and xylanases directly at transcriptional level and shown to be specific and dependent of the carbon source.


A small heat shock protein gene, CpsHsp1, a ubiquitous chaperone in Cryphonectria parasitica, was characterized. The predicted protein sequence of CpsHsp1 gene contains a putative conserved domain, which is alpha crystallin domain (ACD) of alpha-crystallin-Hsp_p23-like superfamily. To characterize biological functions of the CpsHsp1 gene in the C. parasitica, the replacement vector for CpsHsp1-null mutant was designed to favor double crossover integration events. Disruption of the CpsHsp1 protein resulted in retarded growth rate, approximately 78.5% of the radial growth observed in the virus-free strain EP155/2. When the hypovirus CHV1 was transferred to the CpsHsp1-null mutant, all of the virus-containing CpsHsp1-null progeny displayed characteristics of invasive feeding hyphae, neare absence of the typical mycelial mat on the surface, and sparse aerial hyphae. Northern blot analysis showed little accumulation of the CpsHsp1 gene transcript under normal growth conditions. However, the accumulation of the CpsHsp1 gene transcript was induced in modified Bavendamm’s medium, which is a 0.7% tannic acid-supplemented malt extract agar. To examine the viral regulation of the induction, the CpsHsp1 induction pattern in the isogenic hypovirus strain UPE1 was compared with that in the wild-type strain EP155/2. Northern blot analysis of RNA from UPE1 cultured under induction conditions with tannic acid showed that hypoviral infection specifically reduced the level of CpsHsp1 transcript induced by tannic acid. To determine whether CpsHsp1 is induced by cool or heat stress, we additionally observed difference in the expression, and induction pattern of CpsHsp1 between virus-free EP155/2 and virus-infected hypovirulent UPE1 strains by Northern blot analysis and Western blot analysis.
415. Artificial miRNA constructs for Phytophthora sojae transformation. Stephanie B. Bollmann, Felipe D. Arredondo, Noah Fahlgren, James C. Carrington, Niklaus J. Grünwald, Brett M. Tyler. 1) Center for Genome Research and Biocomputing, and Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR; 2) Donald Danforth Plant Science Center, St. Louis, MO; 3) Horticultural Crops Research Laboratory, USDA Agricultural Research Service, Corvallis, OR.

Phytophthora, a genus of fungal-like oomycetes, contains some of the most devastating plant pathogens, causing multi-billion dollar damage to crops, ornamental plants, and natural environments. The genomes of five Phytophthora species, including the soybean pathogen P. sojae, have recently been sequenced, with many more species soon to be completed. Gene regulation by small RNA pathways is highly conserved among eukaryotes, although little is known about small RNA pathways in the Stramenopile kingdom. Two Dicer homologs, DCL1 and DCL2, and one RDR homolog were cloned and annotated from P. sojae, and gene expression analysis revealed only minor changes in transcript levels among different lifestages and infection timepoints. At this point, the role of the two oomycete Dicer homologs is only speculated. This study aims to down-regulate DCL1 and DCL2 expression in order to analyze the contribution of each homolog to small RNA biogenesis. Traditional RNAi, such as overexpression of RNAi complementary to a target miRNA transcript, has been used to knockdown gene expression in Phytophthora, although the effect is most often short-lived. Dicer homologs are involved in the RNAi pathway, therefore this method may not be effective, especially for the homolog involved in the sRNA pathway. Artificial miRNAs, designed from endogenous miRNAs, have recently been used to target transcripts such as these. We designed artificial miRNA constructs based on the conserved Phytophthora miRNA found in P. sojae, targeting both DCL1 and DCL2 as well as the effector Avr1K, the histidine biosynthesis enzyme HISG, and GFP for controls. Analysis of transfectants is currently underway.

416. RNAi-dependent epimutations evolve antifungal drug resistance in the zygomycete fungal pathogen Mucor. Silvia Calo Varela, Cecelia Shertz, Robert J Bastidas, Soo Chan Lee, Piotr Mieczkowski, Joshua A Garnek, Rosa Ruiz-Vazquez, Santiago Torres-Martinez, Maria E Cardenas, Joseph Heitman. 1) Molecular Genetics and Microbiol, DUKE University Medical Center, Durham, NC; 2) High Throughput Sequencing Facility, CGGS, UNC, Chapel Hill, NC; 3) Department of Molecular Genetics and Microbiology, University of Murcia, Murcia, Spain.

Microorganisms evolve via a panopoly of mechanisms spanning aneuploidy, sexual/parasexual reproduction, mutators, Hsp90, and even prions. Mechanisms that may seem detrimental can be repurposed to generate diversity. The pathogenic fungus Mucor circinelloides grows as a hyphae aerobically, but as a yeast in anaerobic conditions or in the presence of the immunosuppressive drug FK506. FKBP12 is a protein folding enzyme conserved throughout eukaryotes that interacts with FK506 and mediates antifungal activity of this drug. The FK506-FKBP12 complex inhibits the protein phosphatase calcineurin and thereby blocks hyphal growth of M. circinelloides.Continued exposure to FK506 yields resistant isolates, which exhibit hyphal growth emerging from the yeast colony. Some isolates harbor a variety of mutations in the fkbA gene that encodes FKBP12. However, other isolates harbor no mutations in the fkbA gene. These unusual epimutant isolates also revert frequently within several generations of vegetative growth in drug-free media and are restored to wild-type (yeast growth in the presence of FK506). Northern and Western analyses revealed a loss of fkbA mRNA and FKBP12 protein in the epimutants. High-throughput sequencing and Northern blot also detected sRNA generated from fkbA in the epimutant strains, revealing a new role for RNAi in the development of transient, reversible resistance to an antifungal drug treatment. RNAi could be triggered via dsRNA production from an overlap in the 3’ regions of the mRNA of fkbA and its neighboring gene patA, which encodes a putative polyamine transporter. Our results reveal a novel epigenetic RNAi-based epimutation mechanism controlling phenotypic plasticity in fungi.

417. Heterochromatic marks are involved in the repression of plant-regulated secondary metabolism in Epichloë festucae and for symbiotic interaction with the host perennial ryegrass. Tetsuya Chugo, Barry Scott. Institute of Molecular Biosciences, Massey University, Palmerston North, New Zealand.

The fungal endophyte Epichloë festucae systemically colonizes perennial ryegrass (Lolium perenne), and produces a range of secondary metabolites to protect the host plant. The alkaloid peramine provides protection against insect herbivory. Protection against mammalian herbivory is afforded by the production of alkaloids, such as ergot alkaloids and lolitremes. Using the E. festucae- perennial ryegrass symbiotic association as a model experimental system we have shown that gene clusters for the synthesis of these biotic/protective metabolites are all preferentially and highly expressed in planta, but not expressed in culture. Recent work showed that disruption of genes encoding either heterochromatin protein-1 (HepA) or the H3K9 methyltransferase (CtD1) in Aspergillus nidulans resulted in enhanced expression of secondary metabolite gene clusters, demonstrating that heterochromatic marks are involved in the repression of these clusters. Thus, we hypothesized that plant-regulated E. festucae secondary metabolite gene clusters have a repressive chromatin structure in culture, and chromatin remodeling is an important component for activation of these gene clusters in planta. To test this hypothesis we have deleted the hepA and ctD1 homologues from E. festucae by targeted gene replacement. Deletion of hepA resulted in a slight reduction in culture radial growth whereas deletion of ctD1 resulted in a severe reduction. Western blot analysis revealed that the level of H3K9 trimethylation (H3K9me3) is dramatically decreased in DctD1 mutants. Expression levels of ltmM & ltmM (cluster 1) and ltmP & ltmP (cluster 2), as measured by qRT-PCR, increased in both the DhepA and DctD1 mutants grown in a defined medium. Introduction of a wild-type allele of either hepA or ctD1 complemented DhepA or DctD1 mutant phenotypes, respectively. In addition, the DhepA mutant has a dramatic host interaction phenotype, inducing severe stunting and premature senescence of the ryegrass host. On the other hand, DctD1 mutant is an infection mutant. These results strongly suggest that heterochromatic marks regulate both secondary metabolite gene expression and the mutualistic symbiotic interaction of E. festucae with its host perennial ryegrass.


To better understand mechanisms of cellulase gene regulation and genome-wide gene regulation enabling robust enzyme secretion, we studied the conservation of gene regulation by cellulase degradation regulator 2 (CLR-2) in Neurospora crassa and Aspergillus nidulans. Misexpression of CLR-2 under normally repressive and non-inducing culture conditions was sufficient for cellulases secretion in N. crassa, but not A. nidulans. We used RNAseq to map the transcriptome in wild-type, deletion and mis-expression strains of both species. We identified a cohort of conserved enzymes with conserved sequence and CLR-2 dependent regulation across evolutionarily divergent ascomycetes, which represent a core of essential enzymes for degradation of complex cellulosic substrates. We also identified non-conserved CLR-2 regulated genes in each species, which may have function specific to a particular substrate or niche. These data suggest that manipulation of CLR-2 has significant potential for improved cellulase production from industrial production strains.

419. The transcriptional repressor CRE-1 regulates glycogen metabolism in Neurospora crassa. Fernanda B. Cupertino, Stela Virgilio, Fernanda Z. Freitas, Thiago S. Candido, Maria Célia Bertolini. Instituto de Quimica,UNESP, Araçarigua, São Paulo, Brazil.

In Neurospora crassa the RCO-1 co-repressor, an orthologue of the yeast Tup1, has been identified as a protein involved in glycogen metabolism regulation in a screening of a transcription factor knockout-enforced strains set. The S. cerevisiae CRE1 Tup1 protein participates in the Tup1-Ssn6
complex, which represses the expression of genes through its interaction with other protein partners, such as the yeast Mig1 repressor, mediator of carbon catabolite repression (CCR). The N. crassa CRE-1 is an orthologue of Mig1p and has been reported to code for a number of genes by binding to the CreA motif 5'-SYGGRG-3'. In this work we investigated if CRE-1 transcription factor regulates glycogen metabolism and whether depends on the RCO-1/RM-1 complex, the orthologue of the yeast Tup1/Ssn6. First, we demonstrated that CRE-1 is involved in catabolic repression in N. crassa. Growth of the wild-type strain in VM medium containing xylose + 1 mM 2-deoxyglucose (2-DG) was drastically reduced, while growth in the cre-1KO was not affected by 2-DG. The cre-1KO, rco-1KO and rcm-1KO mutant strains showed impaired glycogen accumulation when compared to the wild-type strain, being the higher levels observed in the cre-1KO strain. Glycogen accumulated by this mutant strain was much higher than the wild-type strain under both repressed (glucose) and non-repressed (xylose) carbon source, suggesting that the carbon source has no influence in the glycogen accumulated. The DNA motif 5'-SYGGRG-3' was identified in the promoters of genes encoding for synthesis (gpn, gsn, and gbn) and degradation enzymes (gpn, gdn). Binding of the GST::CRE-1 recombinant protein to the CRE-1 motifs in the gpn and gpn promoters was confirmed by DNA gel shift, indicating that both genes must be regulated by CRE-1. Gene expression was analyzed by qRT-PCR and all genes were differently expressed in the mutant strains, and for some genes, gene expression correlated well with the levels of glycogen accumulated. CHIP-PCR assay was performed to confirm in vivo CRE-1 binding to the promoters and the results suggested that the CRE-1, RCO-1 and RCM-1 proteins likely interacted. All results together indicated that the CRE-1 transcription factor acts as a repressor in glycogen regulation, and might require the interaction with RCO-1 and RCM-1 co-repressors. Supported by FAPESP and CNPq.

420. Transcriptional Response to Hypoxia in the Dimorphic Fungus Histoplasma capsulatum. Juwen C. DuBois1,2, A. George Smulian1,2. 1) Pathobiology and Molecular Medicine Graduate Program, University of Cincinnati, Cincinnati, OH; 2) Infectious Disease Division, University of Cincinnati, Cincinnati, OH; 3) Cincinnati VA Medical Center, Cincinnati OH.

Background and purpose: The incidence of life-threatening fungal infections has increased because of a rapidly growing population of immunocompromised individuals. Histoplasma capsulatum (Hc) is a thermally dimorphic fungal pathogen which causes pulmonary and systemic disease in both immunocompetent and immunocompromised individuals. All fungal pathogens encounter microenvironmental stresses as they colonize and infect the mammalian host and their ability to adapt to environmental changes is critical for pathogenicity. While oxygen is essential for the survival of most eukaryotic organisms, low oxygen availability or hypoxia is one microenvironmental stress that is known to occur during infection. Therefore, we aim to determine the mechanism by which Hc is able to survive and adapt to hypoxia by determining the significance of its transcriptional response to hypoxia.

Methods: Hc yeast cells were cultured to mid-log phase then transferred to normoxic (+21%) or hypoxic conditions (<1% O2). After culture under these conditions, Hc viability was determined and RNA extracted. RNA was subjected to high throughput RNA sequencing (RNA-seq) and quantitative real-time PCR analysis of the predicted upregulated genes, HcSREBP and HcUPC2. Selected key genes were downregulated using RNA-mediated silencing and the viability of Hc hypoxia in the absence of expression of these genes was assessed. Results: After exposure to hypoxia, Hc viability remained between 80-90% through all time points. RNA-seq analysis demonstrated a significant increase in expression of NTS0, a swim domain protein-DNA binding protein, a predicted ABC transporter, a NADPH oxidoreductase and the RSP guanine nucleotide exchange factor. Although RNA-seq analysis did not reveal significant increases in SREBP and UPC2, these genes are known hypoxia responsive transcriptional regulators in fungi. Computational transcription factor binding site analysis revealed predicted human SREBP and Aspergillus fumigatus SrBa binding sites upstream of all genes upregulated under hypoxic conditions. RNAi-mediated silencing of HcSREBP and HcUPC2 gene expression impaired the ability of Hc to grow under hypoxia. Conclusion: Creation of Hc strains with impaired survival under hypoxia allows characterization of the role of hypoxic adaptation to Hc intracellular survival.

421. The Neurospora crassa SEB1 transcription factor binds to STRE motif and modulates stress responses through different pathways. F. Freitas, F. Cupertino, R. Gonçalves, M. C. Bertolini. Instituto de Química-UNESP, Araquari, São Paulo State, Brazil.

Glycogen is a polysaccharide widely distributed in microorganisms and animal cells. Its metabolism is highly conserved in eukaryotes, being glycogen synthase (GSS) and glycogen phosphorylase (GPN) the rate-limiting enzymes in the synthesis and degradation, respectively. Previous studies demonstrated that the down-regulation of the gene encoding GSN (gsn) under heat shock might require the two STress Response Element (STRE) existent in its 5'-flanking region. In Saccharomyces cerevisiae the Msn2/4p transcription factors bind to STRE regulating expression of genes under different stress conditions. However orthologues of Msn2/4p were not identified in N. crassa. The ORF NCU02671 product (named here as SEB1) was identified in pull-down assays and mass spectrometry as a protein able to bind to STRE and modulates stress responses. This protein has two C2H2 Zn-finger DNA binding domains and shares 40% and 32% identity with, respectively, the Trichoderma atroviride Seb1 and Aspergillus fumigatus SebA transcription factors. While SebB is the functional orthologue of Msn2p/4p, SebA plays a more complex role, participating in several stress tolerance pathways, such as heat shock and oxidative stresses. N. crassa GST::SEB1 bound specifically in vitro to both STRE motifs of the gsn promoter as demonstrated by EMSA. TTR:SEB1 mutant strain accumulated higher levels of glycogen than the wild-type strain under vegetative growth (30°C) and the glycogen content was drastically reduced after heat stress (45°C). The gsn and gpn gene expression at 30°C was increased in the mutant strain as compared to the wild-type strain. However at 45°C gpn expression in the mutant strain was much higher than that in the wild-type strain, what may explain the reduced levels of glycogen in the mutant strain under the same temperature. Growth of the seb1KO mutant strain under different stress conditions showed that SEB1 is involved in heat, osmotic and oxidative stresses. The mutant strain was unable to grow at 45°C and growth was recovered when the culture was shifted back to 30°C. Moreover, the mutant strain displayed defective growth in the presence of NaCl, sorbitol, and pararacetamol but not in the presence of hydrogen peroxide, suggesting that SEB1 controls gene expression under different stress conditions acting through different signaling pathways. Supported by FAPESP, CNPq, and CAPES.

422. WITHDRAWN

423. Regulation of glycolysis and gluconeogenesis by antisense transcription in Aspergillus nidulans? Michael Hynes1, Koon Ho Wong2, Sandra Murray1.

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The last step in glycolysis is carried out by pyruvate kinase, encoded by pkIA, converting phospho-enol-pyruvate to pyruvate for metabolism to oxaloacetate and acetyl-CoA. The key step in gluconeogenesis is conversion of oxaloacetate to phospho-enol-pyruvate by PEP carboxykinase, encoded by acuf. Simultaneous activity of these enzymes would generate a nasty futile cycle. A number of observations suggests that control of the expression of these two genes involves activation of sense transcription and negative control by activation of antisense transcription. For pkIA, ChIP studies have found binding of the gluconeogenic activators AcuK and AcuM and of the acetate dependent FacB activator in the downstream region. Cognate binding sites are conserved in filamentous ascomycetes. RNA Seq, poll ChIP and RT-PCR analysis indicates antisense transcription during growth on acetate or proline as carbon sources. Old data (de Graaf, van den Broek, Visser; Cur: Genetics 13: 315, 1988) showed that transformation of a construct lacking these 3' sites resulted in inappropriate pkIA expression on acetate. In response to growth on gluconeogenic carbon sources, the acuf gene is activated by AcuK and
AcuM binding to sites in the 5’ upstream region. Studies with an acuF-lacZ gene fusion indicate positive control by AcuM and AcuR but a loss of the glucose repression observed in Northern suggesting negative regulation acting via 3’ sequences in response to growth on glycolytic carbon sources. Support for this is provided by transcription studies. Modulation of the balance between the opposing activities of these two gene products is proposed to result from transcriptional interference involving collision of RNA polymerase molecules.


The cellulose- and cellulose-responsive induction of the cellobiohydrolase I (cbh1) and carboxymethylcellulase 2 (cmc2) genes is not regulated by CINR, a Zn(II)Cys6 transcriptional activator, in Aspergillus aculeatus. We have identified a novel activator containing a Zn(II)Cys6 binuclear cluster motif designated as cellulose-response regulator (CblR), and which is not homologous to CblR, a transcriptional activator controlling cellulose-responsive induction in Neurospora crassa and Aspergillus nidulans. Interestingly, CblR regulates not only the expression of cbh1 and cmc2 but also genes regulated by CINR under the cellulose-inducing condition. However, the CblR overexpression did not increase the expression of all genes under control of CINR. Therefore, we predict that CblR functions cooperatively with other factor(s). This study reports the CblR function and the functional relationships among CblR, CblR-interacting factor, and CblR homolog on cellulose-responsive induction in A. aculeatus. CblR-interacting proteins were screened from a prey library composed of CblR paralogs and transcription factors controlling the expression of glycoside hydrolase genes by yeast two-hybrid method. CblR2, 42% identity to CblR, was so far isolated as a CblR interacting protein. To investigate the correlation between CblR and CblR2 function, gene expression profiles under control of CblR were assessed in the cblR and cblR2 single disruption mutants, and cblR/cblR2 double disruption mutant. The expression of cbh1 and cmc2 decreased to the same level in all three mutants under the cellulose-inducing condition. Furthermore, transcripts of the cbh1 and cmc2 genes drastically decreased in the cblR disruption mutant. This result suggests that CblR regulates gene expression controlled via the XlnR-independent signaling pathway in this fungus. Because the cblR gene is induced by cellulose compounds, we investigated if cblR expression is regulated by CblR and CblR2. In the cblR, cblR2, and cblR/cblR2 mutants, cblR transcripts under the inducing condition reduced to almost the same level as those under the uninducing condition. Taken together, these data demonstrate that CblR and CblR2 regulate the cellulose gene expression in response to cellulose by regulating the cblR gene expression.

425. Expression of a bacterial xylanase in Trichoderma reesei under the egl2 and cbh2 glycosyl hydrolyase gene promoters. Helena Nevalainen1,2, Shingo Miyauchi1,2, Junior Teo1,2, Peter Bergquist1,2,3
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Our main aim in this study was to isolate a suite of promoters, other than cbh1, to establish a gene expression platform in which they could be used synergistically for the expression of recombinant gene products, using a bacterial thermophilic xylanase (XynB) as an example. The egl2 and cbh2 promoters were selected on the basis of their relative strength and functionality under the same cultivation conditions that induce cbh2 so that this promoter, widely used for recombinant expression, could be included in the ‘promoter mix’ when desired in the future. Together, these promoters possess considerable expression capacity that can be harnessed for the synthesis of recombinant gene products in T. reesei. We also explored the mode of glycosylation of the recombinant bacterial xylanase in T. reesei to confirm that the higher molecular weight bands seen in activity zymograms were indeed post-translationally modified by glycosylation and to characterize the sugars attached to the protein. The highest XynB production was achieved from a transformant containing 1-2 copies of the EGL2sigpro vector (egl2 promoter). Best xylanase producers did not show any particular pattern in terms of the number of gene copies and their mode of integration into the chromosomal DNA. Transformants produced multiple forms of XynB which were decorated with various N- and O-glycans. One of the O-glycans was identified as hexuronic acid, whose presence had not been observed previously in the glycosylation patterns of T. reesei.

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Regulation by RNAi of diverse cellular functions in metazoans is largely known. However, although different classes of endogenous small RNAs (esRNAs) have been identified in fungi, their biological roles are poorly described. The Argonaute proteins are the core component of all known RNAi pathways. Here we identified three argonaute genes of the basal fungus Mucor circinelloides and investigated their participation in exogenous and endogenous RNAi. Only ago-1 is required for transgene-induced RNA silencing. Ago-1 is also required for the production of distinct classes of esRNAs derived from exons (exesRNAs), which differ in the silencing proteins required for their biogenesis. Classes I and II esRNAs bind to Ago-1 to control mRNA accumulation of the target protein coding genes. Class III esRNAs do not specifically bind to Ago-1, but require this protein for their production, revealing the complexity of the biogenesis pathways of exesRNAs. We also show that ago-1 gene is involved in the response to environmental signals, since vegetative development and autolysis induced by nutritional stress are affected in ago-1 disruption mutants. Our results highlight the role of exesRNAs in the regulation of endogenous genes in basal fungi and expand the range of biological functions modulated by RNAi silencing. This work was performed with MICYT (BFU2009-07220) and MINECO (BFU2012-32246), Spain.


We previously demonstrated that a novel Zn(II)Cys6 transcriptional activator, AoAtrR, regulates gene expression of the ABC transporters that would function as drug efflux pumps and contributes to the azole resistance in Aspergillus oryzae. Moreover, we showed that a deletion mutant of the AatOrR ortholog (AfatR) in Aspergillus fumigatus was similarly hypersensitive to azole drugs. However, little is known about target genes regulated by AatOrR. In this study, we comprehensively examined the target genes regulated by AatOrR using next-generation DNA sequencing technology (RNA-seq). RNA-seq analysis indicated that AatOrR similarly regulated at least one ABC transporter. In addition, surprisingly, AatOrR also regulated several ergosterol biosynthetic pathway genes including erg11(cyp51A). It has been known that the basic helix-loop-helix transcription factor, SrbA, has a critical role in ergosterol biosynthesis and resistance to the azole drugs in A. fumigatus. Interestingly, the ergosterol biosynthetic pathway genes regulated by AatOrR were nearly identical with those regulated by AatR. Therefore, we investigated difference in function between AatR and SrbA in A. oryzae. The expression of ergosterol biosynthetic pathway genes such as erg11, erg24, and erg25 etc. and three ABC transporter genes was significantly down-regulated in the
disruption mutant of AoatrR. Similarly, the expression of same ergosterol biosynthetic pathway genes was also markedly down-regulated in the disruption mutant of AosrbA, but the expression of ABC transporter genes was not affected. In contrast, the expression of ergosterol biosynthetic pathway genes was not up-regulated in an overexpression strain of AoatrR. These results suggest that AtrR and SrbA coordinately regulate ergosterol biosynthetic pathway genes in aspergilli. On the other hand, the AoatrR disruptant was more hypersensitive to azole drugs compared to the AosrbA disruptant, suggesting that hypersensitivity of the atrR disruptant to azole drugs is attributed not only to lowered ergosterol levels due to down-regulation of ergosterol biosynthetic pathway genes, but also to reduced efflux transport of the drugs due to down-regulation of ABC transporter genes.

428. Effects of intron deletions in production of a heterologous protease in Trichoderma reesei. Maria PalhoIMO, Raol Oy, Rajamäki, Finland.

A protease gene originating from a filamentous fungus Malbranchea cinnamomea was expressed in Trichoderma reesei using the strong T. reesei cbh1 (car7A) promoter. The heterologous protease was produced at relatively high yields. However, when the protease cDNA was included in the (otherwise identical) expression cassette the amount of protease in the culture supernatants of the transformants was very low. The native M. cinnamomea protease gene contains three introns. To further study the effects of the removal of introns in the production of protease, single-copy transformants were constructed that contained expression cassettes in which each of the three introns was separately removed and in which two of the introns (all combinations) were deleted at a time from the genomic protease gene. The expression cassettes were targeted to the native cbh1 locus. Three single copy transformants from each transformation were chosen for further studies. These strains were cultivated in shake flasks and the amount of protease was analysed from the culture supernatants. A clear decrease in the protease activity was detected when any of the introns was removed. However, depending on the intron removed there were differences in the relative decreases of protease activity. Removal of two introns at a time had a cumulative decreasing effect on production of protease. The results obtained are shown and discussed.

429. Novel core promoter elements in the oomycete Phytophthora infestans and their influence on expression pattern detected by genome-wide analysis. Laetitia Poidevin, Sourav Roy, Howard S. Judelson. Department of Plant Pathology & Microbiology, University of California Riverside, USA.

The core promoter is the region flanking the transcription start site (TSS) that directs pre-initiation complex formation. While core promoters have been studied intensively in mammals and yeast, little is known about more diverse eukaryotes including oomycetes. Prior studies of a small collection of cloned oomycete genes proposed that its core promoters contain a 19-nt block bearing both an Initiator-like sequence (INR) and a novel 3' sequence named FPR, but this has not been extended to whole-genome analysis. To learn more about oomycete core promoters, we used expectation maximization to find over-represented motifs near TSSs of Phytophthora infestans, the potato blight pathogen. The motifs corresponded to INR, FPR, and a new element found 25-nt downstream of the TSS called DPEP. TATA boxes were not detected. Assays of DPEP function by mutagenesis were consistent with its role as a core motif. Genome-wide searches found a well-conserved combined INR+FPR in only 13% of genes after correcting for false discovery, contradicting prior reports that INR and FPR are adjacent to each other in genes. INR or FPR were found alone near TSSs in 18% and 7% of genes, respectively. Promoters lacking the motifs had pyrimidine-rich regions near the TSS. The combined INR+FPR motif was linked to higher than average mRNA levels, developmentally-regulated transcription, and functions related to plant infection, while DPEP and FPR were over-represented in constitutive housekeeping genes. The motifs were all detected in other oomycetes including Hyaloperonospora arabidopsidis, Phytophthora sojae, Pythium ultimum, and Saprolegnia parasitica, but only INR seemed present in a non-oomycete stramenopile. The absence of a TATA box and presence of novel motifs show that oomycete core promoters have diverged from that of previously-studied model systems, and likely explains failures in prior heterologous expression studies. The association of the INR+FPR with developmentally-regulated genes shows that oomycete core elements influence stage-specific transcription in addition to regulating pre-initiation complex formation.

430. New insights into the phosphate-sensing network in Neurospora crassa. Antonio Rossi, Gabriela F PersinotA, Nalu T A Peres, Diana E Gras, Nilce M Martinez-Rossi. 1) Bioquímica e Imunologia, FMRP-USP, Ribeirão Preto, SP, Brazil; 2) Genética, FMRP-USP, Ribeirão Preto, SP, Brazil.

The filamentous fungus Neurospora crassa is an excellent model system for examining molecular responses to ambient signals in eukaryotic microorganisms. Inorganic phosphate is an essential growth-limiting nutrient in nature and is crucial for the synthesis of nucleic acids and the flow of genetic information. Numerous ambient signals activate the recruitment of mitogen-activated protein kinase (MAPK) cascades. Thus, to identify genes involved in metabolic responses to exogenous phosphate sensing and the functioning of an MAPK, MAK-2, we performed microarray experiments using a mak-2 knockout strain (Dmak-2) grown under phosphate-shortage conditions by comparing its transcription profile to that of a control strain grown in low- and high-phosphate cultures. These experiments revealed 912 uniquely differentially expressed genes involved in a number of physiological processes related to phosphate transport, metabolism, and regulation as well as posttranslational modification of proteins, and MAPK signaling pathways. Quantitative real-time-PCR gene expression analysis using independent RNA samples of 10 arbitrarily chosen genes validated our microarray results. A high Pearson correlation between microarray and quantitative real-time-PCR data was observed. The analysis of these differentially expressed genes in the Dmak-2 strain provide evidence that the mak-2 gene is a component of the hierarchical phosphate-signaling pathway in N. crassa in addition to its independent involvement in other metabolic routes such as the isoprenylation pathway. In this extended model, MAK-2 is functional regarding transcription of Pi-repressible phosphatases when N. crassa is cultured under Pi shortage and is non-functional under abundant Pi conditions, thus revealing novel aspects of the N. crassa phosphorus-sensing network. Financial support: FAPESP, CNPq, CAPES, FAPEA.

431. Carbon source and light dependent regulation of gene clusters in Trichoderma reesei (Hypocrea jecorina). Doris Tisch, Monika Schmoll. 1) Health and Environment, Bioresources, Austrian Institute of Technology AIT, Tulln, Austria; 2) Vienna University of Technology, Institute of Chemical Engineering, Vienna, Austria.

Trichoderma reesei (anamorph of Hypocrea jecorina) is one of the most prolific producers of plant cell wall degrading enzymes. Regulation of the genes encoding these enzymes occurs in response to the nutrient sources available in the environment and many of them are responsive to light as well. Cellulose as the natural substrate induces the most complete enzyme set, while induction of cellulases also occurs on sophorose and lactose. In contrast, no cellulases are induced on glycerol and the respective genes are repressed on glucose. We therefore investigated the transcriptome on these five carbon sources in light and darkness and aimed to identify genes specifically expressed under cellulase inducing conditions. These conditions are characterized by a significant enrichment of genes involved in C-compound and carbohydrate degradation and transport among the upregulated gene set. Genes down-regulated under inducing conditions show a significant enrichment in amino acid metabolism and energy metabolism. We were further interested whether light dependent regulation is clustered in the genome and if the carbon source is relevant for activation of light dependent clusters. We found that light dependent clustering predominately occurs upon growth on cellulose, with the most significant regulation in a gene cluster comprising env1. This cluster appears on glucose as well, but is not down regulated in mutants of bir1 or bir2. Also cbi2, the arabinofuranosidase gene abf2 and the histone acetyltransferase gene gcn5 are part of light dependent clusters. Hierarchical clustering of gene expression patterns was performed to reveal functional
divergence of gene regulation with respect to light response or carbon specific regulation. Glycoside hydrolase genes follow the whole transcriptome pattern with carbon source being superior to light in terms of regulation. ENV1 in in part the G-protein beta subunit GN1 was found to be crucial for carbon source specific regulation of G-protein coupled receptors, genes involved in secretion, sulphur metabolism and oxidative processes as well as transporters. We conclude that clustered regulation of light responsive genes preferentially occurs upon growth cellulose and that ENV1 and to a lesser extent GN1 play a role in carbon source dependent regulation of specific gene groups in light.


*Trichoderma reesei* (anamorph *Hypocreace jecorina*) is an efficient producer of enzymes degrading cellulose and hemicellulose biomass. The cellulases and hemicellulases produced by the fungus are widely employed in industry, and also in biofuel applications. Various environmental and metabolic factors together with the physiological state of the cell affect the enzyme production of *T. reesei*. Thus, a complex signalling cascade and regulatory network is needed for the accurate timing of hydrolytic enzyme production and to control the pattern of enzyme activities produced. In previous studies, both positively and negatively acting regulatory factors for cellulase and hemicellulase genes have been characterised in *T. reesei*. In this study, an expression microarray data on *T. reesei* cultivated in the presence of different carbon sources was analysed in order to identify additional regulatory genes for cellulase and hemicellulase production. In total, 28 putative regulatory factors for *T. reesei* cellulases and hemicellulases were identified and selected for further studies. The genes were overexpressed in *T. reesei* QM9414. Cultivated modified strains were tested for their ability to produce cellulases, xylanases and total secreted protein. Over-expression of seven of the genes led to increased production of cellulases and/or xylanases.


All living organisms must sense and respond appropriately to different environmental stimuli in order to survive. *Trichoderma atroviride* is a filamentous fungus with wide adaptability to different environmental conditions and is considered a good morphogenetic model because it respond to light and injury producing asexual reproductive structures (*conidia*). The mechanisms used by this fungus to respond to these stimuli have been studied independently and models of perception and signal transduction for each stimulus have been proposed. In our research group, we found that the *Ddc2* mutant, involved in small RNA biogenesis, is affected in conidiation in response to light and injury, indicating that conidiation is regulated by small RNAs. In this work we discovered that when the *Ddc2* mutant receives simultaneously light and injury, it is able to conidiate. Based on this observation, we propose that there is a central core of genes needed to coordinate the response to both stimuli and in the *Ddc2* the signaling pathways act synergistically to achieve the correct expression of these genes in order to conidiate. To test the above hypothesis we analysed the transcriptome in response to light and injury of the wild type (WT), and identified 38 genes that have the same expression profile in response to the two stimuli, 19 of them are induced and 19 are repressed, called the central core genes, most of the up-regulated genes are involved in RNA processing, ribosome biogenesis, chromatin remodeling and other cellular processes indicating that this core regulates gene expression response to the stimulus. While genes that are repressed are mainly involved in lipid, carbohydrate and protein metabolic processes suggesting that a metabolic arrest is necessary to respond to both stresses. The expression analysis of this core of genes in the *Ddc2* revealed some of them are deregulated, but other genes respond similarly to WT in either light or injury in *Ddc2*, so this group of genes is regulated by the RNAi machinery.

434. RNA-mediated Gene Silencing in Candida albicans: Reduction of Fungal Pathogenesis by Use of RNAi Technology. M. Moazeni 1, MR. Khorriramizadeh 2, P. Kordbacheh 3, H. Zeraati 2, F. Noorbakhsh 4. 1) Dept. of Medical Mycology & Parasitology, Tehran University of Medical Sciences, Tehran, Iran; 2) Dep. of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran; 3) Dep. of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; 4) Dept of Biology, Islamic Azad University, Varamin-Pishva Branch, Varamin, Iran; 5) Dep of Molecular Medicine, Biotechnology Research Center, Pasteur Institute of Iran, Iran.

Background: The introduction of RNA silencing machinery in fungi has led to the promising application of RNAi methodology to knock down essential vital factor or virulence factor genes in the microorganisms. Efg1p is required for development of a true hyphal growth form which is known to be essential for interactions with human host cells and for the yeast's pathogenesis. In addition, it is responsible for positive regulation of light responsive genes preferentially occurs upon growth cellulose and that ENV1 and to a lesser extent GN1 play a role in carbon source dependent regulation of specific gene groups in light.

**Material and Methods:**

The expression analysis of this core of genes in the *Ddc2* revealed some of them are deregulated, but other genes respond similarly to WT in either light or injury in *Ddc2*, so this group of genes is regulated by the RNAi machinery.

435. RNAi machinery controls the asexual reproduction induced by light of the filamentous fungus *Trichoderma atroviride*. N. Carreras-Villaseñor 1, EU. Esquivel-Naranjo 2, J.M. Villalobos-Escobedo 2, C. Abreu-Googder 2, A. Herrera-Estrella 1. 1) LANGEBIO-CINVESTAV, Irapuato, Guanajuato, Mexico; 2) Facultad de Ciencias Naturales. Universidad Autónoma de Querétaro, Mexico.

RNA silencing is a mechanism through which RNAs induce the inactivation of genes by mRNA degradation, translation inhibition, chromatin remodeling, and DNA elimination. Dicer, Argonaute and RNA dependent RNA Polymerase (RdRP) are the RNAi components, and participate in the biogenesis and function of most sRNAs. Representatives of the kingdom Fungi possess multiple RNA silencing components in their genome. However, the physiological roles of the RNAi pathway in fungi have been poorly studied. Here, we report the phenotypical and transcriptional analysis in RNAi machinery mutants in the filamentous fungus *Trichoderma atroviride*. We found a defect in conidiation induced by light in the *Ddc2*, *Ddr1Ddc2*, *Drdr3* and *Dag02*. Light responsive genes are different among the dicer mutants and WT, therefore the processes that are turned on and off in each strain are different too. Genes involved in metabolism, chromatin remodeling and growth are differentially expressed in the mutants compared with the WT, which denote the possible

Light regulates developmental and physiological processes in a wide range of fungi. Particularly, Zygomycete fungi have developed complex mechanisms to control the responses to light that await detailed characterization at molecular level. The zygomycete *Mucor circinelloides* is a good model for this purpose because its genome has been sequenced and several molecular tools are available. *Mucor*, like other Zygomycetes, has three white collar-1 genes (mcwc-1a, mcwc-1b and mcwc-1c) that code for proteins which present characteristics of photoreceptors. Each mcwc-1 gene controls a specific response to light. Thus, mcwc-1a and mcwc-1c control phototropism and photocarcotogenesis, respectively, whereas the mcwc-1b function in regulation by light has not been proved. In order to deepen in the regulation by light in *Mucor*, a systematic approach using microarrays was followed to characterize white-light-inducible transcriptional changes in wild-type and knockout mutants for each mcwc-1 gene. Analysis of microarray data revealed that light is mainly a positive signal for transcription in *Mucor*, as in other fungi, since 123 genes were up-regulated in the wild-type strain in response to light, whereas only 26 were down-regulated, considering a threshold of threefold change. Genes strongly induced by light included genes known to be up-regulated by light, like the carotenogenic gene *carB* (74-fold), cryptochrome (45-fold) and mcwc-1c (22-fold), supporting reliability of the microarray data. Although many of up-regulated genes code for proteins implicated in protection against light-induced damage, several of them code for protein involved in signal transduction that could be involved in responses like phototropism. Transcriptomic analysis of mcwc-1 mutants showed that induction of around 60% of the genes is mediated by mcwc-1a, whereas only 1% is mediated by mcwc-1c and none is mediated by mcwc-1b, suggesting that mcwc-1a is the main photoreceptor. Searching for cis-acting regulatory motifs upstream of genes regulated by mcwc-1a identified consensus sequences similar to those found in light regulated genes of *Neurospora crassa*. Moreover, the identification of a small group of genes regulated by the three mcwc-1 genes points out that the three proteins form complexes to regulate gene expression. Funded by MINECO (BFU2012-32246), Spain.

437. Gene expression profiling of the basidiomycetous fungus *Lentinula edodes* after light stimulation. **H. Sano**, Y. Sakamoto, M. Abe, S. Kaneko, M. Nakamura, Y. Miyazaki. 1) Department of Applied Microbiology, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki, Japan; 2) Department of Biological Resources Research, Iwate Biological Research Center, 22-174-4 Narita, Kitakami, Iwate 024-0003, Japan; 3) Forest and Forestry Research Institute, Tokushima Agriculture, Forestry and Fisheries Technology Support Center, 5-69 Nanshocho, Tokushima, Tokushima 770-0045, Japan; 4) Department of Life Science, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsutacho, Midori-ku, Yokohama, Kanagawa 226-8503, Japan.

Light is an important environmental signal for various organisms and is known to regulate their physiological and developmental processes. In fungi, light influences sexual and asexual reproductions, mycotoxin productions and pigmentation. Morphological differentiations of mushroom-forming homobasidiomycetes are also affected by light and stimulation of light is one of the important factors regulating fruiting body formation. The molecular mechanisms regulated by light are well studied in ascomycetes, and a number of light-regulated genes were categorized by Gene Ontology and were assigned by the KEGG pathway mapping. In basidiomycetes, the photoreceptor-encoding genes homologous to *Neurospora crassa* wc-1 have been isolated from several species and those genetic evidences revealed the involvement in fruiting body formation. However, the details of light-sensing systems and light-dependent regulation of genes are unclear. In this study, we performed super serial analysis of gene expression (SuperSAGE) using Illumina/Solexa genetic analyzer and analyzed the change of gene expressions stimulated by light during fruiting body formation in *Lentinula edodes*. The samples for high-throughput SuperSAGE were prepared from mycelia cultivated under darkness and after exposure to light with low temperature treatment. The mycelium which had been exposed to light formed a number of primordium and developed into normal fruiting bodies, whereas the mycelium cultivated under darkness could produce no primordium. The obtained transcriptome data showed that there were many kinds of genes expressed in *L. edodes* after light irradiation (5251 genes), compared with the data under darkness (2876 genes). The comparison analysis revealed that the expressions of 2500 genes were different regulated, considering a threshold of twofold change. Among these, 60% of genes were positively regulated, whereas only 1% is mediated by mcwc-1c and none is mediated by mcwc-1b, suggesting that mcwc-1a is the main photoreceptor. Searching for cis-acting regulatory motifs upstream of genes regulated by mcwc-1a identified consensus sequences similar to those found in light regulated genes of *Neurospora crassa*. Moreover, the identification of a small group of genes regulated by the three mcwc-1 genes points out that the three proteins form complexes to regulate gene expression. Funded by MINECO (BFU2012-32246), Spain.

438. Further Characterization of Surface Recognition Mechanisms in *Magnaporthe oryzae*. **Guanghui Wang**, Xiaoying Zhou, Guotian Li, Jin-Rong Xu. 1) College of Plant protection, Northwest A & F University, Yangling, Shaanxi, China; 2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, USA.

Surface recognition and appressorium penetration are critical infection processes in the rice blast fungus *Magnaporthe oryzae* and many other plant pathogenic fungi. Various chemical and physical surface signals are known to be recognized by germ tubes to activate the Pmk1 MAP kinase that is conserved in fungal pathogens for regulating appressorium formation and penetration. Recently, the Msb2 mucin gene was found to function as a surface sensor upstream from the Pmk1 pathway. However, it is not clear how is Msb2 activated and what is its relationship with other surface sensors. In this study, we found that the cleavage domain and transmembrane helix are essential for Msb2 functions. Site-direct mutagenesis was used to verify two candidate cleavage amino acid sites. In addition, we conducted deletion analysis with the cytoplasmic tail of Msb2 that likely plays a role in intracellular signaling. We also assayed the effects of over-expressing the C-terminal region of Msb2 and identified proteins co-precipitated with it by affinity purification. Because CBP1 and PTH11 are two other putative surface sensor genes, we also generated the msb2 cbp1 and msb2 pth11 double mutants and triple mutants with sho1. The msb2 cbp1 mutant rarely formed appressoria and was non-pathogenic, indicating that Msb2 and Cbp1, the only two mucins in *M. oryzae*, may have overlapping functions in surface recognition. Detailed phenotype characterization of the msb2 pth11 and triple mutants are under the way. A model of Msb2 activation and relationship among different receptors will be presented.

439. Evidence of Microbial Epigenetics; Loss-of-function mutant of the Bck1 Homolog, CpBCK1, from the chestnut blight fungus *Cryphonectria parasitica* resulted in the sectoring accompanied with the changes in DNA methylation. **J.-M. Kim**, S.-H. Yun, K.-Y. Jahng, D.-H. Kim. 1) Department of Bio-Environmental Chemistry, Wonkwang University, Iksan, Jeonbuk, South Korea; 2) Institute for Molecular Biology and Genetics, Center for Fungal pathogenesis, Chonbuk National University, Jeonju, Jeonbuk, South Korea.

*CpbcK1*, encoding a mitogen-activated protein (MAP) kinase kinase kinase from the chestnut blight fungus *Cryphonectria parasitica*, is an ortholog of...
Bck1 from Saccharomyces cerevisiae. Colony morphology of the Cpbck1-null mutants differed dramatically from the wild type that mutants showed the invasive growth pattern characterized by slower growth rate, absence of distinctive aerial hyphae resulting in almost absence of conidia-bearing structure and conidial rotation, sparse mycelial growth on the surface of agar plate with abnormal pigmentation, and irregular mycelial mat within the restricted area. Feeding hyphae growing under the plate showed less branched and relatively slower growth pattern. Interestingly, the Cpbck1-null mutant produced sectors appeared as thick rubbery patches of matted growth without pigmentation and sporulation. Complementation of the Cpbck1-null mutant with a wild-type allele rescued mutant phenotypes indicating that the mutant phenotypes were due to the absence of the Cpbck1 gene. Intracellular structure observed by electron microscopy revealed both invasive growth-type and sector-type showed the occurrence of hypertrophy of cell wall, multiple nuclei within swollen cells and intrahyphal hyphae. DNA methylation, an indicative of epigenetic marker, examined by Southern blot analysis and bisulfite DNA modification of putative target genes revealed that there was difference in the DNA methylation pattern between original Cpbck1-null mutant and sector isolated. This study suggests that epigenetic changes are predisposed by the loss of function mutation of a specific gene Cpbck1 and it will be of interest to determine what decide the transition of the mycelia growth pattern from the invasive and very-sick hyphal growth type to compact-mat type. The Cpbck1-null mutant showed the sectorised phenotype accompanied with the changes in DNA methylation demonstrated that the fungal signaling pathway implicated in the control of epigenetic processes, without which abnormal degeneration such as sectoring occurred.

440. NUP-6 (Importin a) is required for DNA methylation in Neurospora crassa. Andrew D. Klocko1, Michael R. Rountree2, Paula L. Grisafi3, Shan M. Hays2, Eric U. Selker3. 1) Institute of Molecular Biology, University of Oregon, Eugene, OR 97448; 2) Department of Natural and Environmental Sciences, Western State College of Colorado, Gunnison, CO 81231.

Heterochromatic regions on chromosomes are essential for numerous cellular processes, including centromere function and gene silencing. The repetitive DNA found in heterochromatin is highly compacted, frequently A:T rich, and in some species such as Neurospora crassa, methylated at cytosines. This DNA methylation can effectively silence genes. Interestingly, unlike the situation in some eukaryotes, loss of DNA methylation is not required for viability in Neurospora. The dispensability of DNA methylation in Neurospora allows for the identification of defective in methylation (dim) genes that have critical roles in the establishment, maintenance, and/or regulation of DNA methylation. This approach established that, at least in Neurospora, DNA methylation is initiated by the histone methyltransferase activity of a multi-subunit complex, DCDC (DIM-5/-7/-9 CUL-4 DDB-1<sup>dim</sup>-a Complex), which catalyzes tri-methylation of lysine 9 on histone H3 (H3K9me3). While the identification of the components of the DCDC was an important step to understanding heterochromatin formation, much is still unknown about the DCDC, including its detailed function, regulation, and assembly. Here, we characterize the action of a previously unidentified <i>dim</i> mutant, <i>dim-3</i>. We found that <i>dim-3</i> corresponds to the <i>nup-6</i> gene, which encodes the Importin a subunit (NUP-6) for classical nuclear transport. NUP-6<sup>dim-3</sup> virtually abolishes H3K9me3 and significantly reduces DNA methylation, and causes DIM-5 and DIM-7 to be mislocalized from heterochromatin, suggesting DCDC activity is impacted in a <i>dim-3</i> strain. Curiously, nuclear transport of DCDC components in a <i>dim-3</i> strain background appears to be equal to or greater than in a wild type background. The possibility exists that NUP-6 could be important in directing the DCDC to heterochromatin or in assembly of the DCDC, and we will address these hypotheses. In addition, the mutations found in <i>dim-3</i> could prevent its gene product, NUP-6, from facilitating DCDC action.

441. Identification and characterization of a Blastomyces dermatitidis mutant with a bidirectional defect in the phase transition. Amber J. Marty, Gregory M. Gauthier. University of Wisconsin - Madison, 1550 Linden Drive, Microbial Sciences Building, Madison, WI, 53706.

Collectively, the dimorphic fungi are the most common cause of invasive fungal disease worldwide. The ability of these fungi to undergo a shift between mold and yeast is critical for pathogenesis. In the soil (22°C), these fungi grow as mold, which produce infectious conidia. In aerosolized conidia inhaled into the lungs of a host (37°C), convert into yeast and cause pneumonia. Knowledge of the mechanisms used to regulate this phase transition is limited. To uncover genes that control the phase transition, <i>Agrobacterium tumefaciens</i>-mediated DNA transfer was used to mutagenize <i>Blastomyces dermatitidis</i> conidia. We generated and screened 22,000 insertional mutants for defects in the phase transition. We identified a mutant, 11-9-75, with a single site of T-DNA insertion that grew as pseudohyphae at 37°C and 22°C, rather than yeast or mold. Adaptor PCR, DNA sequencing, and reverse transcription PCR (RT-PCR) revealed the T-DNA was located in the 5’ UTR of an uncharacterized gene (referred to as <i>BKY1</i>) that was not annotated in the <i>B. dermatitidis</i> genome. Analysis of cDNA indicated BKY1 was 1546 bp in length, lacked introns, and the ORF was predicted to encode a 156 amino acid protein. BLAST analyses against the NCBI database failed to reveal homologs of <i>BKY1</i> in other fungi. The T-DNA insert altered transcription of <i>BKY1</i> in mutant 11-9-75. BKY1 transcript in the mutant was 35-fold higher versus wild type (WT) by quantitative real-time PCR (qPCR) and truncated at the 5’ UTR when analyzed by rapid amplification of cDNA ends (RACE). RT-PCR supported the qPCR and RACE analyses, and indicated the GAPDH promoter, which is upstream of a hygromycin resistance cassette in the T-DNA was driving increased transcription of truncated BKY1. The T-DNA insert also altered alternative splicing of a gene with unknown function, <i>BdsS94</i>, which was less than 1.2 kb downstream of <i>BKY1</i>. Although transcript abundance of <i>BdsS94</i> in mutant 11-9-75 was similar to WT, the frequency of intron excision was reduced. In conclusion, we have identified an insertional mutant with a bidirectional defect on the phase transition; it grows as pseudohyphae instead of yeast at 37°C or mold at 22°C. The T-DNA insert alters transcription of adjacent genes, <i>BKY1</i> and <i>BdsS94</i>, in a poorly characterized region in the <i>B. dermatitidis</i> genome.
FULL POSTER SESSION ABSTRACTS

442. Transcriptional regulation of peptides in proteinase treatment with the nonsteroidal synthetic drug 2-aminoethanesulfonic acid and dithiothreitol by the necroscopic fungi Paxillus involutus, Firoz Shah2, Francois Rinea2, Tomas Johansson2, Anders Tunlid2. 1) Microbial Ecology Group, Department of Biology, Lund University, SE-22362, Lund, Sweden; 2) Centre for Environmental Sciences, Hasselt University, Building D, Aigoralaan, 3590 Diepenbeek, Limburg, Belgium.

Proteins and amino acids form a major part of the organic nitrogen (N) sources in soils. Though a poorly characterized process, this N is mobilized and becomes available to plants due to the activity of ectomycorrhizal (ECM) fungi. We have examined the role of ectomycorrhizal extracellular peptides and amino acid transporters in the degradation, uptake and transfer of various protein sources (BSA, Gliadin and pollen) as well as plant litter material using the ECM model fungus Paxillus involutus. During N-deprived conditions, all substrates induced secretion of peptide activities. The activity had acidic pH optimum (2.3-3.0), and it was mainly due to aspartic peptides and with minor contribution of metallo and serine peptides. The activity was partly and temporally repressed by low concentrations of ammonium (1mg/l). Transcriptional analysis showed that P. involutus expressed a large array of proteins and enzymes involved in the assimilation of organic N including peptides, N-transporters and enzymes of the N-metabolism. Extensive in-silico analysis revealed the presence of genes encoding 312 peptides, 129 N transporters and 264 enzymes involved in amino acid metabolism. Out of these, 89 peptidases and 57 N-transporters and 199 amino acid metabolism enzymes encoding genes were significantly upregulated during organic N assimilation. The genes were encoding a variety of secreted (23) and non-secreted (20) peptides which were differentially expressed depending on the medium with the highest expression of the aspartic and metallo peptides. Apart from the YAAH/ATO family, upregulated genes were found in all the other families of transporters for amino acids, oligopeptides, ammonium, urea and allantatoe/allantoin. The results shows that the expression levels of peptides and transporters in P. involutus are coordinately regulated during the assimilation of organic N sources.

443. Characterization of genome maintenance components in Neurospora crassa using whole-genome high-throughput approach. Evelina Y. Basenko, Zack Lewis. Department of Microbiology, University of Georgia, Athens, GA.

Eukaryotic DNA is package into a higher order DNA-protein structure also known as chromatin, which can regulate and impact an array of nuclear processes including DNA repair and genome maintenance. Disruptions in genome integrity can lead to serious ailments in humans and also contribute to cancer emergence. DNA repair and genome maintenance have been extensively studied in yeast. We, however, have chosen to investigate genome maintenance in the filamentous fungus Neurospora crassa. N. crassa possesses a much larger genome than budding yeasts, and it also contains approximately 3400 genes which are conserved in higher eukaryotes including humans and are absent in yeasts. We utilized the whole-genome Neurospora knockout library to search for novel regulators of genome maintenance. We screened the knockout library for mutants sensitive to the DNA damage agent methyl methane sulfonate (MMS). We have further confirmed and investigated additional mutagen sensitivities of confirmed MMS-sensitive strains. For our further studies, we have decided to focus on one of the MMS-sensitive mutants, which contains a deletion of a SNF2-like protein. The whole-genome high-throughput approach is a powerful method to identify novel players of genome maintenance. We have identified several hundred mutants sensitive to DNA damage, that fall within various categories of cellular processes, including but not limited to DNA repair, RNA metabolism, and chromatin maintenance. Our findings and current progress will be reported.


Circadian clocks are autonomous timers composed of interconnected transcriptional/transcriptional feedback loops. They are thought to confer a selective advantage to individuals by temporally coordinating several processes and contributing to cellular homeostasis. In Neurospora crassa, a model organism in circadian studies, ~20% of its genes are under circadian control and interestingly, many of them are related to metabolism. Indeed, the idea of a crosstalk between metabolism and the circadian clock has become stronger in the last years, and several examples have been obtained in mammalian systems. However, we still don’t know in Neurospora crassa the actual influence of circadian regulation in its physiology and its real impact in the “real-world”. Moreover, the different transcriptional regulators linking time-of-day information and the expression of genes involved in metabolically relevant processes, like Carbon-Catabolite Repression (CCR) or cellulose degradation remain largely unknown. Thus, we are analyzing glucose repression in a circadian context, using N. crassa as a model. We hypothesize that there is an intimate crosstalk between both regulatory systems over the expression of several rhythmic CCR-controlled genes. We are using gene expression assays and a codon-optimized luciferase transcriptional reporter, to evaluate the role of the transcription factor CRE-1 (carbon catabolite regulation-1), a conserved metabolic regulator, in this potential cross-talk. Also, CRE-1 is a crucial transcription factor involved in several important cellular processes as cellulose degradation and catabolic repression. Further, we are studying how both inputs are integrated to control the expression of target genes. Our results suggest CRE-1 as a link between both pathways, as it appears to be important for both CCR and circadian control of target genes. In addition, we describe for the first time in Neurospora the presence of a functional clock in cellulose (Avicel)-- containing media. This observation strengthens the hypothesis that a circadian clock may regulate the expression of several cellulase-encoding genes, having a real impact in such a physiologically relevant process.

445. Opposing activities of the HCHC and DMM complexes maintain proper DNA methylation in Neurospora crassa. Shinh Ji Hong3, Eun Yu1, Eric Seiker1. 1) University of Oregon, Institute of Molecular Biology, Eugene, OR; 2) University of Fukui, Life Science Unite, Fukui, Japan.

Proper regulation of heterochromatin and DNA methylation is critical for the normal function of cells. We show that heterochromatina and DNA methylation are faithfully controlled in Neurospora by opposing activities of the silencing complex HCHC and the anti-silencing complex DMM. The workings of these two complexes were investigated. HCHC consists of four proteins, the two chromo domain proteins HP1 and CDP-2, the histone deacetylase HDA-1 and the AT-hook motif protein CHAP. We found that histone deacetylase activity is critical for HCHC function but the H3K9me3 binding activity of the CDP-2 chromo domain is not. Instead, CDP-2 serves as an essential bridge between HP1 and HDA-1. CHAP interacts directly with HDA-1, binds in a methylation-independent way to the A-T-rich DNA that forms the cores of methylated regions and is important for stable association of HDA-1 with chromatin. HCHC is involved in the spreading of DNA methylation in dmm mutants. The DMM complex consists of a presumed histone demethylase, DMM-1, plus DMM-2, which is characterized by a fungal-specific Zn(ll)/Cys6 DNA-binding domain ("Zn-Cys"). We found that DMM-2 strongly binds to DNA from euchromatin/heterochromatin junctions, thereby promoting the stable association of DMM-1 at the edge of heterochromatin domains to prevent aberrant spreading of DNA methylation.

446. The transcription factor FL is phosphorlylated and interacts with a trehalose related protein in Neurospora crassa. Carmen Ruger-Herreros 2, Gencer Sancar2, Michael Brunner2, Luis M. Corrochano1. 1) Departamento de Genetica, Universidad de Seville, Spain; 2) BZH, Universität Heidelberg, Germany.

Several environmental cues, including light, promote a developmental transition in Neurospora crassa that leads to the formation of conidia. Conidiation is controlled by FLUQFF (FL), a zinc finger transcription factor. Light activates the transcription of fl through the transient binding of the WC complex to the fl promoter. Light also activates the transcription of several conidiation genes in Aspergillus nidulans, and their Neurospora homologs have been identified.
in the Neurospora genome. We have assayed the activation of the Neurospora homologs of A. nidulans conidiation genes (filA, filC, filD, medA and stuA), and the Neurospora conidiation gene con-10 as a control. Unlike con-10, none of the Neurospora homologs of the A. nidulans conidiation genes were induced by light in vegetative mycelia. However, we found that deletion fl resulted in light-dependent mRNA accumulation for all the conidiation genes. This result indicated that the absence of FL allows the binding of the WC complex to the promoter of these genes to activate transcription in a light-dependent manner. We have assayed the amount of WC proteins in the DFl and wild type strains but we did not find any difference between the two strains. We expect to identify additional genes deregulated by the absence of FL after massive sequencing of total RNA (RNAseq) using a DFl strain and wild-type strain in dark and light conditions. We have investigated the role of FL during conidiation in Neurospora using a tagged version of FL. FL is present in vegetative mycelia but the amount increases after light exposure. We observed several forms of FL due to phosphorylation, and we have determined by mass spectrometry that FL is phosphorylated in several residues. We have immunoprecipitated FL to identify proteins that may interact with FL. We have found a protein that interacts with FL in different growth conditions. This protein has been described in other organisms and plays a role in the ability to grow in the presence of trehalose. Since FL is a transcription factor, we use FL:3XFLAG strain to do ChIPseq in order to identify the putative binding sites of FL to the DNA. We expect that the results from these experiments will help us to understand in more detail the role of FL in the activation of gene transcription during development.


The plant fungal pathogen Fusarium verticillioides can infect various plants worldwide, including maize, and contaminate kernels with mycotoxins of the fumonisin family. Fumonisins B are stable polyketides that resist agrifood processing and are classified as potentially carcinogenic. As such, contamination of food and feeds with these toxic secondary metabolites must be avoided. Numerous factors influence fumonisins B accumulation on maize, including the composition of the grains on which Fusarium develops. In particular, several phenolic compounds were shown to inhibit fumonisin B biosynthesis.

Preliminary analyses showed that free phenolic acids are particularly abundant in immature grains, i.e., at the onset of toxin production, from cereal cultivars on which mycotoxins tend to accumulate less. We tested in vitro the effect of chlorogenic, caffeic, and ferulic acid on fumonisin B production in F. verticillioides. All three compounds inhibit fumonisin B accumulation on maize, including the mechanisms by which these phenolic acids may exert their inhibitory properties and analyzed whole genome expression levels by RNA-seq. Sequenced reads were mapped to the reference genome of F. verticillioides and results were analyzed according to the current annotation available at the Fusarium Comparative Database. Doing so, we identified 175 and 1133 potential new genes and transcripts, respectively. We also found that the genes involved in the fumonisins biosynthetic pathway are all inhibited in the presence of any of the three tested phenolic acids. Finally, we identified sets of genes that are regulated specifically by a given phenolic acid, and others that follow similar patterns in all tested conditions. As a whole, our results show a large re-organization of Fusarium’s transcriptome upon phenolic acid treatment.

448. Differential transcriptome analysis of Zymoseptoria tritici infecting wheat reveals novel effectors. Stefano F.F. Torriani1, Marcello Zala1, Daniel Croll1, Patrick C. Brunner2, Eva H. Stukenbrock2, Dee Carter2, Bruce A. McDonald3. 1) Integrative Biology, ETHZ, Zurich, Switzerland; 2) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany; 3) University of Sydney, Sydney, Australia.

Zymoseptoria tritici (formerly called Mycosphaerella graminicola) is a hemibiotrophic fungus belonging to the Dothideomycetes, the largest class of ascomycetes that includes many plant pathogens. Like other hemibiotrophic pathogens Z. tritici uses different strategies for obtaining nutrition during its life cycle. For the first 10 days post inoculation (dpi) the pathogen colonizes the host as a biotroph without causing visible symptoms. The necrotrophic phase lasts until the affected plant cells have died. Depending on the strain-cultivar interaction, plant cell death occurs from 18 to 20 days after penetration. Z. tritici concludes its life cycle by surviving as a saprotroph on dead leaves for several months. Thus Z. tritici presents a powerful system to study host-pathogen interactions during different stages of disease development. Next generation sequencing technology was used to analyze changes in transcription during the complete infection cycle of Z. tritici on wheat. The total RNA was extracted from inoculated plants at six time points (3-, 7-, 11-, 14-, 21- and 56- dpi). RNA-Seq analyses allowed us to trace the expression profile of 10,251 genes and identify genes that differed in expression between the biotrophic, necrotrophic and saprotrophic stages of infection. About 14% and 34% of the genes showed statistically significant differences in expression from the biotrophic to necrotrophic and from the necrotrophic to saprotrophic stages of infection, respectively. Putative effector genes were preferentially transcribed at 11 dpi during the transition between biotrophy and necrotrophy. Through this screen we identified five putative effector genes for further characterization, using Agrobacterium-mediated transformation to determine their role in pathogenicity. Although recent experimental efforts focused mainly on protease-degrading effectors, we investigated the role of non-protease genes and identified two different clusters of genes (PKS4 and PKS5-related genes) involved in the biosynthetic pathways of different secondary metabolites showed expression patterns similar to the putative effectors. Confirmation of function of the putative virulence genes will be based on gain or loss of virulence in planta using gene knock-outs and knock-ins.


The Aspergillus nidulans xprG gene encodes a transcriptional activator that is a member of the Ndt80 family in the p53-like superfamily of proteins. Previous studies have shown that XprG controls the production of extracellular proteases in response to starvation. We undertook transcriptional profiling to investigate whether XprG has a wider role as a global regulator of the carbon nutrient stress response. Our microarray data showed that the expression of a large number of genes, including genes involved in secondary metabolism, development, and autolysis, were altered in an xprG null mutant. Many of these genes are known to be regulated in response to carbon starvation. We confirmed that sterigmatocystin and penicillin production is reduced in xprG mutants. The loss of fungal mass and secretion of pigments that accompanies fungal autolysis in response to nutrient depletions occurred in an xprG1 gain-of-function mutant and decreased or absent in an xprG- mutant. We found that conidiophore development occurred in carbon-starved submerged cultures of both the xprG1 loss- and xprG1 gain-of-function mutants, though the number of metulae appeared to be reduced. Thus, the reduction of brlA expression observed in the xprG1 mutant is not sufficient to block conidiophore development in response to carbon starvation. However, the xprG1 gain-of-function mutation partially suppresses VeA-mediated repression of conidiophore development and the conidiophore development defect in the fluG701 mutant. These results support the hypothesis that XprG plays a major role in the response to carbon limitation and that nutrient sensing may represent one of the ancestral roles for the p53-like superfamily.

The sirtuins are members of the NAD⁺-dependent histone deacetylase family that contribute to various cellular functions which are affected aging, disease and cancer development. However, physiological roles of the fungal-specific sirtuin family are still poorly understood, especially with regard to their participation in the genomic stability of yeast. Here, we determined the novel function of the fungal-specific sirtuin HstD, which is homolog of yeast Hst4 in Aspergillus oryzae. The deletion of HstD indicated that both conidial development and secondary metabolism were regulated by HstD in A. oryzae. Furthermore, the gene expression of LoeA, which is most studied coordinator for the regulation of secondary metabolism and development, was induced in the DHsD strain, and we found a significant genetic interaction between HstD and LoeA using double-disrupted or overexpression strains. Thus, we concluded the fungal-specific sirtuin HstD coordinates the fungal development and secondary metabolism via the regulation of LoeA gene expression in filamentous fungi. The HstD is fungal-specific, but it is conserved in the vast family of filamentous fungi. Therefore, HstD has great potential as a drug target for mycosis or plant disease, because the fungal development and secondary metabolism are virulence determinants of pathogenic fungi. In addition, our findings are also important for improving the productivity of useful secondary metabolites and developing an attractive host for the production of several heterogeneous secondary metabolites.


The Ehrlich pathway utilizes amino acids to generate higher alcohols with distinctive flavor in three enzymatic steps including a transaminase, a decarboxylase and an aldehyde dehydrogenase. Comparative genomics revealed the absence of key genes of the Ehrlich pathway in Eremothecium cymbalariae whereas these genes were found to be present in the closely related species Ashbya gossypii. A. gossypii produces a very fruity flavor both in liquid culture and on solid media. The biological significance of this is unknown. Here, we present the functional analysis of A. gossypii key genes of the Ehrlich pathway, ARO8a, ARO8b, ARO10, and ARO80. Deletion of any one component resulted in a noticeable reduction of flavor production as determined by GC/MS. In Saccharomyces cerevisiae ARO80 has been described as the main transcription factor regulating other genes of the Ehrlich pathway. Therefore, we analyzed the effect of deletion and overexpression of this gene on flavor production in yeast. As expected, overexpression resulted in a marked increase in flavor production, particularly in isomyl alcohol, a banana-like flavor. Next to chemical analyses we generated a lacZ-based reporter gene assay using AR0-gene promoters. With such a tool we can determine the status of flavor production under various conditions and in a variety of yeast strains. Initial results will be presented.


Aspergillus nidulans is a filamentous fungus known to produce a variety of complex natural products known as secondary metabolites (SM). Regulation of these bioactive SM can occur through cluster specific transcription factors, or through global regulators such as LoeA. Deletion of loeA results in drastically decreased amounts of multiple secondary metabolites. A multi-copy suppressor screen for genes capable of phenotypically returning norsolorinic acid (NOR) production to the DlaeA mutant resulted in identification of 17 plasmids containing inserts ranging from one to four genes. Further analysis of the suppressor plasmids confirmed a subset to increase SM production both in the original loeA deletion strain and in wild type backgrounds.

453. A network of HMGB box transcription factors regulates sexual cycle in the fungus Podospora anserina. J. Aït-Benkhail1,2, E. Coppin1,2, S. Brun1,2,3, T. Martin1, C. Dixielus1, B. Debuchy1,2,1) Univ Paris-Sud, Institut de Génétique et Microbiologie, Orsay, France; 2) CNRS, Institut de Génétique et Microbiologie, Orsay, France; 3) UFR des Sciences du Vivant, Université Paris-7 Diderot, Paris, France; 4) Department of Plant Biology and Forest Genetics, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Uppsala, Sweden.

High-mobility group B proteins are eukaryotic DNA-binding proteins characterized by the HMGB-box functional motif. These transcription factors play a pivotal role in global genomic functions and in the control of genes involved in specific developmental or metabolic pathways. The filamentous ascomycete Podospora anserina contains 12 HMGB box genes. Of these, four have been previously characterized; three are mating-type genes that control fertilization and development of the fruiting-body, whereas the last one encodes a factor involved in mitochondrial DNA stability. Systematic deletion analysis of the eight remaining uncharacterized HMGB-box genes indicated that none were essential for viability, but that seven were involved in the sexual cycle. Two HMGB-box transcription factors display striking features. Pa_1_13940, an ortholog of SpStp11 from Schizosaccharomyces pombe, is a pivotal activator of mating-type genes in P. anserina, whereas Pa_7_7190 is a repressor of several phenomena specific to the stationary phase, most notably hyphal anastomoses. Constitutive expression of mating-type genes in a Dpa_1_13940 strain did not restore fertility, indicating that Pa_1_13940 has additional functions related to sexual development besides activating mating-type genes. RT-qPCR analyses of HMGB-box genes in different HMGB-box deletion strains indicated that Pa_1_13940 is at the hub of a network of several HMGB-box factors that regulate the sexual cycle. Complementation experiments with a strain deleted for mating-type genes revealed that this network control fertility genes in addition to mating-type target genes. This study points to the critical role of the HMGB-box members in sexual reproduction in fungi, as 11 out of 12 members were involved in the sexual cycle in P. anserina. Pa_1_13940 and SpStp11 are conserved transcriptional regulators of mating-type genes, although P. anserina and S. pombe have diverged 1.1 billion years ago. Two HMGB-box genes, SOK9 and its upstream regulator SRY, play also an important role in sex determination in mammals. The mating-type genes and their upstream regulatory factor form a module of HMGB-box genes similar to the SRY/SOX9 module, suggesting it may be ancestral in Opisthokonta.


Sclerotinia sclerotiorum (Lib.) de Bary is an omnivorous, polyphagous, phytopathogenic fungus that relies on the completion of the sexual cycle to initiate most new disease cycles. The sexual cycle is characterized by the development of apothecia that forcibly discharge ascospores for local and, under suitable conditions, long distance dissemination. A strategy for understanding the regulation of apothecial multicellular development is being pursued through functional characterization of the mating type genes in S. sclerotiorum. These genes are hypothesized to encode master regulatory proteins required for aspects of sexual development ranging from fertilization through fertile fruiting body development. Experimentally, gene deletion strategies were performed to create loss-of-function mutants in the two conserved “core” mating type genes common to most ascomycete fungi as well for two lineage-specific genes found only in S. sclerotiorum and closely related fungi. mat 1-1 and mat 1-2-1 mutants are able to form ascogonia but are blocked in all aspects of apothecia development. These mutants also exhibit defects in secondary sexual characters including the production of smaller sclerotia and lower numbers of spermatia. mat 1-2-4 mutants are delayed in apothecia production and form apothecia with aberrant disc morphogenesis and ascospore production. They too produce lower numbers of spermatia and smaller sclerotia and additionally, exhibit a slower hyphal growth rate. Phenotypes of the mat 1-1-5 gene knockouts are under evaluation and will be reported. Our findings demonstrate that mat genes are involved in both sexual fertility and development in S. sclerotiorum.
455. The Sclerotinia sclerotiorum mating type locus (MAT) contains a 3.6-kb region that is inverted in every generation. Patrik Inderbitzin\(^1\), Periasamy Chitrampalam\(^2\), Karunakanar Maruthachalam\(^1\), Bo-Ming Wu\(^3\), Krishna Subbarao\(^1\). \(^1\) Department of Plant Pathology, University of California-Davis, Davis, CA, USA; \(^2\) Department of Plant Sciences, University of Arizona, Tucson, AZ, USA; \(^3\) Department of Plant Pathology, China Agricultural University, 2 West Yumamingyuan Rd., Haidian District, Beijing, China.

Sclerotinia sclerotiorum is a filamentous ascomycete in the Sclerotiniaceae (Pezizomycotina) and a necrotrophic pathogen of more than 400 hosts worldwide, including many important agricultural crops. In California, the biggest lettuce producer in the United States, S. sclerotiorum is a causal agent of lettuce drop that reduces overall annual lettuce yield by 15%. Little is known about the details of sexual reproduction in S. sclerotiorum, but the structure of the S. sclerotiorum mating type locus was previously reported in the master regulator of sexual reproduction in ascomycetes, a signal transducing adaptor protein affecting growth and virulence of Cryptococcus. Bem1 is present in the cytosol of many fungi, Dock180 only activates Rac in both organisms. Despite the proven activation Cdc24 does not interact with any GTPase in yeast too. Cdc24 DHPH domains are able to nucleotide exchange factors (GEFs) Cdc24 and Dock180 belong to different families, strictly polarity of the hyphal growth during the first infection stage which seems to be crucial for the non-fertile, and the inversion segregated in a 1:1 ratio regardless of whether the parent was Inv- or Inv+. This suggested the involvement of a highly regulated process in maintaining equal proportions of Inv- and Inv+, likely associated with the sexual state. The MAT inversion region, defined as the 3.6-kb MAT inversion in Inv+ isolates and the homologous region of Inv- isolates, was flanked by a 250-bp inverted repeat on either side. The 250-bp inverted repeat was a partial MAT-1-1 that through mediation of loop formation and crossing over, may be involved in the inversion process. Inv+ isolates were widespread, and in California and Nebraska constituted half of the isolates examined. We speculate that a similar inversion region may be involved in mating type switching in the filamentous ascomycetes Chromocerca spinulosa, Sclerotinia trifoliorum and in certain Cercospora species.

456. Repression of the phosphor-transmitter gene ypdA resulting in growth defect in Aspergillus fumigatus. Daisuke Hagiwara\(^1\), Hiroki Takahashi\(^1\), Mayumi Nakayama\(^2\), Keietsu Abe\(^3\), Tohru Gonoi\(^1\), Susumu Kawamoto\(^1\). \(^1\) Medical Mycology Research Center, Chiba university, Chiba, Japan; \(^2\) NICHe, Tohoku university, Sendai, Japan.

Two-component system (TCS) is a conserved signal transduction system implicated in cellular responses to a variety of environmental stimuli in fungi. Aspergillus fumigatus has 13 histidine kinases, single HPT (histidine-containing phosphor transmitter), and 3 response regulators, which together constitute a TCS signaling. According to studies of HPT in several fungi, ypdA encoding a single HPT of A. fumigatus has been thought to be an essential gene. In this study, we tested if absence of YpdA leads to cell lethality in A. fumigatus, and investigated what the molecular mechanisms underlying the lethality is. To address these questions, we constructed a conditional TCS signaling. According to the previously known ypdA-ypdA expressing strain by replacing a native promoter of ypdA with thik promoter (Pthik). Pthik is strictly repressed in the presence of thiamine, while moderately expressed in absence of the thiamine. The conditional Pthik-ypdA strain showed severe growth defect on a plate containing thiamine, while the strain grew normally on a plate without thiamine as the wild type stain. We, then, investigated the expression profiles of catA, dprA, and dprB genes, which are regulated under the control of SakA MAPK cascade, since the SakA MAPK cascade is downstream of A. fumigatus TCS. In a liquid culture, expressions of catA, dprA, and dprB were gradually increased after addition of thiamine, suggesting that inactivation of ypdA expression leads to the activation of SakA MAPK cascade. To get more insight into a response to ypdA-repression, the transcriptome profiles were obtained by RNA-seq. Expression levels of each gene at 3h, 6h, and 9h after addition of thiamine were compared to that at 0h. More than 2-fold or less than 1/2-fold of expression changes were regarded as up- or down- regulated, respectively. Through statistical analysis on category of gene ontology, the groups concerning ribosome biogenesis or RNA metabolism were found to be significantly down-regulated after the inactivation of ypdA. Based on these results, we will discuss the cellular responses to YpdA deprivation and try to find out the molecular mechanisms contributed to the lethality.

457. Unravelling the GTPase polarity complex in Claviceps purpurea. Andrea Herrmann\(^1\), Janine Schürmann\(^2\), Britta Tillmann\(^2\), Michael Böcker\(^2\), Paul Tudzynski\(^1\). \(^1\) IBPP, WWU Muenster, Schlossplatz 8, 48143 Muenster, Germany; \(^2\) Philipps-Universität, Karl-von-Frisch-Strasse 8, 35032 Marburg, Germany.

Claviceps purpurea is a plant pathogen infamous for its production of toxic alkaloids on infected host plants like barley. Consumption of infected grains leads to severe symptoms up to the death of the patient. Infection patterns are complex and the topic of intensive research. One interesting aspect is the strict polarity of the hyphal growth during the first infection stage which seems to be crucial for the non-recognition of C. purpurea as a pathogen by the host. To address the question of the importance of polarity the structure and dynamics of the polarity complex are the focus of this work. The guanine nucleotide exchange factors (GEFs) Cdc24 and Dock180 belong to different families, Cdc24 being a member of the Dbl GEF family and Dock180 a CZH GEF. Cdc24-GFP localises cytosolically and to hyphal tips whereas Dock180-GFP is present in small vesicles in the hypha, though concentrated at the tip region, too. Dock180-DPH domains are able to activate the GTPases Rac and Cdc42 of C. purpurea and U. maydis In vitro, whereas the catalytic domain of Dock180 only activates Rac in both organisms. Despite the proven activation Cdc24 does not interact with any GTPase in yeast two hybrid assays. Dock180 shows a weak interaction with Rac and the two p21-activated kinases (PAKs) Ste20 and Cla4. Thus, both GEFs do not share many characteristics apart from their GEF activity. The PAKs Ste20 and Cla4 and the scaffold protein Bem1 are involved in the polarity complex, too. Ste20 localises to cytosolic tip and interacts with Cdc24 in a loading status dependent manner, whereas Cla4 is the main partner of Rac. Other interactions of Ste20 with Dock180 and Cla4 could also be shown. Bem1 is present in the cytosol - concentrated at the hyphal tip - and links most of the proteins of the polarity complex as interactions with Cdc24, Cla4, Ste20 and Dock180 have been detected. Taken together we postulate at least two different polarity complexes, the Rac complex and the Cdc42 complex. Both are gathered by Bem1, but Cla4 is the main partner of Rac, whereas Ste20 plays a special role for Cdc42. Dock180 is mainly linked to Rac, Cdc24 can be active in both complexes. We are interested in the spatial and temporal formation and regulation of these complexes and its influence on polarity and virulence which will be the subject of further studies.

458. Atypical Gb and RACK homolog Gib2 is a signal transducing adaptor protein affecting growth and virulence of Cryptococcus neoformans. Yanli Wang\(^1\), Gui Shen\(^2\), Jinjun Gong\(^2\), Amy Whittington\(^2\), Ping Wang\(^1,3\). \(^1\) Res Inst for Children, Children's Hospital, New Orleans, LA USA; \(^2\) Dept Microbiology, Immunology and Parasitology, LSUHSC, New Orleans, LA USA; \(^3\) Dept Pediatrics, LSUHSC, New Orleans, LA USA.

Virulence in Cryptococcus neoformans is a multifaceted trait underpinned by complex signaling pathways. The atypical G-protein b subunit Gib2 displays versatility in interactions with signaling molecules such as Ga Gpa1 that governs cAMP signaling and intersectin C1n that regulates intracellular trafficking. This and the conserved seven-bladed b-propeller motif are highly suggestive that Gib2 functions as an adaptor protein. We here show that Gib2 binds to...
Gpa1 and Ggp1/Ggp2 in a direct manner, and Gib2 promotes cAMP levels through novel interactions with phosphodiesterase Pde2 and RAS proteins Ras1 and Ras2. Using TAP technology, we have identified additional 42 proteins whose putative function range from signal transduction, energy generation, metabolism, and stress response to ribosomal function. Finally, through establishing a protein-protein interactive network, we illustrate that Gib2 adapts a scaffold role to mediate specific protein-protein interactions that drive the formation of various protein complexes. This includes fostering a heterotrimeric complex with Gpa1 and Ggp1/Ggp2, targeting Pde2 (direct) and adenylyl cyclase Cac1 (indirect) to regulate CAMP levels, and likely serving as a conserved ribosomal core protein facilitating fundamental cellular processes to underlie growth and virulence. Our studies reveal the complexity of the regulatory network in fungi and advocate Gib2 as a novel target for antifungal therapy.

459. Consequences of the loss of transcription factors SreA and HapX on siderophore biosynthesis and iron homeostasis in the perennial ryegrass endophyte, *Epichloë festucae*. Natasha T. Forester1,2, Geoffrey A. Lane1, Ian L. Lamont1, Linda J. Johnson1. 1) Plant Fungal Interactions Team, AgResearch Ltd, Palmerston North, New Zealand; 2) Biochemistry Dept, University of Otago, Dunedin, NZ.

Siderophores are low molecular weight ferric iron chelators that are made by microorganisms to compete for and to sequester iron, an essential micronutrient. *Epichloë festucae*, a fungal endosymbiont of perennial ryegrass, synthesises two siderophores, epichloënin A and ferricrocin to harvest and utilise iron from its host grass. Work by our group has implicated epichloënin A in the maintenance of symbiosis and is described in another abstract. To explore the regulation of siderophore biosynthesis and iron homeostasis processes, we have characterised mutants of two major iron-responsive transcription factors, SreA and HapX that coordinate cellular responses to iron availability. To evaluate the effect of loss of SreA and HapX on siderophore biosynthesis, we measured the production of epichloënin A and ferricrocin by LC-MS/MS. Relative to wild type; both siderophores were over-produced in DsreA mycelia grown in the presence of iron, while ferricrocin was produced in excess in DhapX mycelia grown under iron deprived conditions. Iron-dependent phenotypic deviations from wild type fungal growth were also observed in culture and in planta but neither mutant disrupted the E. festucae - L. perenne association under standard soil conditions. However, under iron-limiting conditions through hydroponic control of symbiotic iron supply, we demonstrated that DsreA mutants can induce chlorosis in their hosts, indicating that DsreA mutants compete for host iron. In planta, the DsreA fungal hyphae are also markedly increased in girth and lack growth in vascular bundles. In DhapX infected plants grown hydroponically in iron deprived conditions, we observed inappropriate fungal growth such as highly convoluted and compressed hyphae and elongated fungal structures which hinted at reduced resource conservation by DhapX under growth limiting conditions. Collectively, these results suggest that Epichloë fungi have a tightly regulated iron management system for niche adaptation and actively set limits on iron withdrawal from the host, presumably to prevent competition with its host to promote mutualistic interactions. Mutations that interfere with deregulated iron management, either by derepressing siderophore synthesis, can destabilise the fungal-plant association.

460. Who is to blame: defining the host responses that lead to ToxA-induced susceptibility. Iovanna Pandelova, Viola Manning, Ashley Chu, Lynda Ciuflietti. Botany and Plant Pathology, Oregon State Univ, Corvallis, OR.

Pathogenicity by the necrotrophic pathogen of wheat, *Pyrenaphora tritici-repentis* (Ptr), is attributed to the production of host-selective toxins (HSTs). Understanding the mode-of-action of HSTs is essential for a complete characterization of how these pathogenicity factors condition plant disease susceptibility. One of the proteinaceous HSTs produced by *Ptr*, PhtoxA (ToxA), induces necrosis in sensitive cultivars. Several studies suggest that ToxA interacts with a high affinity receptor, enters mesophyll cells and localizes to chloroplasts. Additionally, ToxA acts as an elicitor of defense responses by increasing production of phenolic compounds and by the up-regulation of genes involved in jasmonic acid and ethylene production pathways. After ToxA treatment and incubation in constant light, there is a decrease in photosystem (PS) I and II transcripts observable already at 9 and 14 hours post infiltration (hpi), which is followed by the drastic reduction in levels of both PSI- and PSII-complex proteins. It is proposed that photosystem dysfunction leads to light-dependent accumulation of reactive oxygen species (ROS) and the development of necrosis. To better understand the role of ROS, photosynthesis and defense responses in necrosis development induced by ToxA, plants were incubated in light (presence of ROS) or in dark (absence of ROS). In order to determine the impact of ToxA on gene regulation and to establish when early changes in protein content of PS complexes occur, both biochemical and microarray analyses were performed. Some defense-related genes are up-regulated in ToxA-treated leaves incubated in the dark (ToxA/dark), although the number of probesets was considerably less compared to ToxA-treated leaves incubated in light (ToxA/light). Furthermore, ethylene biosynthesis genes, that play a role in symptom development in ToxA/light treatment are not significantly up-regulated in ToxA/dark-treated leaves. Finally, only a small fraction of PSI- and II-related and chlorophyll a/b-binding genes are down-regulated in ToxA/dark compared to ToxA/light treatment. These data suggest that only certain defense-related pathways are involved in ToxA-induced necrosis development, and help to identify those genes whose differential regulation by ToxA is light and/or ROS-dependent.

461. RNA silencing of * pacC increases aflR transcript levels under alkaline pH conditions in *Aspergillus flavus*. Benesh M Somai, Kyle W van der Holst, Essa Suleman. Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, Port Elizabeth, 6031, Eastern Cape, South Africa.

*Aspergillus flavus* produces aflatoxin B1 which is an important hepatocarcinogen, especially amongst the developing third world countries which have a large number of poor, rural, subsistence communities with little access to fungicides. The master regulator of aflatoxin production is aflR which, in turn, appears to be negatively regulated by *pacC*. However, until now, there were never any direct measurements of the relative aflR/pacC transcript ratios produced under aflatoxin conducive and non-conducive conditions. In the current study, *pacC* was down-regulated in two transformants by a synthetic *pacC*RNAi construct under the control of a thiamine inducible promoter. Expression of *pacC* and *aflR* transcripts was then measured via RT-qPCR in cultures grown under alkaline or acid conditions. At pH 4, between *pacC*RNAi inducing and repressing conditions, an aflR/pacC transcript ratio of 1.09 relative to the reference gene was obtained indicating the production of an equal abundance of aflR and pacC mRNA. It is generally accepted that at acidic pH the majority of pacC mRNA is unprocessed, remains untranslated and non-functional thereby being incapable of repressing AFLR protein production. This stimulates aflatoxin production at acidic pH. Between pH 8 and pH 4, when pacC was suppressed, the aflR/pacC ratio was 0.2 indicating that pacC production was higher than that of aflR. aflR transcript levels were reduced between 76% and 80% therefore explaining the normal lack of aflatoxin detection at pH 8. Between pH 8 and pH 4, when pacC was induced, the aflR/pacC ratio was between 1.77 and 13.21 indicating that at alkaline pH, suppression of pacC allowed a large increase in AFLR which stimulated aflatoxin production. It is concluded that pacC is produced at acidic pH, but remains largely non-functional. Furthermore, at pH 8, aflR production decreases only by about 80% and therefore it is possible that the remaining 20% of transcripts still stimulates aflatoxin production. Finally, via RNAi silencing it is conclusively proved that pacC negatively regulates aflR production at pH 8.


Most filamentous pathogens have complex life cycles in which gene expression networks orchestrate the formation of cells specialized for dissemination.
or host colonization. In the oomycete Phytophthora infestans, the potato late blight pathogen, we identified major shifts in mRNA profiles during developmental transitions using microarrays. We then used those data with three search algorithms to discover more than 100 motifs that are over-represented in promoters of genes up-regulated in hyphal, sporangia, sporangia undergoing zoosporogenesis, swimming zoospores, or germinated cysts forming appressoria. Most of the putative stage-specific transcription factor binding sites (TFBSs) thus identified had features typical of TFBSs such as position or orientation bias, palindromy, and conservation in related species. Each of six motifs tested in P. infestans transformants using the GUS reporter gene conferred the expected stage-specific expression pattern, and were shown to bind nuclear proteins in gel-shift assays. Several motifs linked to the appressorium-forming stage were over-represented in promoters of genes encoding effectors and other pathogenesis-related proteins. To understand how promoter and genome architecture influence expression, we also mapped transcription patterns to the P. infestans genome assembly. Adjacent genes were not typically induced in the same stage, including genes transcribed from a small shared promoter region. Analyses of global expression, however, demonstrated that co-regulated gene pairs occurred more than expected by random chance. These data help illuminate the processes regulating development and pathogenesis, and will enable future attempts to purify the cognate transcription factors. Our approach should be applicable to both oomycetes and fungi.

463. Cooperative regulation of Aspergillus nidulans cellulase genes by transcription factors McmA and ManR/ClrB. Tetsuo Kobayashi. Nuo Li1, Miki Aoyama2, Yohsi Yamakawa1, Masahiro Ogawa2, Yasuji Koyama1. 1) Grad Sch of Bioagricultural Sci, Nagoya Univ, Nagoya, Aichi, Japan; 2) Kikkoman Corp, Noda, Chiba, Japan.

Expression of the endoglucanase A gene (eglA) in A. nidulans is inducible by cellulose. Previously, the cis-element responsible for the inductive expression, designated CeRE (Cellulose Responsive Element), was identified based on mutational analysis of the eglA promoter. CeRE contained the binding consensus of SRF-MADS proteins, suggesting involvement of McmA, the sole SRF-MADS protein in A. nidulans. While two Zn2Csys transcription factors in Aspergillus were recently reported to be essential to cellulase induction, one is ManR in A. oryzae, which regulates both mannanase and cellulase genes, and the other is ClrB in A. nidulans, a homolog of the cellulase regulator CLR-2 in N. crassa. Since these factors were orthologous sharing 63% identity, we use the name ManR/ClrB. In this presentation, we provide evidences that McmA and ManR/ClrB cooperatively regulate induction of cellulase genes. Effects of mcmA mutation and mnrR/cle deletion on expression of cellulase genes were examined by qRT-PCR. Expression of eglA, eglB, and cbhA was highly induced by cellulose in the wild type strain. The induction was significantly impaired by the mcmA mutation and abolished by the mnrR/cle deletion, indicating that the cellulase genes under control of McmA and ManR/ClrB are overlapped. Binding of His-tagged McmA and Flag-tagged ManR/ClrB-DDDB (DNA Binding Domain), which were produced in E. coli and purified, to the CeRE containing region of the eglA promoter was examined by EMSA. McmA gave two shifted bands corresponding to single and double occupation of the binding sites, which lay within and just upstream of CeRE. McmA/ClrB-DDDB alone showed very weak binding to the region. While both McmA and ManR/ClrB-DDDB were added, a slower-migrating and more abundant shifted band was appeared, which suggested cooperative binding of McmA and ManR/ClrB-DDDB. Presence of McmA and ManR/ClrB-DDDB in the shifted band was confirmed by supershift assay with the anti-his-tag and anti-flag-tag antibodies. These results indicated that inductive expression of eglA is regulated by cooperative binding of McmA and ManR/ClrB to its promoter, and suggests that regulation of eglB and cbhA would be similar. This work was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry.


For economically feasible production of liquid fuels and other value-added compounds from lignocellulosic plant material, strategies are required to boost cellulolytic and hemicellulolytic enzyme production by industrially relevant fungi. One promising approach is to modulate the transcriptional control mediating release from carbon catabolite repression (CCR) and induction of cellulase, hemicellulase and xylanase gene expression. To better understand the underlying molecular dynamics during induction, we characterized nucleo-cytoplasmic shuttling of the two transcription factors carbon catabolite repressor 1 (CRE1) and xylanase regulator 1 (XYR1) of Trichoderma reesei by means of live-cell imaging. In submerged cultures, nuclear import and export of CRE1 upon repression and induction, respectively, occurred within minutes and therefore was generally faster than shuttling of XYR1. Under CCR conditions XYR1 expression levels were very low, and its nuclear signal required up to one hour to significantly increase upon replacement into an inducing carbon source. Cultured directly under inducing conditions, nuclear accumulation of XYR1 was detectable after about 20h post inoculation, and strongly increased within the following 24 hours. CRE1 under the same conditions was localized exclusively to the cytoplasm. In plate cultures, nuclear recruitment of CRE1 and XYR1 differed within the central area, the subperiphery and the periphery of the colony depending on the provided carbon source. Most interestingly, under inducing conditions we found evidence for increased nuclear recruitment of CRE1 in the central area, correlating with strong nuclear import of XYR1 in the same region. Notably, the cytoplasmic signal of CRE1 was usually elevated in leading hyphae, whereas XYR1 was never significantly recruited to the colony periphery. Taken together our data provide the first temporal resolution of transcription factor shuttling during cellulase induction. One is ManR in A. nidulans, a homolog of the cellulase regulator CLR-2 in N. crassa. Since these factors were orthologous sharing 63% identity, we use the name ManR/ClrB. In this presentation, we provide evidences that McmA and ManR/ClrB cooperatively regulate induction of cellulase genes. Effects of mcmA mutation and mnrR/cle deletion on expression of cellulase genes were examined by qRT-PCR. Expression of eglA, eglB, and cbhA was highly induced by cellulose in the wild type strain. The induction was significantly impaired by the mcmA mutation and abolished by the mnrR/cle deletion, indicating that the cellulase genes under control of McmA and ManR/ClrB are overlapped. Binding of His-tagged McmA and Flag-tagged ManR/ClrB-DDBD (DNA Binding Domain), which were produced in E. coli and purified, to the CeRE containing region of the eglA promoter was examined by EMSA. McmA gave two shifted bands corresponding to single and double occupation of the binding sites, which lay within and just upstream of CeRE. McmA/ClrB-DDBD alone showed very weak binding to the region. While both McmA and ManR/ClrB-DDBD were added, a slower-migrating and more abundant shifted band was appeared, which suggested cooperative binding of McmA and ManR/ClrB-DDBD. Presence of McmA and ManR/ClrB-DDBD in the shifted band was confirmed by supershift assay with the anti-his-tag and anti-flag-tag antibodies. These results indicated that inductive expression of eglA is regulated by cooperative binding of McmA and ManR/ClrB to its promoter, and suggests that regulation of eglB and cbhA would be similar. This work was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry.

465. Trichophyton rubrum ap-1 gene expression in response to environmental challenges. Nalu TA Peres, Gabriela F Persinoti, Larissa G Silva, Tiago R Jacob, Antonio Rossi, Nilce M Martinez-Rossi. School of Medicine, University of Sao Paulo, Ribeirao Preto, Brazil.

Several families of transcription factors (TF) are found in fungal cells, which contribute for the broad range of cellular responses triggered by environmental changes in these organisms. These TF regulate the expression of genes involved in different cellular processes allowing cell survival under stressful as well as physiological conditions. The AP-1 TF belongs to the bZIP family (basic leucine zipper), and is involved in the conidiation process, response to oxidative stress, multidrug resistance, and pathogenicity of some fungi. In dermatophytes, a group of keratinophilic fungi, little is known about this TF and the processes in which it is required. Trichophyton rubrum is the major etiologic agent isolated from clinical cases of cutaneous mycoses in humans, and studies of the responses of this fungus to several environmental conditions allow a better understanding of its physiology and pathogenicity, thus providing information of how to decrease its growth and to establish more efficient therapeutic measures. Here, we evaluated the ap-1 gene expression profile during growth of T. rubrum in several nutrient sources (keratin, ex vivo skin and nail), exposure to antifungal drugs, and oxidative/osmotic stresses. An up-regulation of ap-1 gene expression was observed during ex vivo infection and keratin utilization, compared to growth in a glucose containing medium. In response to different antifungal agents, ap-1 was up-regulated, while osmotic and oxidative stress did not alter its expression level. These results provide insights into the regulation of the ap-1 TF gene expression, suggesting its involvement in T. rubrum pathogenicity, possibly regulating several genes that allow the utilization of proteins from the host tissues as nutrient sources, and also protecting the cell against the damages caused by antifungal drugs. Financial Support: FAPESP, CNPq, CAPES, and FAPEA.
466. Vib-1 is required for cellulose utilization in Neurospora crassa. Yi Xiong, Jianping Sun, N. Louise Glass. Department of Plant and Microbial Biology, Univ California, Berkeley, Berkeley, CA.

Vib-1 (vegetative incompatibility blocked) encodes a transcriptional regulator that is required for nonself recognition and heterokaryon incompatibility in Neurospora crassa. It is also required for the production of extracellular proteases upon carbon and nitrogen starvation. We found that vib-1 null mutant was severely defective in utilizing cellulose as a carbon source and this growth defect could be rescued by constitutively expressing clr-2, an essential transcription factor for cellulase gene expression. Our transcriptional profiling with RNA-seq showed that most genes that were induced in wild type on cellulose were not induced in the vib-1 null mutant, but were expressed in vib-1 null mutant under non-inducing conditions when clr-2 transcription factor was constitutively expressed. Our data suggests that vib-1 functions in a signaling pathway upstream of clr-2 for cellulose utilization. By comparing the transcriptomes of vib-1 and clr1/clr-2 mutants versus wild type in response to different carbon sources including sucrose and cellulolytic materials as well as carbon and nitrogen starvations, we differentiated vib-1 dependent gene expression into several categories. We propose that vib-1 may play an important role in carbon starvation signaling thus positively regulating preparation for utilization of cellulotic materials.

467. The stringency of start codon selection in the filamentous fungus <em>Neurospora crassa</em>. Jiawei Wei1, Ying Zhang1, Ivaylo P. Ivanov2, Matthew S. Sachs1.

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In eukaryotic cells, initiation may occur from near-cognate codons that differ from AUG by a single nucleotide. The stringency of start codon selection impacts the efficiency of initiation at near-cognate codons and the efficiency of initiation at AUG codons in different contexts. We used a codon-optimized firefly luciferase reporter initiated with AUG or each of the nine near-cognate codons in a preferred context to examine the stringency of start codon selection in the model filamentous fungus <em>Neurospora crassa</em>. In vivo results indicated that the hierarchy of initiation at start codons in N. crassa (AUG >> CUG > GUG > ACG > AUA > UUG > AUU > AUC) is similar to that in human cells. Similar results were obtained by translating mRNAs in a homologous N. crassa in vitro translation system or in rabbit reticulocyte lysate. We next examined the efficiency of initiation at AUG, CUG and UUG codons in different contexts in vitro. The preferred context was more important for efficient initiation from near-cognate codons than from AUG. These studies demonstrated that near-cognate codons are used for initiation in N. crassa. Such events could provide additional coding capacity or have regulatory functions. Analyses of the 5'-leader regions in the N. crassa transcriptome revealed examples of highly conserved near-cognate codons in preferred contexts that could extend the N-termini of the predicted polypeptides.

468. A temperature-dependent complex transcriptional network controls cell shape and virulence in Histoplasma capsulatum. Sinem Beyhan1, Matias Gutierrez2, Mark Voorhies3, Anita Sii1,2. 1) Microbiology and Immunology, University of California, San Francisco, San Francisco, CA; 2) Howard Hughes Medical Institute, Chevy Chase, MD.

<i>Histoplasma capsulatum</i>, which is a respiratory fungal pathogen of humans, is endemic in the United States. Depending on the exposure dose and the immune status of the host, the infection can lead to mild to life-threatening and systemic disease. <i>H. capsulatum</i> has a dimorphic life cycle, switching from an infectious filamentous form in the soil to a pathogenic yeast form in mammalian hosts. This morphological switch, which requires a dramatic shift in the gene expression profile of the cells, can be easily recapitulated in the laboratory simply by changing the temperature from room temperature to 37°C. We previously identified three regulators, Ryp1, Ryp2 and Ryp3, which are required for the yeast-phase growth. ryp1, ryp2 and ryp3 mutants are unable to respond to change in temperature and grow constitutively in the filamentous form even at 37°C. Ryp1 belongs to a conserved family of fungal proteins that regulate cellular differentiation in response to environmental signals. The best-studied member of this family of proteins is WOR1, which is a master regulator of white-to-opaque switching in <i>Candida albicans</i>. Ryp2 and Ryp3 are orthologues to WorsA and VehB, respectively, which are development regulators in <i>Aspergillus nidulans</i>. In this study, using transcriptional profiling and chromatin immunoprecipitation (ChIP) experiments, we explored complementary and unique roles of Ryp1, Ryp2, and Ryp3 in regulating yeast-phase growth. Our results reveal that Ryp1, Ryp2 and Ryp3 physically interact and associate with DNA throughout the genome. Additionally, we identified a fourth transcription factor, Ryp4, which is a direct target of Ryp1, Ryp2 and Ryp3, as a novel regulator of yeast-phase growth in <i>H. capsulatum</i>. Further transcriptional profiling and ChIP experiments show that Ryp4 regulates and associates with the upstream regions of a subset of Ryp1, Ryp2, and Ryp3 targets, which are involved in morphology and virulence in <i>H. capsulatum</i>. Finally, we identified two distinct cis-regulatory elements that are utilized by Ryp1 or the Ryp2/Ryp3 complex to facilitate gene expression. Our results reveal a tightly regulated and interwoven transcriptional network that controls the ability of a pathogenic fungus to cause disease in response to host temperature.


Coprinopsis cinerea is a mushroom of limited edible value and is extensively used as a model organism to study the development of homobasidiomycete fungi. Unraveling the molecular basis of the fungus developmental processes would contribute to evolutionary studies and lead to improvement in the breeding and cultivation of edible mushroom homobasidiomycete mushrooms. MicroRNA (miRNA) is a group of endogenous non-coding regulatory RNAs of ~22 nt that regulate gene expression in various biological processes such as cell differentiation, development regulation and heterochromatin formation. Dicer is a key enzyme involved in the biogenesis of miRNAs and is highly conserved through eukaryotes. A miRNA-like RNA cannot be defined as a miRNA unless a Dicer (or Dicer-like) protein is found participating in its biogenesis. There are three Dicer homologs (CC1G_00230, CC1G_03181, CC1G_13988) identified in the C. cinerea genome. In order to gain an insight into the roles of Dicer proteins in C. cinerea and to investigate whether Dicer is involved in miRNA biogenesis, we employed a comprehensive phylogenetic analysis of the Dicer protein family in all of the three kingdoms under Eukaryota - animal, plant and fungus - and highlighted the results of Dicer homologs in C. cinerea. We showed that Dicer genes duplicated and diversified independently in early animal, plant and fungus evolution, coincident with the origins of multicellularity. Besides, identified a group of Dicer homologs that are specific to mushroom-forming fungi. We also showed that changes in one of the Dicer domains, the double-stranded RNA binding domain (dsRBD), alone may lead to diversification of Dicer proteins. As a whole, we revealed a dynamic picture in which the evolution of Dicer proteins has driven elaboration of parallel RNAi functional pathways in the animal, plant and fungus kingdoms.


Genetic transformation of the basidiomycete <i>Coprinopsis cinerea</i> has first been described by Binninger et al. in 1987 (1). For the transfer of genetic material, chromosomal integrative vectors are used, which contain a selectable marker gene and/or a gene of interest. During transformation the genetic material integrates at ectopic sites into the host chromosomes. Binninger et al. (1987) created the vector pC1001, by cloning a 6.5 kb PstI genomic
fragment, with the tryptophan synthetase gene (trp1) of C. cinerea, into the CoEl vector pUC9. The inserted gene allows the complementation of trp1 auxotrophs and can be used as a selection marker. Several transformation experiments using this vector reveal a surprising phenomenon. Single-transformation with solely pCC1001 gives only low numbers of transformants, whereas co-transformation with an additional plasmid yields about 2x more transformants. To explore this phenomenon, the length of the trp1 harboring fragment was changed and the existing replicon was replaced by a modified CoEl replicon. All single-transformations resulted in the same observations. An alternative selection marker (paB1 encoding) and different relative vector- vector concentrations were tested in several co-transformation experiments. The obtained results lead to the conclusion that tryptophan feedback inhibition might be responsible for the reduced transformation efficiencies in single-transformations of trp1 vectors. (1) Binninger et al. (1987). DNA-mediated transformation of the basidiomycete Coprinus cinereus. EMBO J 6: 835-840.

471. The first promoter for conditional gene expression in Acremonium chrysogenum: iron starvation-inducible mir1, Fabio Gselle1, Michael Blatzer1, Beate Abt1, Markus Schrettl1, Herbert Lindner1, Hubertus Haas1. 1) Christian Doppler Laboratory for Fungal Biotechnology, Division of Molecular Biology, Medical University of Innsbruck, Austria; 2) Sandoz GmbH, Kundl, Austria; 3) Division of Clinical Biochemistry, Medical University of Innsbruck, Austria.

The filamentous fungus Acremonium chrysogenum is of enormous biotechnological importance as it represents the natural producer of the beta-lactam antibiotic cephalosporin C. However, a limitation in genetic tools, e.g. promoters for conditional gene expression, impedes genetic engineering of this fungus. Here we demonstrate that in A. chrysogenum iron starvation induces the production of the extracellular siderophores dimeruc acid, coprogen B, 2-N-methylcoprogen B and dimethylcoprogen as well as expression of the putative siderophore transporter gene, mir1. Moreover, we show that the promoter of mir1, mir1', is suitable for conditional expression of target genes in A. chrysogenum as shown by mir1'-driven and iron starvation-induced expression of genes encoding green fluorescence protein and phleomycin resistance. The obtained iron-starvation dependent phleomycin resistance indicates the potential use of this promoter for selection marker recycling. Together with easy scorable siderophore production, the co-regulation of mir1 expression and siderophore production facilitates the optimization of the inducing conditions of this expression system. This work was funded by Sandoz GmbH (Kundl, Austria) and the Christian Doppler Society (Vienna, Austria).

472. Mutagenic effect of high-LET ion beam irradiation in Neurospora crassa. Liqiu Ma1,2, Yusuke Kazama2, Tomoko Abe2, Shuuitsu Tanaka2, Shin Hatakeyama1. 1) Regulation Biol, Saitama Univ, SAITAMA, Japan; 2) Radiation Biology Team, RIKEN, SAITAMA, Japan.

Heavy ion beams cause great damages to cellular components particularly generating severe DNA damages. DNA double strand breaks (DSBs). We examined the biological effect and mutagenesis of irradiation of high-LET ion beam (Fe-ion) to DSBR repair defect mutants in filamentous fungus Neurospora crassa. Fe-ion beam (24He+, 90 MeV/u, LET=641 kev/mm) was irradiated to two DSBR repair deficient mutants and wild-type strain. By lower doses (<100 Gy), all of the strains showed same survival rates. In the case of higher doses (>100 Gy), sensitivity to irradiation of the mus-52 strain (non-homologous end-joining deficient) is higher than that of the wild type, whilst lower than that of the mei-3 strain (homologous recombination deficient). Frequency of forward mutation occurred in the ad-3 loci was similar to previously examined C-ion beam irradiation, i.e. mei-3 > wild type > mus-52 strains. However, characteristic difference of mutation was observed as the scale of deletions; large deletions were frequently in the Fe-ion beam irradiated wild type strain, comparing to that 1 bp-deletions were mainly observed in the C-ion irradiation. Differences of mutagenesis and killing effect between the irradiation of two heavy ions, Fe-ion and C-ion, were discussed based on types of DNA damages.


The zygomycete Phycomyces blakesleeanus responses to light include phototropism of the fruiting body, activation of beta-carotene biosynthesis, and regulation of fruit body development. These photoresponses require the Mad complex, a protein complex composed of proteins MadA and MadB. These proteins are homologous of WC-1 and WC-2 from Neurospora crassa and presumably play a similar role in the regulation by light of gene expression. MadA and MadB have a zinc finger domain at the carboxyl end, and MadA has a LOV domain that should serve as the binding site for a flavin chromophore. In Phycomyces, the Mad complex should operate as a photoreceptor and transcription factor complex. The Phycomyces genome contains two additional wc-1 homologs, wcoA and wcoB, and three additional wc-2 homologs, wctB, wctC, and wctD, but their function is unknown. We have expressed MadA and MadB in E. coli, and we have shown that these proteins bind the promoter of the light-regulated gene hspA by electrophoresis mobility shift assays (EMSA). Protein binding to the hspA promoter was observed with each isolated protein or with the two proteins associated in the Mad complex. The binding site to the hspA promoter will be identified by DNA footprinting analysis. We are performing similar assays with the other Phycomyces Wc proteins and we hope that the results will help us to understand the role of the multiple Wc proteins in light-dependent gene regulation in Phycomyces.

474. Down Regulation of sidB Gene by Use of RNA interference Technology in the Filamentous Fungi Aspergillus nidulans, S. Rezaie1,2, H. Eslami1, M.R. Khorramizadeh1, M.R. Pourmand1, M. Moazeni1. 1) Medical Biotechnology Dept, Tehran University of Medical Sciences, PhD; 2) Div. of Molecular Biology, Dept. of Medical Mycology and Parasitology, Tehran University of Medical Sciences, PhD.

Background: RNA interference (RNAi) is a natural process by which short double-stranded RNA (siRNA) silences the expression of complementary target RNAs by inducing RNA cleavage and subsequent reduction in protein expression levels. Introduction of the RNA interference machinery has guided the researchers to discover novel methodologies for knocking down essential vital factor or virulence factor genes in the microorganisms such as fungi. In filamentous fungi, Aspergillus nidulans, the gene sidB plays an essential role in septation, conidiation and vegetative hyphal growth. In the present study, we benefited from the RNA interference strategy for down-regulating of a vital gene in the fungus Aspergillus nidulans. Materials and Methods: The 21-nucleotide siRNA was designed on the basis of the CDNA sequence of the sidB gene of A. nidulans. Transfection was performed via uptaking siRNAs from medium by germinated spores. After 18 hours of incubation, total RNA was extracted and quantitative changes in expression of the sidB gene were analyzed by measuring the cognate sidB mRNA level by use of a quantitative real-time RT-PCR assay. Results: In the presence of 25 nM of siRNA, a significant inhibition in germ tube elongation was observed compared with positive control samples (21 VS 42 mM). In addition, at the concentration of 25 nM, a considerable decrease in sidB gene expression was revealed. Conclusion: Usage of RNA interference as a kind of post-transcriptional gene silencing methods is a promising approach for designing new antifungal agents and discovering new drug delivery systems.

475. SmallRNA mediated meiotic silencing of a transposable element in Neurospora crassa, Yizhou Wang, Jason E. Stajich. Plant Pathology & Microbiology, Univ. of CA, Riverside, Riverside, CA.

Meiotic silencing of unpaired DNA plays an important role in protecting the genome integrity of Neurospora crassa. It is thought to fight against the invasion of virus and endogenous transposable elements. Our previous work has shown that a 10 KB MULE (mutator-like element)-related DNA
transposable element, named sly-1, uniquely exists in the wild type strain OR74A (FGSC#2489) of N. crassa. Here we show that in the cross between OR74A and D60 (FGSC#8820), a strain lacking sly-1, the unpaired sly-1 induced the production of small RNAs 4 days after fertilization. The small RNAs were generated from both strands of the sly-1 region and demonstrated typical Dicer-processed smallRNA features in Neurospora crassa: 25bp long with a strong preference for uridine at the 5’ end. An RNA-dependent RNA polymerase (SAD-1) was found to be required for such small RNA production (1). We generated draft genome sequencing of D60 with Illumina HiSeq and compared it to the OR74A genome to identify additional unique regions where meiotic silencing of unpaired DNA may have occurred. These unique regions were also found to produce smallRNA with the same features as those from sly-1. These results provide strong support for the endogenous silencing role of meiotic silencing against a natural intact transposable element and describe the RNA interference pathway-involved silencing pattern of meiotic silencing. 1) Shiu PK, Raju NB, Zickler D, Metzenberg RL. Cell 2001; 107(7):905-16.

Pathogenic and Mutalistic Interactions


The filamentous fungus Magnaporthe oryzae is the most destructive pathogen of rice worldwide. It is described as having two distinct lifestyles within the host plant: a biotrophic phase during the early stages of infection followed by a necrotrophic phase characterized by host cell death and lesion formation. To identify candidate effector proteins that may contribute to pathogenesis, the genome of M. oryzae strain 70-15 was mined for predicted proteins that contain a signal peptide, have greater than 3% cysteine content, and are less than 250 amino acids in length. These criteria were selected based upon the characteristics of known effectors from other plant-pathogenic fungi and oomycetes. To investigate the roles of these candidates in the biotrophic or necrotrophic phases of infection, they were transiently expressed in Nicotiana benthamiana leaves via agroinfiltration. When expressed within plant cells, candidate effectors that induce necrosis in the N. benthamiana leaves could potentially act as inducers of host cell death during the necrotrophic phase of infection. Conversely, candidate effectors that prevent necrosis when co-infiltrated with known inducers of host cell death are potentially involved in suppressing host plant defenses and therefore may contribute to the biotrophic phase of infection. Of 70 candidate effectors tested to date, 10 were found to induce necrosis when transiently expressed in N. benthamiana. In addition, to test for suppression of host cell death, candidate effectors are currently being co-agroinfiltrated with the BAX gene, a known inducer of host cell death in both plant and mammalian cells, or a known necrosis inducer from M. oryzae. Those candidates that show an interesting phenotype will be selected for further characterization as potential effectors by analyzing their expression in planta and activity when expressed within rice protoplasts.

477. Penetration-specific effectors from Phytophthora parasitica favour plant infection. Edouard Evangelisti1, Benjamin Gouveto2, Naima Minet-Kebdani1, Marie-Line Kuhn2, Agnes Attard3, Franck Panabieres1, Mathieu Gourgues1. 1) UMR Institut Sophia Agrobiotech, INRA/CNRS/Université de Nice, Sophia Antipolis, France; 2) Institut Méditerranéen de Biodiversité et d’Écologie marine et continentale (IMBE), CNRS-INEE - IRD -Aix Marseille Université - Université d’Aix-Marseille - Institut Pytheas.

Oomycetes are major crop pests which cause million dollars losses every year. To date only a few efficient chemicals are available against these filamentous microorganisms. A better understanding of the molecular events occurring during plant-oomycete interactions will help to propose new strategies for crop protection. We performed a transcriptional analysis in order to identify oomycete penetration-specific genes and identified a set of penetration-specific effectors (PSE) bearing a RXLR motif. This motif was previously shown to promote effector import into plant cells during biotrophic stage in feeding structures called haustoria. Here we report the functional analysis of three candidate genes, referred to as PSE1, PSE2 and PSE3. The three effectors were able to abolish plant defense responses when transiently expressed in Nicotiana plants. Moreover, constitutive expression of PSE1 and PSE3 in A. thaliana led to an enhanced susceptibility to F. parasitica infection suggesting a role for these proteins in F. parasitica pathogenicity. Transgenic Arabidopsis lines accumulating PSE1 protein showed several developmental perturbations that were associated with altered auxin physiology. Root growth inhibition assays showed that auxin signaling pathway is not altered by PSE1 accumulation. Nevertheless, the coiled-root phenotype and the enhanced susceptibility of PSE1-expressing lines to P. parasitica were reverted by synthetic auxin 2,4-D supply, or treatment with the auxin efflux inhibitor TIBA suggesting that a reduced auxin accumulation is responsible for these phenotypes. This hypothesis was confirmed by a reduced activity of the pDRS auxin sensitive promoter at the root apex. The alteration of the expression pattern observed for two auxin efflux carriers, PIN4 and PIN7 suggests that a perturbation of auxin efflux could be responsible for the PSE1 associated defects. We proposed that PSE1 could favour P. parasitica virulence by interfering with auxin content. Our results show that penetration specific effectors can modulate general plant functions to facilitate plant infection. Perturbation of hormone physiology was previously reported for other plant pathogens, including nematodes and bacteria, supporting the hypothesis that infection strategies from distant pathogens species could converge onto a limited set of plant targets.


Rice blast disease is caused by the fungus Magnaporthe oryzae and is the most destructive disease of cultivated rice. The pathogen elaborates a specialized infection structure called the appressorium. The morphological and physiological transitions that lead to appressorium formation of M. oryzae during plant infection are stimulated through perception of environmental signals including surface hydrophobicity and hardness, and the presence of cutin monomers and leaf surface waxes. The fungus perceives and internalizes these stimuli by a variety of intracellular MAP kinase signaling pathways. The homeobox and C2/H2 Zn finger domain transc...
480. Functional analysis of Nbs1 of Magnaporthe oryzae. K. Sasaki, K. Amano, T. Sone, M. Narukawa, T. Kamakura. 1) Applied Biological Science, Tokyo Univ. of Science, Noda, Chiba, Japan; 2) Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan.

The filamentous fungus Magnaporthe oryzae causes rice blast, the most serious disease that affects global rice production. On the surface of host plant, a specialized infection structure called appressorium is formed on tip of germ tube. Induction of the development of appressorium requires several external stimulants and a complete cycle of cell division. Although many studies have revealed some of process of appressorium formation in M. oryzae, the complete mechanism is still obscure. We selected Nbs1 from germ tube expressing cDNA library and made Nbs1 disruptants. The cDNA library mainly contains the genes that express in the period of germ tube development and/or appressorium formation. Nbs1 is presumed to have forkhead associated (FHA) domain, which is contained in many proteins that are involved in DNA repair and cell cycle. In our previous study, Nbs1 disruptants showed growth delay, abnormality of conidia formation and nuclear division, reduction of germination rate and appressorium formation rate, abnormal pigmentation and high sensitivity to DNA-damaging agents. Although Neurospora crassa knock-out mutants of rcaA, which share sequence similarities with Nbs1, showed similar phenotypes to Nbs1 disruptants, rcaA did not seem to contain FHA domain. Toward further study of the function of Nbs1, we induced a plasmid carrying an rcaA (pNB51) or FHA domain-deleted Nbs1 (pCB51dF) into Nbs1 disruptants. Consequently, pNB51 and pCB51dF were able to partially complement phenotypes of Nbs1 disruptants. This result suggested that rcaA has at least partial similar functions of Nbs1 in N. crassa and another functional domain exists in Nbs1.

481. Influence of hypoxia on antifungal susceptibility, sterole pattern and biomarker release of Aspergillus spp. Ulrike Binder, Elisabeth Maurer, Christoph Müller, Franz Bracher, Cornelia Lass-Flörl. 1) Division of Hygiene and Medical Microbiology, Medical University Innsbruck, Innsbruck, Tyrol, Austria; 2) Department of Pharmacy, Ludwig Maximilians University Munich, Germany.

Invasive aspergillosis (IA) is a major life-threatening disease in immunocompromised patients, with mortality rates from 40% up to 90% in high-risk populations. The most common species causing aspergillosis is Aspergillus (A.) fumigatus, accounting for approximately 90% of infections. Depending on regional distinctions, A. flavus and A. terreus are frequently reported. During infection, fungal pathogens must adapt to microenvironmental stresses, including hypoxia as well as high CO2 levels. Such oxystress conditions are usually not taken into account in in vitro models of infection, the assessment of antifungal sensitivities or the release of biomarkers used for diagnosis. Therefore, we compared the in vitro activity of amphotericin B (amb), different azoles and echinocandins in hypoxic conditions (1% O2, 5% CO2) to their activity in normoxic conditions against isolates of A. fumigatus and A. terreus and other aspergilli. Using Etest strips, we found a reduction of the minimal inhibitory concentration (MIC) for amb for all aspergilli in hypoxic conditions. Similarly, a significant reduction in the MIC for all tested azoles was demonstrated for A. terreus isolates, while for A. fumigatus isolates differences were less pronounced. For echinocandins, little or no change in the MEC (minimal effective concentration) was detected between hypoxic and normoxic conditions for all isolates. Most interestingly, A. terreus strains, that are resistant to amb in normoxia, exhibited sensitivity to amb in hypoxic conditions, defining a breakpoint of > 2 mg/ml. Notably, for none of the strains tested, MIC/MEC values increased in hypoxia. Currently we are investigating if changes in the sterole pattern or the amount of ergosterol contribute to these changes in antifungal susceptibility in hypoxia. The detection of circulating fungal antigens in serum for Aspergillus galactomannan or b-glucan has become an accepted diagnostic strategy. However, sensitivity and specificity vary extremely and the reasons are only partially clear; therefore, we are currently checking whether hypoxia influences the physiological kinetics of GM and b-glucan release.

482. Sit and wait: Special features of Aspergillus terreus in macrophase interactions and virulence. M. Brock, I.D. Jacobsen. 1) Microbial Biochemistry/Physiology, Friedrich Schiller University and Hans Knöll Institute, Jena, Germany; 2) Molecular Pathogenicity Mechanisms, Hans Knöll Institute Jena, Germany.

While Aspergillus fumigatus is known as the main cause of invasive pulmonary aspergillosis in immunocompromised patients, Aspergillus terreus is an emerging pathogen prevalent in some local hot spots. When tested in embryonated egg or murine infection models A. terreus required substantially higher infectious doses compared to A. fumigatus to cause high mortality rates. Furthermore, when A. fumigatus and A. terreus infections were followed by in vivo imaging using bioluminescent reporter strains, germination and tissue invasion of A. terreus was significantly delayed. To elucidate differences in more detail, the interaction of A. terreus and A. fumigatus with macrophages was compared. A. terreus was phagocytosed significantly faster, which appears mainly due to higher exposure of galactomannan and glucans on the surface of conidia. Additionally, although phagocytosis of both species resulted in phagolysosome maturation, A. fumigatus efficiently inhibited acidification, which was not the case for A. terreus. However, within this acidic environment of phagolysosomes A. terreus showed long-term persistence without significant inactivation of conidia. Further analyses revealed that inefficient blocking of acidification by A. terreus was due to differences in the spore colour pigment of both species. Recombinant production of a naphthopyrone synthase from Aspergillus nidulans enabled A. terreus to inhibit the acidification to a similar extent as observed for A. fumigatus. This alteration of the phagolysosomal environment resulted in an increased escape from macrophages and was accompanied by increased virulence in a murine infection model. We speculate that the long-term persistence of A. terreus wild-type strains in acidified phagolysosomes might be responsible for high dissemination rates observed in infected human patients, because A. terreus might hitchhike inside immune effector cells to reach secondary sites of infection.

483. Identification and characterization of an RXLR-like effector family from medically relevant fungi. Shiv D. Kale, M.C. Drews, H. Clark, Hua Wise, Vincenzo Antignani, Tristan A. Hayes, Christopher B. Lawrence, Brett M. Tyler. 1) Virginia Bioinformatics Institute, Virginia Tech., Blacksburg, VA; 2) Department of Biological Sciences, Virginia Tech., Blacksburg, VA; 3) Department of Biochemistry, Virginia Tech., Blacksburg, VA; 4) Department of Botany and Plant Pathology, Virginia Tech., Blacksburg, VA.
Fungal infections have become an increasingly significant problem for immunocompromised individuals, transplant recipients, the elderly, several cases involving healthy individuals. There is a significant growth in incidences of morbidity and mortality associated with medically important fungi, specifically Aspergillus species. Aspergillus fumigatus virulence has been attributed to production of pigments, adhesins on the surface of the cell wall, secreted proteases, and mycotoxins. Current treatments consist of oral corticosteroids, antifungal medications, and/or surgery to remove aspergillosis. Many of these treatments have substantial shortcomings. Detection and diagnosis is also weghty problem as most clinical tests take weeks for results allowing the infection to proceed. Appropriately, the paradigm for human fungal interactions has been focused on the host deficiencies mediating virulence of opportunistic pathogenic fungi. There has been substantial progress in identifying and characterizing secreted proteins (effectors) from bacterial, oomycete, and fungal pathogen. A subset of these effector proteins are able to enter host cells and modulate host intracellular functions. Using our bioinformatics pipeline we have been able to identify a family of secreted proteins from A. fumigatus sharing a conserved N-terminal RXLR-like motif. This family is expanded amongst primary fungal pathogens. The RXLR and RXLR-like motifs from known intracellular effectors of plant pathogenic and mutualistic oomycetes and fungi have been shown to facilitate effector entry into plant cells via binding external phosphorylated isothiocyanate-3-phosphate (Pi3P). Here we describe AF2, a candidate effector from A. fumigatus that contains a N-terminal RXLR-like motif. Through the use of confocal microscopy and flow cytometry AF2 is rapidly able to enter several primary and immortalized mammalian cell lines. Through the use of isothermal titration calorimetry and liposome binding assays we show AF2 has nanomolar binding affinity for Pi3P, and does not bind other mono or poly-PiPs that we have tested thus far. Based on our bioinformatics and biochemical analysis we postulate AF2 is a secreted effector protein capable of rapidly translocating into mammalain cells. We will present our latest findings on the physiological relevance of AF2.

484. A role for PalH-mediated signal transduction in A. fumigatus virulence and cell wall integrity: An exploitable target for combination therapy? M. Bertuzzi1, C.M. Grice2, L. Alcataz-Fuoli3, A.M. Calcagno-Pizzarelli1, J. Kalischmidt1, S. Gill4, K. Fox4, A. Cheverton1, Hong Liu5, V. Valiante1, E.A. Espeso1, S.G Filler1, A. Brakhage6, E.M. Bignell1. 1) Centre for Molecular Bacteriology & Infection, Imperial College London, London (UK); 2) Mycology Reference Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III, Madrid (Spain); 3) David Geffen School of Medicine at UCLA, Division of Infectious Diseases, Los Angeles Biomedical Institute at Harbor-UCLA Medical Center (USA); 4) Leibniz-Institute for Natural Product Research and Infection Biology Hans Knöll Institute, Molecular and Applied Microbiology, Jena (Germany); 5) Dept. of Cellular and Molecular Biology, Aspergillus Molecular Genetics Unit, Centro de Investigaciones Biológicas (C.S.I.C.), Madrid (Spain).

Adaptation to host-imposed stress is a crucial requirement for persistence of Aspergillus fumigatus in the mammalian lung. In Aspergillus species, PacC signalling promotes tolerance of alkaline environments via signal-dependent proteolytic processing of the transcription factor PacC. The aim of this study was to test the requirement for A. fumigatus PalH during infection and to decipher its role in PacC-mediated signalling. The role of PalH in alkaline-mediated PacC processing was tested using electrophoretic mobility shift assay, and A. fumigatus virulence was examined in a murine neutropenic model of pulmonary aspergillosis. To probe the mechanistic basis of PalH-mediated signalling, we utilised a split-ubiquitin Membrane Yeast Two-Hybrid (MYTH) assay to assess protein interactions amongst candidate A. fumigatus signalling proteins of this pathway. A. fumigatus isolates expressing epitope-tagged PalH protein were constructed to assess the relevance of PalH oligomerisation. Analysis of PacC processing identified the requirement for PalH to initiate alkaline-mediated PacC signalling. A DpalH mutant is somewhat sensitive to alkaline pH, and attenuated for virulence in a murine model of pulmonary aspergillosis. The mutant is also sensitive to cell wall-perturbing agents, and in the presence of the cell wall-active antifungal caspofungin undergoes extensive hyphal branching and ballooning compared to the parental and reconstituted strains. In the absence of PalH A. fumigatus-mediated damage of epithelial cells is abrogated in vitro. By using a MYTH assay a significant interaction between A. fumigatus PalH and PalF was detected in Saccharomyces cerevisiae. In A. fumigatus PalH-mediated PacC signalling, likely implemented in a (PalF) arrestin-like manner, commands a central role in the expression of virulence-determining functions. The impairment of PacC signalling exerts a synergistically inhibitory effect upon fungal viability in the presence of cell wall-active antifungal drugs and therefore represents an attractive target for the development of novel antifungal mono- and combination therapies. Our results support a scenario whereby PalH is an oligomerising receptor, responsible to extracellular pH, and required for virulence and echinocandin tolerance. Future studies will focus upon the mechanism of PalH-mediated pH sensing.

485. Aspergillus fumigatus trehalose-6-phosphate regulates innate immune responses and virulence through modulation of fungal cell wall composition. Srisombat Puttikamonkul1, Vishu K. Amananda1, Jean-Paul Latge1, Kelly M. Shephardson2, John R. Perfect3, Nora Grahl4, Bridget M. Barker1, Robert A. Craemer1. 1) Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH; 2) Immunology and Infectious Diseases, University of California, San Francisco; 3) Immunochemistry and Infectious Diseases, Montana State University; 3) Unite des Aspergillus, Institut Pasteur; 4) Medicine, Division of Infectious Diseases, Duke University Medical Center.

Mechanism(s) behind the attenuated fungal virulence of trehalose biosynthesis pathway mutants are not fully understood. We observed previously that TPS2/OrfA, a key enzyme in TPS1/TPS2 trehalose biosynthesis is required for cell wall integrity and fungal virulence in A. fumigatus. In this study, we tested the hypothesis that the significant in vivo attenuated virulence and in vitro impaired cell wall integrity of DorIA is due to accumulation of Trehalose-6-Phosphate (T6P). Our data suggest that the mechanism behind the attenuated virulence of the A. fumigatus TPS2 null mutant, DorIA, in a murine model of X-linked chronic granulomatous disease (X-CGD) is mediated by an increased susceptibility of DorIA to polymorphonuclear leukocyte (PMN) killing. In the absence of PMNs in the xCGD murine model, DorIA exhibited restored fungal burden and virulence similar to wild-type inoculated animals. Null mutations in putative trehalose biosynthesis proteins TsA and TsB in the DorIA background were able to ameliorate T6P accumulation and restore cell wall integrity and virulence strongly suggesting that accumulation of T6P is the key factor associated with DorIA virulence. Our results identify a previously unknown mechanism of immune modulation by the fungal carbohydrate metabolite T6P that has significant implications for targeting trehalose biosynthesis as an antifungal drug target.

486. Fungal lipoxigenases: a novel instigator of asthma? Gregory J. Fischer1, Katharyn Affeldt2, Erwin Berthier2, Nancy P. Keller1. 1) Department of Genetics, University of Wisconsin-Madison, Madison, WI; 2) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI; 3) Department of Bacteriology, University of Wisconsin-Madison, Madison, WI.

Statement of Purpose: Fungi have long been associated with asthmatic diseases, yet the exact mechanism(s) by which fungi induce asthma is unknown. We propose that fungal lipoxigenase enzymes and their eicosanoid products are involved in asthmatic diseases. Human 5-lipoxigenase derived leukotrienes induce inflammation, mucus secretion, vasodilatation, and bronchial constriction. We hypothesize that the fungal pathogen Aspergillus fumigatus is capable of secreting a 5-lipoxigenase homolog, LoxB, that participates in eicosanoid production, including leukotrienes. This secreted homolog is translocated into lung epithelial cells, participates in the production of leukotriene and other eicosanoids, and exacerbates asthmatic responses, such as bronchoconstriction. Together, this work will help delineate the role fungal products play in asthmatic diseases. Methods: We are
assessing fungal interactions with lung epithelial cells using a microfluidic in-vitro platform followed by murine asthma model research. To assess the effects of LoxB overexpression, mass spectrometry was used to identify eicosanoid oxylipins within culture supernatants. Results: We have identified an *Aspergillus fumigatus* lipoxigenase, LoxB, with high identity to human 5-lipoxigenase. Moreover, we have identified a motif in LoxB that may mediate entry into lung epithelial cells. To fully understand the impact of LoxB in asthma, we have developed an *Aspergillus fumigatus* strain that overexpresses LoxB. Overexpression of LoxB results in increased levels of various eicosanoids that are known to cause airway hyperresponsiveness and increased mucus production. Future work will focus on characterizing the effect these eicosanoid products have on the airway and whether fungal effector translocation result in increased leukotriene levels.

487. F-box protein 15 (Fbx15) links virulence of Aspergillus fumigatus to protein degradation and stress response. Bastian Jöhnk, Özgür Bayram, Oliver Valerius, Thorsten Heinke, Ilse D. Jacobsen, Axel A. Brakhage, Gerhard H. Braus. Institute for Microbiology and Genetics, Department of Molecular Microbiology and Genetics, Georg August University, Göttingen, Germany; Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKG) and Friedrich Schiller University, Jena, Germany; Department for Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology (HKG) and Friedrich Schiller University, Jena, Germany.

Rapid adaptation to a versatile host represents a challenge for the opportunistic human pathogen *Aspergillus fumigatus* for successful infection. F-box proteins are the adaptor subunits of E3 SCF (Skp1 cullin-1 F-box protein) ubiquitin ligases. They recognize target proteins, which are marked by the SCF complex for degradation in the 26S proteasome. Here we have identified Fbx15 as an F-box protein, which links *A. fumigatus* virulence to protein degradation. *A. fumigatus* deletion strains that have lost fbx15 are unable to infect immunocompromised mice in a murine model of invasive aspergillosis. Fbx15 is required for growth during stress including increased temperature, oxidative stress and amino acid starvation. Fbx15 is also required for controlling the synthesis of the antipathogenic glutoxin. Fbx15 interacts in the nucleus with the linker protein Skp1/SkpA suggesting that SCF[^fbx15] primarily targets nuclear proteins. Four nuclear subunits of the COP9 signalosome are putative Fbx15 interaction partners. We propose an interdependent stabilization of Fbx15 and the COP9 signalosome, which is required to link protein degradation and stress response to virulence.

488. The sfp-type phosphopantetheinyl transferase, PPTA, is critical for the virulence of Aspergillus fumigatus. A. E. Johns, P. A. Warn, P. Bowyer, M. J. Bromley. Inflammation and repair, Univ. of Manchester, Manchester, United Kingdom.

Aspergillus fumigatus is the leading cause of invasive aspergillosis (IA), a fungal disease which is increasing annually on a global scale. IA poses as a common threat to patients with a weakened immune response due to disorders such as leukaemia, HIV, AIDS and also persons undergoing chemotherapy treatments. The ability of *A. fumigatus* to produce a wide array of secondary metabolites is thought to contribute to the pathogenicity of this organism. We have identified an enzyme, PPTA that plays a key role in secondary metabolism in *A. fumigatus*. PPTA is a sfp-type phosphopantetheinyl transferase and is required to activate non-ribosomal peptide synthases, polyketide synthases and a protein required for lysine biosynthesis aminoadipate reductase (AARA). Disruption of pptA prevents the production of most secondary metabolites and renders the fungus avirulent in both insect and murine infection models. To investigate which aspects of pptA activity are essential to virulence a series of knock out mutant strains were generated; DaarA, DpskP and DsidA. These genes play a vital role in lysine, melanin and siderophore biosynthesis pathways respectively. The sidA gene proved vital to virulence in the insect model whereas the DaarA and DpskP mutants were unaffected. The pathogenicity of both the pptA and sidA knock out strains was restored by co-injecting larvae with iron. We postulate that, at least in the larval model, it is PPTA role in siderophore biosynthesis and not the activation of other secondary metabolism pathways that is critical for the virulence of *A. fumigatus*.


*R. commune* is the causal agent of barley leaf scald. This disease is a persistent threat and widespread in particular in cool and moist barley-growing areas of the world. Yield losses as high as 35-40% have been reported, but a yield loss of only 5% may already lead 2012 to an economic loss of >700 Mio € in Europe. *R. commune* colonizes the leaves of its host plants by growing beneath the cuticle, mainly in the pectic layer of the outer epidermis cell walls, without directly contacting the plant plasma membrane. Therefore, the fungus needs to secrete effectors to manipulate the host physiology. Previous studies have shown that three secreted necrosis-inducing proteins (NIP1, NIP2, NIP3) affect fungal virulence in a quantitative manner depending on the host genotype. NIP1 was also identified as the avirulence factor that is recognized by barley resistance gene Rs1.

After obtaining the genome sequence of *R. commune* it turned out that NIP2 and NIP3 are encoded by single genes. In contrast, a small family of highly homologous NIP2 genes was identified, precluding a simple targeted deletion strategy for further functional analysis of NIP2. In addition, deletion of one NIP2 homolog affected the expression of the others. For further investigations an approach to simultaneously silence all members of the NIP2 family is being followed using a recombination-based cloning strategy. To this end, a plasmid expressing an intron-containing hairpin RNA (ihpRNA) was constructed. Transfection of *R. commune* with the ihpRNA plasmid and qRT-PCR-based assessment of the transcriptional down-regulation of NIP2 homologues are in progress. Establishing a gene silencing system will be of great value for future functional studies of fungal effectors involved in plant-pathogen interactions.

490. Molecular and genetic basis guiding the establishment of a mutualistic relationship between Epichloë festucae and perennial ryegrass. Sladana Bec, JinGe Liu, Christopher L. Schardl. Dept Plant Pathology, Univ Kentucky, Lexington, KY.

The relationship established between *Epichloë festucae* and perennial ryegrass (*Lolium perenne*) is a model system for studying mutualism between endophytes and cool season grasses. *E. festucae* colonizes all above-ground plant organs, growing by intercalary hyphal extension in elongating grass leaves. During the reproductive phase of growth, the fungus exhibits a dual nature: retaining its benign endophytic growth and seed transmission, or forming external stromata and suppressing seed production on affected tillers. From our previous work regarding the genes involved in the switch between benign plant colonization and formation of stromata, we have identified a number of genes encoding small secreted proteins (ssp) that are highly up-regulated in benign infected inflorescences. Two of those genes, ssp8 and sspX, are located in a subtelomeric region, and preliminary evidence suggests that they may play a role in host specificity. Although *E. festucae* is reported to be compatible with two related host species, *L. perenne* and *Lolium pratense* (meadow fescue), strains generated from a series of crosses and backcrosses showed a range of compatibility with *L. perenne*, but consistently were compatible with *L. pratense*. One such strain, E2368, had low compatibility with *L. perenne*, whereas a subculture (variant E4844) showed improved compatibility with this host. Genomes of E4844 and E2368 were compared, revealing that the variant had lost the subtelomeric region containing ssp8 and sspX, and of other gene losses and genomic changes in the variant, are under investigation. Also, the parents and full siblings of strain E2368 are being tested for SNPs segregating for phenotypes related to the establishment of stable mutualistic symbioses with *L. perenne*. The set of progeny strains has been screened for the establishment of host specificity with perennial ryegrass, and is slated for

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491. *Puccinia graminis* and *Brachypodium distachyon*: contrasting profiles of host-pathogen incompatibility. **Melania Figueras**, **Sergei Filichkin**, **Sean Gordon**, **Henry Priest**, **John Vogel**, **David Garvin**, **Todd Mockler**, **William Pfenfer**. 1) Oregon State University, Corvallis, OR; 2) USDA-ARS, WRRC, Albany, CA; 3) Donald Danforth Plant Science Center, St. Louis, MO; 4) USDA-ARS, PSRU, St. Paul, MN. The causal agent of stem rust, *Puccinia graminis*, is a devastating pathogen that affects the production of cereals and temperate-zone grasses. Some important examples of *P. graminis* and their typical hosts are *P. graminis* f. sp. *tritici* (*Pg-tr*) on wheat and barley, *P. graminis* f. sp. *loli* (*Pg-lo*) on perennial ryegrass and tall fescue, and *P. graminis* f. sp. *pheii-pratensis* (*Pg-pp*) on timothy grass. The biological and evolutionary attributes of *Brachypodium distachyon* have led to its development as a model to study cereals and grasses. To assess the applicability of *Brachypodium* to investigate non-host resistance to stem rust, disease severity caused by *Pg-tr*, *Pg-pp* and *Pg-lo* was evaluated across a collection of *Brachypodium* inbred lines. The different fungal isolate/Brachypodium line combinations demonstrated significant variation in stem rust resistance and revealed the contrasting pathogenic/virulent characteristics among these stem rust isolates. Given the distinct phenotypes obtained when inoculating with *Pg-tr*, *Pg-lo* or *Pg-pp*, *Brachypodium* line Bd1-1 was selected for further analyses. Histological analysis of the early infection events (first 68 h of infection) indicated that *Pg-lo* and *Pg-pp* are more efficient than *Pg-tr* in establishing a biotrophic interaction, and that Bd1-1 exhibits post-haustorial resistance to *Pg-tr* and post-haustorial resistance to *Pg-lo* and *Pg-pp*. A comparative transcriptome analysis (RNA-Seq) of the early responses of Bd1-1 to *Pg-lo*, *Pg-pp* and *Pg-tr* was performed. Gene expression profiles were determined to capture the transcriptional events in response to 1) appressorium formation (12 hpi, hours post-inoculation), and 2) fungal penetration (18 hpi) and initial stages of fungal growth in the plant mesophyll for each fungal isolate. The data show distinctive profiles for each fungal isolate/Brachypodium combination. Our results demonstrate a significant transcriptional re-programming that leads to the activation of early plant defenses associated with quantitative resistance (i.e., phenylpropanoid pathway, cytochrome P450s, and different types of transcription factors). Additionally, several receptor-like proteins and uncharacterized proteins were identified as putative players in pathogen recognition.


Pathogens secrete effector proteins into host tissue to suppress immunity and cause disease. Pathogenic bacteria have evolved several distinct secretion systems to target specific effector proteins during pathogenesis, but it was not previously known if fungal pathogens require different secretory mechanisms. We present evidence that the blast fungus *Magnaporthe oryzae* possesses distinct secretion systems for delivering effector proteins during biotrophic invasion of rice cells. *M. oryzae* secretes cytoplasmic effectors targeted for delivery inside rice cells and apoplastic effectors targeted to the extracellular space. Cytoplasmic effectors preferentially accumulate in the biotrophic interfacial complex (BIC), a novel in planta structure located beside the tip of the initially filamentous invasive hypha and then remaining next to the first differentiated bulbous invasive hypha cell. In contrast, apoplastic effectors remain in the extracellular compartment uniformly surrounding the invasive hypha inside the invaded cell. Disruption of the conventional ER-Golgi secretion pathway by Brefeldin A (BFA) treatment blocked secretion of apoplastic effectors, which were retained in the ER, but not secretion of cytoplasmic effectors. Fluorescence Recovery After Photobleaching experiments confirmed that cytoplasmic effectors continued to accumulate in BICs in the presence of BFA. Analysis of mutants showed that the BIC is associated with a novel form of secretion involving exocyst components, Exo70 and Sec5, and the t-SNARE Sso1, which are required for efficient delivery of effectors into plant cells and are critical for pathogenicity. By contrast, effectors which function between the fungal cell wall and plant plasma membrane are secreted from invasive hyphae to the apoplast by the ER-Golgi secretory pathway conserved in eukaryotes. We propose a model for the distinct secretion systems that the rice blast fungus has evolved to achieve tissue invasion.


Establishment of root symbiosis is one the key drivers of biocontrol success for members of the fungal genus **Trichoderma**. This root symbiosis is described as a two-step process, whereby *Trichoderma* species colonise the soil surrounding the root (rhizosphere) and then penetrate the root tissue and establish an endophytic relationship. The ability to colonise and then proliferate over time within the rhizosphere is termed rhizosphere competence (RC). There have been numerous reports of *Trichoderma* biocontrol strains which persist within the rhizosphere for the growing season of the crop plant. Our results strongly suggest that RC is widespread among members of the genus *Trichoderma* and that RC interactions are strain and host plant specific. For endophytes and their host plants to maintain a mutualistic relationship requires a constant molecular dialogue between the organisms involved. For example, the fungal-derived phytohormone, indole acetic acid (IAA), plays an important role in signalling between *Trichoderma* and the model plant *Arabidopsis thaliana*. There are however, additional, currently unknown, chemical signals which may be even more important for a positive interaction between *Trichoderma* and plants. By using a soil-maize-*Trichoderma* as a model system in in situ sterile conditions we are currently analysing the RC and endophytism transcriptomes of two *Trichoderma* species: *T. virens* and *T. atroviride*. Using a combination of bioinformatics, quantitative RT-PCR (for stage specific genetic markers from *Trichoderma*) and fluoro-labelled *Trichoderma* strains we are currently identifying and analysing promising *Trichoderma* candidates involved in endophytism and RC. A comprehensive panorama of the *Trichoderma*-soil-plant interaction will be discussed in this conference.

494. **Ustilago bromivora** - *Brachypodium distachyon*: a novel pathosystem. **Franziska Rabe**, **Regine Kahmann**, **Armin Djamei**. Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany.

The *Ustilago maydis* - *Zea mays* pathosystem is a well established model system to investigate basic principles of biotrophic plant-pathogen interactions. However, due to the long generation time, space requirements, and difficulties in transformation of maize studying the plant side is demanding. Recently, it has been shown that the yet uncharacterized smut fungus *Ustilago bromivora* infects *Brachypodium distachyon*, a model grass species. Short generation time, small size, sequenced diploid genome, and accessible reverse genetics make this monocot highly suitable for the analysis of biotrophic interactions with focus on the pathogen as well as the plant side. The primary goal of this study is therefore the characterization of *U. bromivora* and the interaction with its host in order to evaluate the suitability of the *U. bromivora* - *B. distachyon* interaction as a new host-pathogen model system. We could show that haploid *U. bromivora* strains obtained after spore germination can be cultivated and transformed with self-replicating plasmids used for *U. maydis* transformation. A hallmark of smut fungi is that the pathogenic form is the dikaryon which arises after mating of compatible haploids. Haploid cells are produced when diploid spores germinate and undergo meiosis. Usually after germination of a single teliospore, cells with compatible
mating types can be isolated. After germination of U. bromivora spores (kindly provided by T. Marcel, INRA-AgroParisTech, France) all haploid progenies were of the a1 mating type. This suggests a mating type bias where the a2 mating type might be linked to a deleterious recessive allele making the isolation of strains harboring this mating type under laboratory conditions impossible. Hence, the genomes of several strains harboring the a1 mating type and of diploid spore material containing both mating types were sequenced via an Illumina-Next-Generation Sequencing approach. Based on these sequences we plan to reconstruct the a2 mating type and to generate a strain containing this mating type as well as a solopathogenic haploid strain.


A collection of mutants of the grey mould fungus Botrytis cinerea has been constructed in order to provide the support for the identification of both fungal functions that are essential for the pathogenic development and/or plant defence traits raised in the host plant. A random insertional mutagenesis strategy based on the Agrobacterium tumefaciens-mediated transformation (ATMT) is used to enlarge an existing mutant library (2367 lines, Giesbert et al. 2011). 2144 additional T-DNA integrated transformants have been generated. The insertion sites of the T-DNA are being determined using TAIL-PCR and capacity to infect the host plant is assayed. These data are organized into a genome-orientated database of tagged genes and will be soon available for the scientific community. One exploitation of this mutant library focuses on the characterization of mutants whose parasitic development in plants is hampered. We present here an example of the study of a new gene encoding a Dnai domain protein. The T-DNA mutant exhibited a drastic alteration of the infectious process on bean leaves. Deletion of the studied gene confirmed this phenotype and revealed that colonization process was also altered on different host plants. A defect in penetration and an abnormal infection cushion formation were registered. A dramatic reduced conidiation and an abnormal hyphal morphology were also observed. Resistance/sensitivity to ROS, formation of ROS, organic acids and cell wall degrading enzymes secretion were investigated. Finally, proteomic analyses are currently developed in order to attribute a function to this gene.

496. The NADPH Oxidase Complexes in Botrytis cinerea. Ulrike Siegmund, Jens Heller, Sabine Giesbert, Paul Tuzdynski. IBPP, WWU Muenster, Muenster, Germany.

Reactive oxygen species (ROS) are generated in all aerobic environments and therefore play a major role in many organisms depending on oxygen. For example they act as messenger molecules for intercellular signaling or play a role during defense mechanisms against pathogens (Takemoto et al., 2007). One good example is the oxidative burst; plants rapidly produce large amounts of ROS as the first defense reaction towards pathogen attacks. NADPH oxidases (Nox) are the most common enzymatic system to produce these ROS. Nox are enzyme complexes, which transport electrons through biological membranes and therewith reduce oxygen to superoxide. In fungi they are shown to be involved in differentiation processes and pathogenicity and are therewith in our focus to gain insights into plant - fungi interactions. In the phytopathogenic fungus Botrytis cinerea two NADPH oxidases (BcNoxA and BcNoxB) as well as their putative regulator (BcNoXR) were previously identified (Segmueller et al., 2008). Besides their involvement in pathogenicity and sclerotia production, deletion studies have revealed that BcNoxA and BcNoXR are also involved in hyphal germination fusions (Roca and Weichert et al., 2011). Recent analyses show a localization of the catalytical subunits BcNoxA and BcNoxB to the ER and partly to the plasma membrane of hyphae, while the regulator BcNoXR is localized in vesicles and at the hyphal tips. Nox are multi-enzyme complexes, whose regulatory process and the participating proteins are well described in mammals. However, in fungi not all components have been identified, yet. For B. cinerea interaction studies with potential candidates identified the small GTPase Rac, the GEF BcCdc24, the scaffold protein BcBem1 and the PAKs BcCla4 and BcSte20 as interacting proteins within the BcNox complex. Roca M.G. and Weichert M. et al., (2012) Fungal Biol 116(3): 379-387. Segmueller N. et al., (2008) Mol Plant Microbe Interact 21: 808-808-819. Takemoto D. et al., (2007) Fungal Genet Biol 44(11): 1065-1076.

497. A putative function of small RNAs in the plant pathogen Botrytis cinerea. Arne Weiberg, Ming Wang, Hailing Jin. Plat Pathology & Microbiology, UC Riverside, Riverside, CA.

Small RNAs (sRNAs) are a class of non-coding transcripts that regulate gene expression. sRNA-directed gene regulation is a common phenomenon in eukaryotes, and in fungal systems function in differentiation, genome defense, and heterochromatin formation has been described. However, it is unknown in any systems whether sRNAs play an important role in fungal pathogenicity. To study sRNAs in the plant pathogen Botrytis cinerea we are currently undertaken a dual approach: 1) sRNA deep sequencing was performed analyzing RNA profiles of fungal in vitro culture samples and Botrytis-infected plant tissues using two host systems, the model plant Arabidopsis thaliana and tomato (Solanum lycopersicum). The goal is to identify infection-specific B. cinerea-derived sRNAs (Bc-sRNAs). II) Genetic analysis of important sRNA biogenesis factors in B. cinerea is currently piloted. B. cinerea possesses all relevant RNA components including two Dicer-like genes (Bc-DCL1 and Bc-DCL2) and two Argonaute-like genes (Bc-AGO1 and Bc-AGO2). Targeted gene disruption by homologous recombination of Bc-DCL1 and Bc-DCL2 led to growth retardation on artificial media and to delay of massive conidiaospore production. In planta, no reduction in virulence was observed. However, a dcl1dcl2 double mutant was strongly impaired in virulence and was unable to produce a set of Bc-sRNAs. Taken our observations together, it is proposed that B. cinerea expresses Bc-sRNAs during infection in order to regulate important processes to facilitate pathogenesis.

498. The Role of Quorum-sensing Molecules in Interactions between Candida albicans and its Host. Jessica C. Hargarten1, Thomas M. Petro2, Kenneth W. Nickerson2, Audrey L. Atkin1. 1) School of Biological Sciences, University of Nebraska, Lincoln, Lincoln, NE; 2) Department of Oral Biology, University of Nebraska Medical Center, Lincoln, NE.

Candida albicans is a polymorphic fungus that is capable of causing the life threatening disease Candidiasis once it reaches the bloodstream of a susceptible host. The capability to switch between morphologies, and its ability to synthesize and secrete the quorum sensing molecule (QSM) farnesol are known virulence factor. Previously, we showed that C. albicans mutants that produced less farnesol are less pathogenic to mice than their parental strain in a tail vein assay. Also, oral administration of farnesol to the mice prior to infection increased mortality. In contrast, farnesol blocks the yeast to mycelia transition in vitro, which should have a protective effect. These observations pose the dilemma of finding a mechanism whereby a molecule which blocks the yeast to mycelia transition can also act as a virulence factor. We hypothesize that farnesol functions as a virulence factor by modulating the host innate immune response. Distinct Candida morphologies elicit different host immune responses. Both white and opaque cells stimulate leukocyte movement, but only white cells secrete a small molecular weight chemoeattractant that draws the leukocyte directly towards the white cell and stimulates engulfment by mouse macrophages. The white cells are also less susceptible to killing by human macrophages and neutrophils than opaque cells, possibly due to their increased capabilities of escape once phagocytosed. The chemical identity of this chemoeattractant is currently unknown, but the reason
behind its continued secretion by white cells is intriguing. One likely candidate is farnesol because opaque cells, unlike white cells, do not accumulate detectable levels of farnesol. Macrophages are capable of detecting and responding to exogenous farnesol. Earlier our group reported that farnesol stimulates the expression of both pro-inflammatory and regulatory cytokines by mouse macrophage. The production of these warning signals is an important indicator of how the body ultimately hopes to clear the infection. Others have shown that farnesol suppresses the anti-Candida activity of macrophages through its cytotoxic effects, thus making it all the more difficult to eliminate the fungus early in infection. Here we report the in vitro role of farnesol and other known QSM in macrophage chemotaxis and relative phagocytosis of C. albicans.

499. The Role of ISW2 for in vitro and in vivo Chlamydospore Production in Candida albicans. Ruvini U. Pathirana1, Dhammika H. M. L. P. Navarathna2, David D. Roberts3, Kenneth W. Nickerson1. 1) School of Biological Sciences, University of Nebraska - Lincoln, Lincoln, NE; 2) Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD.

The production of chlamydospores is an unusual feature in the medically important opportunistic pathogen Candida albicans which is commonly used as an in vitro diagnostic tool. These thick walled spherical structures arise from a filament tip which is termed a suspensor cell. In the process of evolution, it is hard to believe that C. albicans makes a spore that does not contribute to its biology and thus the function of chlamydospores is of interest. Upon careful observation of the chronic stage of C. albicans colonization in mouse kidneys, we often find large cells similar in appearance to chlamydospores. We characterized these large cells using sucrose gradients and compared them with in vitro induced chlamydospores. The in vivo cells had the same buoyancy and were physiologically similar to in vitro chlamydospores. So we hypothesized that chlamydospores may promote the persistence of these pathogens during pathogenesis, particularly in kidneys. To test the role of chlamydospores during host infection, we used the wild type strain SC5314 and created a ISW2 knockout mutant. An ISW2 knockout out mutant. An ISW2 knockout out mutant was introduced into the mouse model of disseminated candidiasis and also failed to induce chlamydospores in mouse kidneys during pathogenesis. in vitro studies confirm the abnormal behavior of these mutants for normal filamentous growth, but they failed to produce typical chlamydospores from suspensor cells. However, after three weeks they produced chlamydospore-like structures that differed from normal chlamydospore production by the complete absence of suspensor cells. As an essential ATP dependent chromatin remodeling factor in yeasts, ISW2 affects the regulation of transcription, recombination, and DNA repair. Our findings suggest that ISW2 may also down regulate the genes for suspensor cell formation but not the genes for chlamydospore formation indicating that these are two independent processes. Further, our investigation into in vivo role of chlamydospores and suspensor cells suggest that ISW2 could be a future drug target. Further studies on gene regulation by ISW2 in C. albicans will be paramount to our understanding of development and regulatory steps for chlamydospore formation and their contribution to host infection.

500. Nutrient immunity and systemic readjustment of metal homeostasis modulate fungal iron availability during the development of renal infections. Joanna Potrykus1, David Stead1, Dagmar S Urgast1, Donna MacCallum1, Andrea Raab1, Jörg Feldmann1, Alistair JP Brown1. 1) Aberdeen Fungal Group, University of Aberdeen, Aberdeen, United Kingdom; 2) Aberdeen Proteomics, University of Aberdeen, Aberdeen, United Kingdom; 3) Trace Element Speciation Laboratory, University of Aberdeen, Aberdeen, United Kingdom.

Iron is a vital micronutrient that can limit the growth and virulence of many microbial pathogens. Here we show, that in the murine model of disseminated candidiasis, the dynamics of iron availability are driven by a complex interplay of localized and systemic events. As the infection progresses in the kidney, Candida albicans responds by broadening its repertoire of iron acquisition strategies from non-heme iron (FTR1-dependent) to heme-iron acquisition (HMX1-dependent), as demonstrated in situ by laser capture microdissection, RNA amplification and qRT-PCR. This suggested changes in iron availability in the vicinity of fungus. This was confirmed by 56Fe iron distribution mapping in infected tissues via laser ablation-ICP-MS, which revealed distinct iron exclusion zones around the lesions. These exclusion zones correlated with the immune infiltrates encircling the fungal mass, and were associated with elevated concentrations of murine heme oxygenase (HO-1) circumventing the lesions. Also, MALDI Imaging revealed an increase in heme and hemoglobin alpha levels in the infected tissue, with their distribution roughly corresponding to that of 56Fe. Paradoxically, whilst iron was excluded from lesions, there was a significant increase in the levels of iron in the kidneys of infected animals. This iron appeared tissue bound, was concentrated away from the fungal exclusion zones, and was accompanied by increased levels of ferritin and HO-2. This iron accumulation in the kidney correlated with defects in red pulp macrophage function and red blood cell recycling in the spleen, brought about by the fungal infection. Significantly, this effect could be replicated by selective chemical ablation of splenic red pulp macrophages by clodronate. Collectively, our data indicate that systemic events shape micronutrient availability within local tissue environments during fungal infection. The infection attenuates the functionality of splenic red pulp macrophages leading to elevated renal involvement in systemic iron homeostasis and increased renal iron loading. Simultaneously, localized nutrient immunity limits iron availability around foci of fungal infection in the kidney. In response, the fungus modulates its iron assimilation strategies.


Identification of the microorganisms that establish themselves inside and outside the human body is crucial to explore how the microbiome impacts human health. The recent Human Microbiome Project provides an initial compilation and identification of the gut microbiome ecosystem. It is well researched and understood that a large part of the gastrointestinal microbiota spans across the prokaryotic domain, but few studies have investigated the contribution of fungi to the human gut microbiome. Factors such as diet, genetics, and environment can play an influential role in explaining why differences in microbiota exist between human hosts. Expanding on work from our lab, this study examines the effects of nonconventional diets (e.g. vegetarians, vegans, gluten-free and lactose-free) on the GI tract fungi. DNA from fecal samples of healthy human subjects was isolated and fungal-specific ITS primers were used to target fungal DNA to obtain a baseline of data for gut fungi. Candida tropicalis and C. albicans were both detected, with C. tropicalis more prevalent. This relative abundance of C. tropicalis is in keeping with our earlier studies in people with conventional diets, and may be a regional phenomenon.

502. The mutational landscape of gradual acquisition of drug resistance in clinical isolates of Candida albicans. Jason Funt1, Darren Abbey2, Luca Issi3, Brian Oliver4, Theodore White5, Reeta Rao6, Judith Berman7, Dawn Thompson8, Aviv Regev1,2. 1) Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02142; 2) Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Ave, Cambridge, MA 02140; 3) Seattle Biomedical Research Institute, Seattle, WA; 4) School of Biological Sciences, University of Missouri at Kansas City, MS; 5) Worcester Polytechnic Institute, Department of Biology and Biotechnology, 100 Institute Road, Worcester MA 01609; 6) Tel Aviv University, Ramat Aviv, 69978 Israel; 7) University of Minnesota, Minneapolis MN 55455 USA.

Candida albicans is both a member of the healthy human microbiome and a major pathogen in immunocompromised individuals. Infections are most...
commonly treated withazole inhibitors of ergosterol biosynthesis. Prophylactic treatment in immunocompromised patients,2,3 often leads to the development of drug resistance. Since C. albicans is diploid and lacks a complete sexual cycle, conventional genetic analysis is challenging. An alternative approach is to study the mutations that arise naturally during the evolution of drug resistance in vivo, using isolates sampled consecutively from the same patient. Studies in evolved isolates have implicated multiple mechanisms in drug resistance, but have focused on large-scale aberrations or candidate genes, and do not comprehensively chart the genetic basis of adaptation.5 Here, we leveraged next-generation sequencing to systematically analyze 43 isolates from 11 oral candidiasis patients, collected sequentially at two to 16 time points per patient. Because most isolates from an individual patient were clonal, we could detect newly acquired mutations, including single-nucleotide polymorphisms (SNPs), copy-number variations and loss of heterozygosity (LOH) events. Focusing on new mutations that were both persistent within a patient and recurrent across patients, we found that LOH events were commonly associated with persistent and recurrent point mutations in over 150 genes may be related to the complex process of adaptation to the host. Conversely, most aneuploidies were transient and did not directly correlate with changes in drug resistance. Our work sheds new light on the molecular mechanisms underlying the evolution of drug resistance and host adaptation.


During the infection process C. albicans has to respond to various stresses imposed by the host environment including oxidative and osmolality stress generated by phagocytes such as macrophages and neutrophils, and also the cell wall stress agents such as exposure to caspofungin and other antifungal antibiotics. These stress responses area orchestrated through the activation of multiple stress pathways including the cAMP-PKA, several MAPK pathways and the Ca2+-calcineurin pathway influence the cell wall shape and composition. We are investigating the effect of the activation or inhibition of these pathways on immune recognition mechanisms. We therefore determined the importance of the MAPK, cAMP-PKA and Ca2+-calcineurin pathways on the fungal innate immune response by examining uptake, phagocytosis, and cytokine profile induced by mononuclear and polyuretic lymphocytes in response to a library of mutants in each of the above pathways under stressed and non-stressed conditions. We find that the activation and inhibition of these pathways plays a important role in remodeling of cell wall and hence the immunological profile. For example, deletion of TPK1 and CNA1 resulted in lower pro-inflammatory cytokine production. Immune-recognition was also affected by the exposure of C. albicans signaling mutants with Calcofluor-white, caspofungin, oxidative and osmotic stress and changes in temperature. These results suggest that stress signaling pathways act in a co-ordinated fashion to regulate yeast-hypha morphogenesis and the changes in the cell wall which in turn affects the immunological signature of the cell. Thus exposure to different microenvironments significantly modifies the immunological response to fungal cells, suggesting that responses to local stresses makes the fungal cell surface is a moving target for immunological surveillance.

504. GPI Pbgp1 of Paracoccidioides brasiliensis is a surface antigen that activates macrophages and mast cells through the NFkB signaling pathway. C. X. R. Valim, L. K. Arruda, P. S. R. Coelho, C. Oliver, M. C. Jamur. Faculidade de Medicina de Ribeirão Preto, USP, Ribeirão Preto, São Paulo, Brazil. Paracoccidioides brasiliensis is the etiologic agent of paracoccidioidomycosis (PCM), one of the most prevalent mycosis in Latin America. P. brasiliensis cell wall components interact with host cells and influence the pathogenesis of PCM. Pbgp1 is a GPI anchored protein that is up-regulated in the yeast pathogenic form. GPI anchored proteins are involved in cell-cell and cell-tissue adhesion and have a key role in the interaction between fungal and host cells. Pbgp1 is an O-glycosylated protein that is localized on the yeast cell surface. Recombinant Pbgp1 (rPbgp1) induces nitric oxide (NO) production and TNF-α release in murine peritoneal macrophages (Valim et al. PloS One, 2012). In the present study, rPbgp1 was able to activate NFkB in macrophage-like RAW cells that had been transfected with NFkB luciferase as well as in a reporter cell line for NFkB activation derived from RBL-2H3 mast cells. The results show that like macrophages, rPbgp1 also activates the transcription factor NFkB in mast cells. However, rPbgp1 does not activate NFAT nor it is able to induce liberation of beta hexosaminidase. The lack of beta hexosaminidase release suggests the Pbgp1 is not able to activate RBL-2H3 mast cells via the high affinity IgE receptor. Mast cell activation by rPbgp1 does result in activation of the transcription factor NFkB suggesting stimulation of cytokine production. Taken together these results indicate that the surface antigen Pbgp1 may play an important role in PCM pathogenesis by activating macrophages and mast cells.

505. Cladosporium fulvum effector Ecp6 outcompetes host immune receptor for chitin binding through intrachain LysM dimerization. Andrea Sánchez-Vallejo1, Raspudin Saleem-Batcha1, Anja Kombrink2, Guido Hansen2, Dirk-Jan Valkenburg3, Jeroen R. Mesters3, Bart P.H.J. Thomma1. 1) Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands; 2) Institute of Biochemistry, Center for Structural and Cell Biology in Medicine, University of Lübeck, Lübeck, Germany.

Successful pathogens secrete effector proteins to deregulate host immunity which is triggered upon detection of pathogen-associated molecular patterns (PAMPs). Several fungal pathogens employ Lymys effectors, such as Ecp6 from Cladosporium fulvum, to sequester fungal cell wall-derived chitin oligomers which act as PAMP and would otherwise be recognized by host immune receptors and trigger defense responses. The mechanism by which Lymys effectors scavenge chitin molecules remained unknown thus far. Based on crystal structure analysis of Ecp6, we reveal a novel mechanism for chitin binding by intrachain LysM dimerization, leading to a binding groove in which chitin is deeply buried in the effector protein. Isothermal titration calorimetry experiments show that the concerted action of two Lymys domains mediates a single chitin binding event with ultra-high (pM) affinity.

506. Genotypic and phenotypic characterization of Setosphaeria turcica reveals population diversity and a candidate virulence gene location. Santiago Mideros1, Chia-Lin Chung1, Jesse Poland2, Gillian Turgeon1, Rebecca Nelson1. 1) Cornell University, Dept. of Plant Pathology and Plant-Microbe Biology, Ithaca, NY, USA; 2) Cornell University, Dept. of Plant Breeding and Genetics, Ithaca, NY, USA; 3) National Taiwan University, Dept. of Plant Pathology and Microbiology, Taipei, Taiwan; 4) USDA-ARS, Hard Winter Wheat Genetics Research Unit, Kansas State University, Manhattan, KS, USA.

The dothideomycete maize pathogen Setosphaeria turcica (anamorph Exserohilum turcicum) causes Northern Leaf Blight, one of the most common fungal diseases of maize worldwide. Little is known about the genetic basis of virulence and aggressiveness in this pathogen, although several races have been described based on their compatibility with maize resistance genes H1, H2, H3 and H10. To study the genetic basis of virulence and aggressiveness, we generated a F1 population consisting of 221 monosporic progeny of a cross between a race 1 strain and a race 23 strain. Genotyping-by-sequencing (GBS) was conducted on the population and an additional 13 diverse isolates that included the parental lines. We obtained between 341,000 and 428,000 sequence tags for each of the 234 isolates. Alignment to the S. turcica Et28A v1.0 genome sequence (http://genome.jgi.doe.gov/Settu1/Settu1.home.html) yielded 27,174 single nucleotide polymorphisms (SNPs) at a density of 0.63 SNPs per kb. In the 13 isolates, using 9,526 filtered SNPs, we found an average nucleotide diversity (p) of 0.297. Using 564 polymorphic markers with less than 35% missing calls, we created a high-density genetic map that resulted in 23 linkage groups and a total length of 1,686 cM. The Et28A sequence has 407 scaffolds, four scaffolds formed a single linkage group in our genetic map. The rest of the genome remains fragmented. To identify genomic regions controlling virulence
and aggressiveness, isolates were characterized for in vitro and in planta phenotypes. For the former, mycelial abundance, colony diameter, pigmentation, and sporulation were rated in replicated trials. For the latter, incubation period, primary diseased leaf area and a qualitative differential score were rated on maize near isogenic lines with and without 

507. The secretome is linked to virulence in the yeast pathogen Cryptococcus. Leona Campbell\textsuperscript{1}, Anna Simonin\textsuperscript{1}, Janna Ferdous\textsuperscript{1}, Matthew Padula\textsuperscript{1}, Elizabeth Harry\textsuperscript{2}, Ben Herbert\textsuperscript{3}, Dee Carter\textsuperscript{1}. 1) School of Molecular Bioscience, University of Sydney, Sydney, NSW. Australia; 2) IThree Institute, University of Technology, Sydney, NSW, Australia; 3) Department of Chemistry & Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia. 

The disease caused by pathogenic Cryptococcus spp. begins after inhalation of infectious propagules leading to infection of the lung. In some cases the pathogen disseminates to the central nervous system, resulting in meningocencephalitis, which can be fatal if left untreated. However, closely related strains of Cryptococcus gattii and its sibling species C. neoformans can exhibit significantly different degrees of pathogenesis in the mammalian host. As fungi utilize absorptive nutrition producing a range of secreted degradative enzymes, and as these may invoke a host response, the fungal secretome is likely to be very important in modulating host-pathogen interactions. To investigate this the secretome was determined for a hypovirulent and a hypervirulent strain of C. gattii, R272 and R265 respectively, and a virulent strain of the opportunistic pathogen C. neoformans, KN99a. All strains were grown under conditions designed to mimic those encountered in vivo. Secreted proteins were analysed using two different mass spectrometry-based techniques: 1D nanoLC-MS/MS and Imaging Mass Spectrometry (IMS). The three strains secreted significantly different protein cohorts. A total of 70 proteins were identified with 47, 13 and 22 identified from R272, R265 and KN99a respectively. Only one protein was shared by all strains, a putative glycosyl hydrolase. The secretomes of R265 and KN99a primarily included uncharacterized proteins, and bioinformatic analysis suggested these proteins contained catalytic roles in roles in carbohydrate degradation. In contrast the less virulent R272 strain secreted a more diverse set of proteins including canonical cytosolic proteins such as enolase and transaldolase. These proteins have been described as fungal allergens that bind IgE. These findings indicate that virulence and the secretome are linked in Cryptococcus. The secretion of proteins with a putative role in nutrient scavenging by virulent strains R265 and KN99a suggest they can source nutrients from a range of available substrates, which may allow them to exploit a wider range of ecological niches including the mammalian host. In contrast, the potentially allergenic proteins secreted by strain R272 suggest this strain triggers a more effective immune response, leading to clearance of the pathogen. 

508. Post-Transcriptional Regulation of the ER Stress Response in Cryptococcus neoformans. Virginia E. Havel, John C. Panepinto. Microbiology and Immunology, University at Buffalo, Buffalo, NY. 

Cryptococcus neoformans is one of a small number of fungi able to make the transition from ambient environmental temperatures to human core body temperature. We have previously reported that the ER stress response plays an important role during host temperature adaptation. Deletion of the RNA binding protein, PuF4, results in temperature sensitivity and increased resistance to the ER stress inducing drug tunicamycin, leading us to hypothesize that PuF4 post-transcriptionally regulates the ER stress response during host temperature adaptation. The ER stress response is initiated by the transcription factor Hac1 (Hxl1 in C. neoformans). Hac1 translation requires unconventional splicing of the pre-spliced HAC1 mRNA to the translated HAC1 mRNA at the ER surface by Ire1. Time courses measuring HXL1 mRNA splicing during a shift to 37°C demonstrate a delay in the splicing of HXL1 in puF4\textsuperscript{0} when compared to wild type. The delay in HXL1 splicing in puF4\textsuperscript{0} results in a delayed and persistent induction of ER stress response transcripts in puF4\textsuperscript{0} compared to wild type as measured by northern blot. We hypothesize that PuF4 is required for localization of the HXL1 transcript to the ER outer surface where it is cleaved by Ire1, thereby promoting the induction of the ER stress response. We have also shown through EMSA analysis and RNA-immunoprecipitation experiments that PuF4 is able to bind to ALG7 mRNA. Alg7 is involved in protein glycosylation at the ER surface and is the target of tunicamycin. Based on the observation that puF4\textsuperscript{0} has increased resistance to tunicamycin, and results demonstrating that PuF4 is able to bind ALG7 mRNA we hypothesize that PuF4 may regulate ALG7 mRNA by repressing translation. In our model PuF4 has a bi-modal mechanism of regulating the ER stress response. ER stress response initiation requires PuF4-mediated localization of pre-spliced HXL1 mRNA to the ER surface. During the attenuation phase of the ER stress response, we hypothesize that ER transcripts are translationally repressed by PuF4, resulting in attenuation of the ER stress response and allowing the cell to return to homeostasis. Despite the well-studied mechanism of unconventional splicing by Hac1 and Hac1 homologs in yeast and other model systems, this study is the first to identify a RNA binding protein potentially involved its activation. 

509. A morphogenesis regulator controls cryptococcal neurotropism. Xiaorong Lin\textsuperscript{1}, Bing Zhai\textsuperscript{2}, Karen Wozniak\textsuperscript{3}, Srijana Upadhyay\textsuperscript{1}, Linqi Wang\textsuperscript{1}, Shuping Zhang\textsuperscript{1}, Floyd Wormley\textsuperscript{1}. 3) Biology, Texas A&M University, TAMU-3258, TX; 2) Biology, the University of Texas at San Antonio, San Antonio, Texas, USA; 3) Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, USA. 

Cryptococcus neoformans is the major causative agent of cryptococcal meningitis, a disease that is responsible for more than 600,000 deaths each year. This ubiquitous environmental pathogen enters host lungs through inhalation and typically establishes asymptomatic latent infections. However, extrapulmonary dissemination often occurs in individuals with weakened immunity and Cryptococcus has a predilection for the central nervous system, resulting in meningoencephalitis, which can be fatal if left untreated. However, closely related strains of Cryptococcus gattii and its sibling species C. neoformans can exhibit significantly different degrees of pathogenesis in the mammalian host. As fungi utilize absorptive nutrition producing a range of secreted degradative enzymes, and as these may invoke a host response, the fungal secretome is likely to be very important in modulating host-pathogen interactions. To investigate this the secretome was determined for a hypovirulent and a hypervirulent strain of C. gattii, R272 and R265 respectively, and a virulent strain of the opportunistic pathogen C. neoformans, KN99a. All strains were grown under conditions designed to mimic those encountered in vivo. Secreted proteins were analysed using two different mass spectrometry-based techniques: 1D nanoLC-MS/MS and Imaging Mass Spectrometry (IMS). The three strains secreted significantly different protein cohorts. A total of 70 proteins were identified with 47, 13 and 22 identified from R272, R265 and KN99a respectively. Only one protein was shared by all strains, a putative glycosyl hydrolase. The secretomes of R265 and KN99a primarily included uncharacterized proteins, and bioinformatic analysis suggested these proteins contained catalytic roles in roles in carbohydrate degradation. In contrast the less virulent R272 strain secreted a more diverse set of proteins including canonical cytosolic proteins such as enolase and transaldolase. These proteins have been described as fungal allergens that bind IgE. These findings indicate that virulence and the secretome are linked in Cryptococcus. The secretion of proteins with a putative role in nutrient scavenging by virulent strains R265 and KN99a suggest they can source nutrients from a range of available substrates, which may allow them to exploit a wider range of ecological niches including the mammalian host. In contrast, the potentially allergenic proteins secreted by strain R272 suggest this strain triggers a more effective immune response, leading to clearance of the pathogen. 

510. Extracellular and intracellular signaling orchestrates morphotype-transitio and virulence in human pathogen Cryptococcus neoformans. Linqi Wang, Xiuyun Tian, Rachana Gaywali, Xiaorong Lin. Biology, College Station, TX. 

Interactions with the environment and divergent species drive the evolution of microbes. To sense and rapidly respond to these dynamic interactions, “simple” microbes developed bet-hedging social behaviors, including the construction of heterogeneous biofilm communities and transition between different morphotypes. The human fungal pathogen Cryptococcus neoformans can undergo morphotype transition between the yeast and the filamentous form. Most recently, we demonstrated that the zinc-finger regulator Znf2 bridges the bi-directional yeast-hypha transition and virulence in this pathogen. 

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One of ZnT2 downstream targets is extracellular protein Cfl1. Cfl1 is a cell-wall bound adhesin and a signaling molecule when it is released. This matrix protein Cfl1 plays a similar but less prominent role than ZnT2 in orchestrating morphogenesis and virulence in C. neoformans. Through transcriptome analyses and screening ZnT2 downstream targets by overexpression, we identified an additional player in the control of morphogenesis and biofilm formation. This factor is an intracellular RNA-binding protein Pum1. As expected, Pum1 affects filamentation in a ZnT2 dependent manner. However, the effect of Pum1 on morphogenesis is independent of Cfl1. The pum1Δ cfl1Δ double mutant shows a more severe defect in filamentation than either of the single mutant, indicating that Pum1 and Cfl1 act in two parallel pathways. Two of Pum1’s targets, Fad1 and Fad2, form a Cryptococcus-specific adhesin family. Like Cfl1, these two extracellular adhesins show regulatory roles in conducting morphogenesis and virulence in C. neoformans and thus may be involved in extracellular signaling transduction. Our results indicate that complex regulatory cascades composed of extracellular and intracellular circuits may be responsible for mediating morphological transition in response to the cues in the environments and the host.

511. Evidence for alkaloid diversity and independent hybridization events of Elymus endophytes. Nikki D. Charlton1, Juan Pan2, Daniel G. Panaccione3, Christopher L. Scharf4, Carolyn A. Young5. 1) Forage Improvement Division, The Samuel Roberts Noble Foundation, Ardmore, OK; 2) Department of Plant Pathology, University of Kentucky, Lexington, KY; 3) Division of Plant & Soil Sciences, West Virginia University, Morgantown, WV.

The epilobeae form mutualistic symbioses with cool-season grasses and have been shown to impart biotic and abiotic fitness benefits to their hosts. Endophyte-infected plants often have greater resistance to biotic stresses such as mammalian and insect herbivory due to the presence of fungal synthesized alkaloids. Four classes of bioprotective alkaloids have been described, which include ergot alkaloids, indole-diterpenes, a pyrrolopyrazine (peramine), and saturated aminopyrrolizidines (lolines). Elymus species, such as Elymus canadensis and E. virginicus, are cool-season bunchgrasses native to much of North America and are known to harbor Epichloë endophytes. Three species are able to associate with Elymus: the non-hybrids Epichloë aestivalis and Epichloë elymi and Epichloë canadensis, a hybrid with E. elymi and E. canadensis ancestral progenitors. The distribution and alkaloid genotypic variation of these fungi was examined to determine endophyte variation that may provide ecological benefits to the host. Endophyte infection frequencies from natural populations and germplasm resources ranged from uninfected to highly infected. Analyses of microsatellite loci and mating type genes characterized the prevalence and distribution of hybrid and non-hybrid endophytes among and between Elymus populations. Overall, non-hybrids were more prevalent than hybrids in the northern region of the U.S., whereas hybrids were more ubiquitous in the southern region. Genotypic analysis based on presence and absence of key alkaloid biosynthesis genes provided information about the potential alkaloid diversity within these populations. Thirteen unique alkaloid genotypes were identified that showed variation within the EAS (ergot alkaloid), LOL (loline) and PER (peramine) loci that indicates some genotypes are likely to accumulate pathway intermediates. Evaluation of the mating-type idiomorphs from the hybrid E. canadensis indicates this species has resulted more than once through independent hybridization events thus explaining variation found among the alkaloid genes. Chemical analyses of representative endophyte-infected plants are being conducted to correlate alkaloid predictions with actual alkaloid production. Chemotype diversity will be evaluated to determine how this translates into differences in fitness and persistence of the host.


The ascomycete fungus Zymoseptoria tritici (synonym: Mycosphaerella graminicola) emerged as a new pathogen of cultivated wheat during crop domestication about 11,000 years ago. To understand the molecular basis of host specialization in this pathogen we have sequenced complete genomes of Z. tritici and closely related species infecting wild grasses. Evolutionary genomic analyses allowed us to identify 17 genes that show strong evidence of positive selection between Z. tritici and the closely related sister species Zymoseptoria pseudotritici. We hypothesize these evolved in a co-evolutionary arms race with different hosts. None of the genes encode proteins with known function. In this study we focus on three candidate genes Mgr80707, Mgr89160 & Mgr103264 and investigate their role in Z. tritici and its two closest relatives Z. pseudotritici and Z. ardeabiae during host infection. Quantitative Real time PCR experiments from the three fungal species infecting four different grass species show that the three genes are strongly up-regulated in planta and that candidate gene expression differs over a time course of 28 days supporting a role in host pathogen interaction. In addition, we show that three different host species (wheat, Elymus repens and Lolium multiflorum) differentially induce gene expression in the fungi. Confocal Laser Scanning Microscopy conducted at different time points reveals clear differences between Zymoseptoria species during infection and within host development in wheat and Brachypodium distachyon. Deletion strains for each candidate gene have been created by Agrobacterium tumefaciens mediated transformation. The single deletion of two candidate genes Mgr80707 & Mgr103264 led to a reduced virulence of Z. tritici on wheat. The deletion of the third gene Mgr89160 led to a hyper-virulence phenotype suggesting an avirulence function of the gene product. Our study confirms that genes involved in host specialization can be identified based on predictions of natural selection.


Coniferous forests of Pakistan are rich in mycodiversity. However, only a few scientific researches have been conducted in these forests. This paper aims to document diversity of Suillus in these forests. During a survey conducted during 2008-2010, a total of thirty two (32) basidiomata were collected. Most of them were found associated with Pinus wallichiana and Abies pindrow. Only a few were found with Cedrus deodara, Populus ciliata and Quercus spp. These basidiomata were characterized morphologically as well as by molecular analysis by amplifying rDNA. Fungal specific primers ITS3 & ITS5R and ITS2 & ITS8F were used to amplify the ITS1 and ITS2 along with partially 5.8S gene. Out of these, twelve (12) different Suillus species were found. Among these (2) species seem undescribed and three (3) as new records for Pakistan. Their Phylogenetic relationships have also been discussed.

514. Saprolegnia species can switch hosts to cause infection: a new insight into host pathogen interaction. Mohammad N. Sarwar1*, A. Herbert van den Berg2, Debbie McLaglan1, Mark Young3, Pieter van West4. 1) Institute of Medical Sciences, Foresterhill, Aberdeen, AB25 2ZD, United Kingdom; 2) Department of Zoology, University of Aberdeen, Aberdeen, AB24 2TZ, United Kingdom.

Saprolegnia species are destructive oomycete pathogens of many aquatic organisms and are found in all parts of the world. Phylogenetic analysis has shown that Saprolegnia strains isolated from different aquatic organisms have a close relationship to fish pathogenic Saprolegnia species. We have now demonstrated, for the first time, that Saprolegnia spp. can actually switch hosts. Saprolegnia australis, Saprolegnia hypogyna and 2 strains of Saprolegnia diclina were isolated from insects. We also isolated other oomycete species, including S. australis, S. ferax, Pythium pachycaule and Pythium sp., in water of a medium to fast running river. The ITS region of all these isolates was sequenced. Four isolates collected from the aquatic insects together with isolates of S. parasitica (collected from salmon), S. diclina (collected from trout eggs) and S. ferax (collected from an amphibian) were tested for pathogenicity on nymphs of a stonefly (Perla bipunctata), Atlantic salmon eggs and frog (Xenopus leavis) embryos. Most of the isolates were highly pathogenic on all tested aquatic animals. These results suggest that Saprolegnia spp. are capable of switching host, which may be related to seasonal variation of host availability in
fresh water environments.

515. The Plant-Microbe Interfaces project: defining and understanding relationships between *Populus* and the rhizosphere microbiome. **Christopher Schadt**1, Dale Pelletier1, Timothy Tschaplinski1, Edward Uberbacher1, Hurst Gregory1, E. Peter Greenberg1, Caroline Harwood1, Amy Schaefer1, Ryta Vilgalys1, Francis Martin1, Mitchel Doktycz1, Gerald Tuskan1, and other PMI researchers (http://pmi.ornl.gov). 1) Bioscience Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA; 2) Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA; 3) Department of Microbiology, University of Washington, Seattle, WA, USA; 4) Department of Biology, Duke University, Durham, NC, USA; 5) Institut National de la Recherche Agronomique, Nancy, FRANCE.

Microbial interactions benefit plant health by affecting nutrient uptake, hormone signaling, water and element cycling in the rhizosphere and/or conferring resistance to pathogens. The model tree species *Populus* provides an opportunity for microbiome research relevant to bioenergy, carbon-cycle research, and other ecosystem processes. In an effort to define *Populus* microbiome, root and rhizosphere samples from *P. deltoides* in the eastern US and *P. trichocarpa* in western US were subjected RNA pyrosequencing and an isolate collection of over 5000 bacteria and 500 fungi obtained. We show that the rhizo- and endo-sphere environments feature highly developed, diverse and to a large degree often exclusive communities of bacteria and fungi. Endophytic bacterial diversity was found to be highly variable, but on average tenfold lower than the rhizosphere, suggesting root tissues provide a distinct environment supporting relatively few species. Fungal endophytic species were more numerous, but also less than rhizosphere spp. Both fungal and bacterial rhizosphere samples showed distinct phylogenetic composition patterns compared to the more variable endophyte samples. Contrary to initial expectations, both *Populus* spp. have low natural levels of colonization by ectomycorrhizal (ECM) and arbuscular mycorrhizal fungi, but high levels of presumed fungal endophytic taxa such as *Nectria, Mortierella*, and members of the *Atractiellales*. Select isolates are being studied at a whole-genome level to enable comparative work on the basis for observed symbioses. Thus far ~43 bacterial and 5 fungal isolates have been sequenced. Initial comparative genomics of these isolates suggest highly divergent physiological and molecular mechanisms of the interactions, even within rather closely related species. Efforts to understand ECM interactions have shown that host defense networks and the ability to bypass such networks through small protein and phytoc hormone signals has a large effect on the ability of *Populus* to form ECM relationships. *Laccaria bicolor* is able to modulate host defense response in *P. trichocarpa*, yet unable to do so in *P. deltoides*. Mycorrhizal Helper Bacteria from the genus *Pseudomonas* partially alleviate this colonization weakness through yet unknown molecular mechanisms, illustrating the value of integrated microbiome wide studies.

516. Do the fungal homologs of *Verticillium dahliae* effector Ave1 act as virulence factors? **Jordi C. Boshoven**1, Melvin D. Bolton2, Bart P.H.J. Thomma1. 1) Laboratory of Phytopathology, Wageningen University, 6708 PB Wageningen, Netherlands; 2) Agricultural Research Service, Northern Crop Science Laboratory, US Department of Agriculture, Fargo, ND 58102.

*Verticillium* species cause vascular wilt disease in over 200 plant hosts, including economically important crops. In tomato, the Ve1 immune receptor confers resistance to race 1 strains of *V. dahliae*, but not to race 2. By population genome sequencing of race 1 and race 2 strains, the effector that is recognized by Ve1 was recently identified as Ave1 (*Avirulence on Ve1* tomato). Ave1 has homology to plant natriuretic peptides that are regulators of homeostasis, and acts as a virulence factor on tomato plants that lack Ve1 as well as on *Arabidopsis*. In addition to plants, Ave1 homologs were also found in a few fungal pathogens, including *Fusarium oxysporum*, *Cercospora beticola* and *Colletotrichum higginsianum*, as well as in the bacterial plant pathogen *Xanthomonas axonopodis*. Co-expression of *V. dahliae* Ave1 and tomato Ve1 in *Nicotiana tabacum* resulted in the activation of a hypersensitive response. Remarkably, also co-expression of some *Ave1* homologs with Ve1 activated a hypersensitive response. Here, we evaluate whether the various pathogen-derived Ave1 homologs are virulence factors. Expression of the Ave1 homologs of *Fusarium*, *Cercospora* and *Colletotrichum* during infection on tomato, sugarbeet, and *Arabidopsis*, respectively, was analysed. To investigate the potential role of the Ave1 homologs in virulence, a *V. dahliae* Ave1 deletion mutant was complemented with the *Ave1* homologs of *Fusarium*, *Cercospora* and *Colletotrichum*, and tested for full aggressiveness on tomato. Finally, targeted gene deletion was pursued in *Fusarium*, *Cercospora* and *Colletotrichum* and the corresponding deletion strains were inoculated on tomato, sugarbeet, and *Arabidopsis*, respectively.

517. The candidate effector repertoire of closely related *Venturia* pathogens of the Maloideae revealed by whole genome sequencing and RNA sequencing analyses. **Cecilia Deng**1, Daniel Jones1, Bruno Le Cam2, Kim Plummer2, Carlos Mesarch1, Matthew Templeton1, Joanna Bowen1. 1) Plant & Food Research, Auckland, New Zealand; 2) La Trobe University, Melbourne, Australia; 3) IRHS, INRA Angers, France; 4) Wageningen University, The Netherlands.

The genus *Venturia* includes pathogens that infect members of the Rosaceae. The most widely researched of these is *V. inaequalis* that causes the disease apple scab. Related species cause disease of other woody hosts closely related to Malus; eg *V. pirina* that infects European pear. Certain isolates that are classified as *V. inaequalis* are unable to infect *Malus* but instead infect different hosts belonging to the subfamily Maloideae, such as loquat. Host-cultivar specificity is also demonstrated by some *V. inaequalis* that infect *Malus*; 17 gene-for-gene pairings between effectors (pathogen proteins that enhance disease) and resistance gene products have been identified to date. Thus the effector repertoire of isolates of *V. inaequalis* determines their cultivar specificity and most probably host specificity. Efforts have yet to be cloned from *V. inaequalis*. Draft whole genome sequences (WGS) of three *V. inaequalis* isolates (two from apple, one from loquat) and an isolate of *V. pirina* have been assembled and candidates that share the characteristics of fungal effectors (small, secreted proteins) have been identified. Of the 13333 predicted genes in the WGS of *V. inaequalis* isolated from apple, 1088 encode putatively secreted proteins identified using algorithms to detect secretion signals and putative cellular location. The expression (measured by transcriptome analysis) of 119 of these is up-regulated, with a false discovery rate less than 0.05 and a log-fold change greater than 2, in planta compared with *in vitro* at both 2 and 7 days post inoculation (dpi), 73 at 2 dpi, and 54 at 7 dpi. Of these 246, only 43 have similarities (~e-05) to genes in publicly available databases with a large proportion encoding proteins with similarities to plant cell wall-degrading enzymes. There are a further 627 genes up-regulated in planta that are not predicted to be secreted; these include numerous genes putatively involved in secondary metabolism. By comparing WGS of isolates differing specificities candidate effectors determining host range have been identified; 84 are unique to *V. pirina*, 6 unique to a *V. inaequalis* isolate specific to loquat and 145 present in all the apple-infecting isolates but absent from those able to infect loquat and *V. pirina*. These candidates are currently being characterised with respect to functionality.

518. Thermodynamic characterization of RXLR and RXLR-like effectors binding to phosphatidylinositol-phosphates. **Kelly Drews**1,2, Gloria Trivett1,2, Tian Zhou1,2, Samantha Taylor1,2, Helen Clark1,3, Brett Tyler1,3, Shiv Kale1. 1) Virginia Bioinformatics Institute, Virginia Tech., Blacksburg, VA; 2) Department of Biological Sciences, Virginia Tech., Blacksburg, VA; 3) Department of Biochemistry, Virginia Tech., Blacksburg, VA; 4) Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR; 5) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR.

Microbial-host interactions encompass a wide array of components to mediate symbiosis. The study of small-secreted proteins capable of manipulating host cellular machinery is currently a core-theme of microbial-host interactions. A number of bacterial symbionts deliver these effector molecules into
cells via three type three systems, a syringe like apparatus that directly penetrates the host cell plasma membrane. Currently, there is no evidence for such translocation machinery being present or utilized by fungal and oomycete symbionts to deliver effector proteins. Several bacterial and fungal toxins utilize external glycosphinolipids to mediate translocation into cells. Upon internalization a subset of these toxins escape endosomal compartments via retrograde transport to the Golgi-endoplasmic reticulum trans network, while others are capable of flipping across the endosomal membrane. The genus Phytophthora contains a number of highly destructive plant pathogens. Comparative genomics and the sequence availability of known intracellular effectors resulted in the discovery of a highly conserved N-terminal motif RXLR-d-DEER that defines an expansive super family of secreted proteins. The RXLR-d-DEER motif of PsAVr1b, PsAVr1k, and PsAVr5 has been shown to facilitate effector entry into a plant and animal cells in the absence of any pathogen encoded machinery. Entry is believed to be mediated by binding cell surface phospholipid, phosphatidylinositol3-phosphate. Using isoform titration calorimetry we have characterized the phospholipid binding properties of the RXLR effectors PaAVr1b, PaAVr3a, and several RXLR-like effectors to mono and poly phosphatidylinositol-phosphates in a variety of experimental conditions. As controls we have characterized the phospholipid binding properties of 2xFYVE and Vam7 PX, two known PI3P binding domains.

519. Investigating virulence effectors in the poplar-poplar rust pathosystem. Sebastien Duplessis1, Benjamin Petre1, Hugo Germain2,3, Arnaud Hecker1, Stéphane Hacquard1, David I Joly1, Armand Séguin1, Nicolas Rouhier1. 1) UMR1136 IAM, INRA, Champenoux, France; 2) Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Quebec, QC, Canada; 3) Université du Québec à Trois-Rivières, Trois-Rivières, QC, Canada; 4) Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC, Canada.

Foliar rust caused by Melampsora larici-populina is a major disease affecting poplar plantations throughout the world. The obligate biotrophic status of the fungus and the perennial status of the host plant, make molecular investigation of this interaction a real challenge. However, availability of both Populus trichocarpa and Melampsora larici-populina sequenced genomes has allowed for setting an emerging model pathosystem to decipher the molecular bases of disease resistance in trees and of biotrophic growth in rust fungi. Comprehensive analyses of rust transcripts encoding small secreted proteins expressed in planta identified putative effector candidates and we focused our attention on a few candidates that show evidence of purification between paralogs. In particular, two gene families encode modular SSP with a conserved N-terminal part and a C-terminal part evolving under positive selection except highly-conserved cysteine residues. Gene family 5464 contains 13 members, homologs of Melampsora lini AvrP4 avirulence factors whereas H1 family gather 31 genes specific to M. larici-populina. Some of these genes present a transient peak of expression during the biotrophic growth of the fungus in poplar leaves, and the corresponding proteins are able to enhance bacterial growth when delivered in Arabidopsis thaliana from Pseudomonas syringae pv tomato DC3000. Currently, we combine a range of approaches including biochemical and structural characterization of recombinant proteins, yeast two-hybrid and pull-down assays to characterize these candidate effectors.


Aphanomyces euteiches is an oomycete infecting roots of various legumes species such as pea, alfalfa and the model legume Medicago truncatula. The genus Aphanomyces, which belongs to the group of Saprolegniales, is phylogenetically distant from the well known Phytophthora genus and comprises both animal pathogen and plant pathogen species. The first genome draft of Aphanomyces euteiches (ATCC201684, 57 Mb) will be soon released and genome sequencing of zoo- and phytopathogen species are under progress. A. euteiches genome mining revealed the expansion of CRNs (Crinkling and Necrosis) genes, initially identified in Phytophthora. These modular proteins contain a conserved N-terminal characterized by the presence of a LFLAK amino acid motif implicated in the protein translocation from the pathogen to the host cell, whereas the modular C-terminal effector domain is highly diverse. The proposed role of the CRNs effectors is to suppress plant defense or to modulate other host cell processes that increase susceptibility and enhance pathogen virulence. In A. euteiches, the active translocation LYLAK motif was detected, and conserved, as well as original effector subdomains, were identified. Functional studies conducted on two types of A. euteiches CRNs, AeCRNs and AeCRN13, showed that both proteins are highly induced during infection of M. truncatula roots. In planta expression of both proteins revealed host nucleus localization and cell-death induction or alteration of roots architecture when expressed in plant cells. Such observations suggest that A. euteiches CRNs are virulence proteins exerting their function through the interaction with nuclear compounds. Latest results regarding the putative function of AeCRNs will be presented.

521. Participation of effector proteins from Trichoderma spp. in interaction with Arabidopsis thaliana. P. Guzman-Guzman1, M.J. Aleman-Duarte2,3, L.J. Delaye-Arredondo1, A. Herrera-Estrella2, V. Olmedo-Monfíl3. 1) Biology Dept.,University of Guanajuato, Guanajuato, Guanajuato, Mexico; 2) CINVESTAV, Langebio Unit, Irapuato Unit, Irapuato, Guanajuato, Mexico; 3) CINVESTAV, Irapuato Unit, Irapuato, Guanajuato, Mexico.

When a plant-pathogen interaction is established, plant activates its immune response system, recognizing pathogen’s virulence factors. In plant response are involved several phytohormones. Studies on plant pathogenic processes triggered by different organisms have shown the involvement of some pathogen proteins that are capable of altering the function and structure of the host cell, facilitating their entry and affecting overall host physiology, these proteins are known as “effectors”. The effectors activity inside the host takes place through conserved mechanisms of molecular interaction, such as effectors that have the RXLR translocation motif, which is highly conserved among different pathogens, directing its entry into the host cells. In plant-pathogen interactions, effectors are recognized, activating the plant response system. However, some of these molecules have been involved in the establishment of plant interactions with non-pathogenic organisms; little is known about the mechanisms controlling the establishment of this beneficial interaction. Fungi of the genus Trichoderma establish beneficial interactions with plants, promoting their growth and defense systems. To better understand this biological process, it is important to identify effector-like molecules in species like Trichoderma, and to establish their role in promoting plant development and defense systems activation. To achieve this goal, we will test the interaction of Trichoderma species with Arabidopsis thaliana, focusing on the selection and identification of effector-like molecules that are expressed during this interaction process. Additionally, we will generate null Trichoderma mutants on these effector-like candidates to evaluate their participation in the plant-fungus interaction. Until now, we have established interaction conditions, confirming the effect of the fungus presence over plant biomass production and root growth, showing interesting morphologic changes in the root system, such as a significant increase in lateral root formation. Based on conserved characteristics and bioinformatics tools, we selected 16 encoding sequences for effector-like molecules among T. virens and T. atroviride genomes, and we confirmed the differential expression of 7 of them in interaction with A. thaliana, by RT-PCR analysis.

522. The role of LysM effectors in fungal fitness. Anja Kombrink1, Jason Rudd2, Dirk-Jan Valkenburg1, Bart Thomma1. 1) Phytopathology, Wageningen University, Wageningen, Netherlands; 2) Department of Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Hertfordshire, United Kingdom.

LysM effector genes are found in the genomes of a wide range of fungal species. The encoded LysM effectors are secreted proteins that contain a varying
number of LysM domains, which are carbohydrate-binding modules. Ecp6, secreted by tomato leaf mould fungus Cladosporium fulvum, is the first characterized LysM effector. We demonstrated that Ecp6 specifically binds chitin, the major constituent of fungal cell walls that acts as a microbial-associated molecular pattern (MAMP) and triggers immune responses upon recognition by the host. Ecp6 outcompetes plant receptors for chitin binding, and thus prevents the activation of immune responses. Many fungal genomes, including saprophytes, carry multiple LysM effector genes that share only low sequence conservation and encode a varying number of LysM domains. We speculate that fungal LysM effectors might bind different carbohydrates and exert various functions in fungal fitness. In the fungal wheat pathogen Mycosphaerella graminicola, two LysM effectors were identified. Mg3LysM, but not Mg1LysM, suppresses chitin-induced immune responses in a similar fashion as Ecp6. Interestingly, unlike Ecp6, both Mg1LysM and Mg3LysM inhibit degradation of fungal hyphae by plant chitinases, revealing an additional function for LysM effectors in pathogen virulence. We recently observed that Mg1LysM binds to the bacterial cell wall constituent peptidoglycan. Similarly, a LysM effector from the saprophytic fungus Neurospora crassa showed peptidoglycan binding. We hypothesize that peptidoglycan binding by LysM effectors plays a role in the interaction of fungal species with bacterial competitors. The soil-borne fungal pathogen Verticillium dahliae contains seven LysM effectors genes of which one (Vd2LysM) is induced during tomato infection. Inoculation with two independent knock-out mutants revealed that Vd2LysM is required for full virulence of V. dahliae. However, Vd2LysM does not specifically bind chitin and does not function in a similar fashion as previously characterized LysM effectors. Thus, its function in virulence remains unclear.

523. WITHDRAWN

524. Functional analysis and localization of SnTox1, a necrotrophic effector produced by the wheat pathogen Stagonospora nodorum. Zhaozhui Liu1, Weilin Shelver2, Justin Faris2, Timothy Friesen1. 1) Department of Plant Pathology, North Dakota State University, Fargo, ND; 2) USDA-ARS, Biosciences Research Laboratory, Fargo, ND; 3) USDA-ARS, Northern Crop Science Laboratory, Fargo, ND.

SnTox1 is one of the necrotrophic effectors produced by the fungus Stagonospora nodorum, the causal agent of wheat Stagonospora nodorum blotch. It interacts, directly or indirectly, with the product of the wheat gene Snn1 to induce host cell death and promote disease. Previously, we showed that SnTox1, a cysteine-rich protein, triggers programmed cell death-like responses in the host and plays an important role in fungal penetration. In the present work, we are investigating the biochemical and molecular function of SnTox1 as well as its mode of action. Based on a Prosite motif search of SnTox1, multiple predicted sites including a putative chitin binding domain were targeted for site-directed mutagenesis. SnTox1 activity was significantly reduced when mutations were produced at a casein kinase II phosphorylation site and a predicted helical region where lysine residues are abundant. Using a fungal strain expressing an SnTox1-GFP fusion protein, we examined the location of the SnTox1 protein during fungal growth and infection. SnTox1 was observed in higher concentrations on several fungal structures, including the surface of conidia and mycelium, hyphal septa, and hyphal tips. The accumulation of SnTox1-GFP is particularly obvious at hyphal regions where new hyphae are arising. This observation suggests a protection mechanism of SnTox1 that is similar to that of chitin binding proteins in other fungal pathogens. In planta, SnTox1 is highly expressed in the hypophodia where the penetration is initiated, providing further evidence that SnTox1 plays a role in penetration. The cellular localization of SnTox1 was also investigated using fluorescent labeled SnTox1 in combination with cytological methods and preliminary data has indicated that SnTox1 is likely not internalized into mesophyll cells but remains in the apoplast. Interestingly, SnTox1 is able to induce host cell death by directly spraying onto the leaf surface of sensitive lines. We are currently investigating if SnTox1 is transported through epidermal cell layer.

525. Host-targeting protein 3 (SpHtp3) from the oomycete Saprolegnia parasitica translocates specifically into fish cells in a pH and tyrosine O-sulfate-dependent manner. Lars Lõbach1, Stephan Wawra2, Irene de Brujin2, Aleksandra Toloczko3, Tim Rasmussen4, Christopher Secombes1, Pieter van West1. 1) Scottish Fish Immunology Research Centre, University of Aberdeen, School of Biological Sciences, Aberdeen, Scotland, UK; 2) Aberdeen Oomycete Laboratory, University of Aberdeen, School of Medical Sciences, Foresterhill, Aberdeen, Scotland, UK; 3) University of Aberdeen, School of Medical Sciences, Foresterhill, Aberdeen, Scotland, UK.

The success of eukaryotic oomycete pathogens depends largely on effector proteins, molecules which manipulate or interfere with host defence mechanisms in the extracellular space or inside their host cells. One economically important oomycete parasite is the fish pathogen Saprolegnia parasitica, which is the causal agent of the disease Saprolegnia. S. parasitica is responsible for devastating losses in the aquaculture industry worldwide. In order to effectively fight any pathogen it is crucial to understand the key molecular mechanisms that lead to the disease. With the focus on putative effector proteins, we screened the genome of S. parasitica in the present study for potential effector candidates. Analysis identified a novel putative secreted S. parasitica effector protein, which we named host-targeting protein 3 (SpHtp3). Gene expression analyses showed that mRNA levels of SpHtp3 are highest in mycelium, sporulating mycelium and during the later stages of infection. Recombinant SpHtp3 was able to translocate specifically into fish cells in a tyrosine O-sulfate and pH-dependent manner. SpHtp3 was found in vesicular structures inside fish cells and was released from these upon infection of the cells with S. parasitica. Interestingly, SpHtp3 possesses an N-terminal RLR tetra-peptide sequence at a similar location as found in RxLR-effectors from plant pathogenic oomycetes. However, this RLR-sequence was not required for the fish cell translocation property of SpHtp3. These findings suggest that SpHtp3 from S. parasitica is a novel intracellular protein that might play an important role in Saprolegniosis.

526. Ave1-like orthologs in Venturia: another expanded effector family emerges. Adam Taranto1, Daniel Jones1, Jason Shiller1, Shakira Johnson2, Nathan Hall1, Ira Cooke1, Gert Talbo1, Carl Mesarchic2, Bart Thomma3, Jordi Boshoven2, Joanna Bowen1, Cecilia Deng1, Matthew Templeton1, Kim M. Plummer1. 1) Department Botany, La Trobe Univ, Melbourne, Victoria, Australia; 2) Laboratory of Phytopathology, Wageningen University, The Netherlands; 3) Plant & Food Research, Auckland, New Zealand.

Effectors are secreted by pathogens to modify plant physiology and establish disease. Plant immune receptors have evolved to recognize effector molecules that are unique to specific pathogens or species, or correspond to physiological races within a species. The availability of many whole genome sequences has revealed that some effectors are found in a discontinuous distribution within the fungal kingdom; a few phytopathogenic fungi (Colletotrichum higginsianum, Cerrospora beticola, Fusarium oxysporum) possess an ortholog of Ave1 from Ventricillium dahliae, an effector that activates Ve1-mediated resistance in tomato. A subset of these orthologs were shown to activate Ve1-mediated resistance in tomato. However, Ave1 also shares similarity to an ortholog in the phytopathogenic bacterium Xanthomonas axonopodis, as well as to a widespread family of plant natriuretic peptides and expansins, involved in plant homeostasis and plant cell wall modification (de Jonge & van Esse et al. 2012). We have identified an expanded Ave1-like gene family in apple and pear scab fungi, Venturia inaequalis and V. pirina. These species also have expanded gene families with similarity to the Leptosphaeria maculans effector AvrLm6. V. pirina has 14 unique hits (best,1.43e-16) to VdAve1. V. inaequalis has 17 unique hits (best,1.07e-16) to VdAve1. The distribution of Ave1 orthologs is suggestive of one or more cross-kingdom gene transfer events. We are characterising Venturia Ave1-like genes to investigate the mode of gene multiplication; seek evidence of horizontal gene transfer; and determine the role
of Ave1-like genes in pathogenicity. Ave1-like genes from non-Venturia fungi (and the bacterial gene) do not contain predicted introns, however, several (not all) V. inaequalis Ave1-like genes are predicted to contain introns. Codon usage bias among fungal, plant, and bacterial Ave1 orthologs, are being compared with the aim of inferring the kingdom of origin of the Venturia Ave1 orthologs. At least two V Ava1 orthologs are up-regulated during infection of apple. To determine whether the Venturia Ave1 proteins also activate a Ve1-mediated hypersensitive response, each has been co-expressed with tomato Ve1 in Nicotiana tabacum, using an Agrobacterium tumefaciens-mediated transient transformation assay.


Plant roots are constantly approached by a myriad of microorganisms and are thus challenged to recognize friends from foes. Most plant roots engage in a mutualistic association with fungi from the Glomeromycota Phylum forming the arbuscular mycorrhiza (AM) symbiosis. The establishment of this beneficial association requires an intensive signal exchange including the down-regulation of PAMP triggered responses. We have shown that secretion and delivery of the effector proteins contribute to the manipulation of the plant defense response. In a previous work we showed that one of these proteins travels to the plant nucleus and interacts with a plant transcription factor to down-regulate plant defenses. In order to identify further mechanisms of how symbiotic effectors function we are investigating new plant targets of mycorrhizal effector proteins and how do they differ from targets from pathogenic fungi. Progress in this area will be presented.

528. Structural basis for interactions of the Phytophthora sojae RXLR effector Avh5 with phosphatidylinositol 3-phosphate and for host cell entry. Furong Sun,1,2, Shiv Kale,1,2, Hugo Azurmendi,1,3, Dan Li,1, Brett M. Tyler1,4, Daniel Capelluto1. 1) Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR; 2) Dept of Biological Sciences, Virginia Tech, Blacksburg, VA 24061; 3) Dept of Chemistry, Virginia Tech, Blacksburg, VA 24061; 4) Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA 24061.

Oomycetes, such as Phytophthora sojae, employ protein effectors that enter host cells to facilitate infection. Entry of some effector proteins into plant cells is mediated by conserved RxLR motifs in the effectors and phosphoinositides (PIPs) resident in the host plasma membrane such as phosphatidylinositol 3-phosphate (PtdIns(3)P). Recent reports differ regarding the regions on RxLR effector proteins involved in PIP recognition. To clarify these differences, we have structurally and functionally characterized the P. sojae effector, avirulence homolog-5 (Avh5). Using NMR spectroscopy, we demonstrate that Avh5 is helical in nature with a long N-terminal disordered region. Heteronuclear single quantum coherence titrations of Avh5 with the PtdIns(3)P head group, inositol 1,3-bisphosphate (Ins(1,3)P2), allowed us to identify a C-terminal lysine-rich helical region (helix 2) as the principal lipid-binding site in the protein, with the N-terminal RxLR (RFLR) motif playing a more minor role. Mutations in the RFLR motif slightly affected PtdIns(3)P binding, while mutations in the basic helix almost abolished it. Avh5 exhibited moderate affinity for PtdIns(3)P, which increased the thermal stability of the protein. Mutations in the RFLR motif or in the basic region of Avh5 both significantly reduced protein entry into plant and human cells. Both regions independently mediated cell entry via a PtdIns(3)P-dependent mechanism. Our findings support a model in which Avh5 transiently interacts with PtdIns(3)P by specific electrostatic interactions mainly through its positively charged helix 2 region, enabling the RFLR domain to promote PIP-mediated host entry. This study, including the identification of the PtdIns(3)P-binding site, provides an improved and updated model for how RxLR effector proteins recognize phosphoinositides and for the contributions of the RFLR motif and basic-rich C-terminal regions to the internalization process.


The mycotoxin deoxynivalenol (DON) is associated with Fusarium head blight (FHB). This disease causes vast losses by reducing grain quality. Our study was aimed at analyzing cell wall changes in the host model grass B. distachyon infected with the mycotoxin deoxynivalenol (DON) itself can induce cell wall changes.

530. How Oomycete Pathogens Exploit PI3P to Target Secreted RXLR Effectors into Host Cells. Q. Wang,1 S. Ferrer,2 J. Carlough,2 F. Arredondo,2 S. Kale,2 B. Tyler3. 1) Botany and Plant Pathology, OREGON STATE UNIVERSITY, Corvallis, OR. 97330; 2) Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA 24060.

EFector proteins from diverse oomycetes and fungi can enter plant cells to facilitate infection. Recent research suggests that phosphoinositides (PIPs) resident in the host plasma membrane such as phosphatidylinositol 3-phosphate (PI3P) mediate the entry of some oomycete RXLR effectors. The PI3P recognition domain of these effectors is still controversial. Current evidence shows that either the RXLR domain or positive residues in the C-terminal domains (Ct) of some effectors such as the P. sojae effectors Avr1b and Avh5 can bind PI3P, it has been unresolved which of these domains, if either, or both, are involved in cell entry during natural pathogen infection. Here we have used heterologous PI3P-binding proteins, such as the yeast VAM7p PX domain to replace the RXLR or Ct domains of Avr1b in P. sojae transformants. Our results reveal that the VAM7p PX domain can replace the RXLR domain of Avr1b in carrying the C-terminal domain of Avr1b into soybean cells, conferring an avirulent phenotype on the transformants. Mutations that abolish the binding of VAM7p to PI3P substantially reduce but do abolish avirulence conferred by the construct. Mutations in the PI3P-binding residues of the Avr1b Ct also substantially reduce avirulence, while the double mutant cannot confer avirulence at all. These results strongly support the hypothesis that PI3P-binding is essential for Avr1b cell entry during natural infection, and further suggest that efficient entry by Avr1b may require two PI3P binding sites.
FULL POSTER SESSION ABSTRACTS

Phytophthora sojae is a notorious oomycete pathogen producing a great loss on global soybean production annually. The disease outcome between soybean and P. sojae depends on whether hosts could recognize pathogen avirulence effectors. Recently identified oomycete avirulence effectors are characterized by N-terminal host entry motif (RxLR motif), sequence and transcriptional polymorphisms between virulent and avirulent strains. Benefit from 454 genome sequencing and solexa transcriptome sequencing of P. sojae strains, eight RxLR effectors are bioinformatically identified, genetic mapping suggested that two of them perfectly matched Avr3b and Avr1d phenotype respectively. Transient expression of the ORF from avirulence strain on soybean specifically triggered Rps3b and Rps1d mediated program cell death, respectively. confirming that they encodes avirulence effector Avr3b and Avr1d. Transient expression of Avr3b and Avr1d on Nicotiana benthamiana could promote the infection of Phytophthora capsici, suggesting both avirulence effectors could suppress plant immunity and contribute to pathogen infection. Silencing of Avr3b impaired the virulence of Phytophthora sojae. Our progress in elucidating the mechanism under the inhibiting plant immunity by these effectors will be presented.

532. Evaluating the translation- and phospholipid binding abilities of the Phytophthora infestans AVR3a and Phytophthora sojae Avr1b RXLR-leaders. Stephan Wawra1, Armin Djamei2, Isabell Küfner3, Thorsten Nürnberg4, Justin A. Boddey5, Stephen C. Whisson5, Pieter van West6, 1) Sch Med Sci, Univ Aberdeen, Aberdeen, United Kingdom; 2) Department of Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Germany; 3) Department of Plant Biochemistry, University Tübingen, Germany; 4) Department of Medical Biology, University of Melbourne, Australia; 5) Cell and Molecular Sciences, James Hutton Institute, Dundee, UK.
Plant pathogenic oomycetes have a large set of secreted effectors that are directed into their host cells during infection. One group of these effectors are the RxLR-effectors found in plant pathogenic oomycetes. These RxLR-effectors are defined as putative secreted proteins that contained a conserved tetrameremic amino acid sequence motif, Arg-Xaa-Leu-Arg. This motif has to be within 40 amino acids C-terminal of the predicted cleavage sites of canonical signal peptides. Often this sequence is followed by a Glu-Glu-Arg (EER) motif. It has been shown, in a few cases, that the RxLR-motif is important for the delivery of these proteins into host cells. However, how these proteins translocate into the cytoplasm of their host is currently the object of intense research activity and debate. One model suggests that the RxLR-leader sequences of these effectors are sufficient to translocate the respective effectors into eukaryotic cells through binding to exposed phosphoinositol-3-phosphate. However, analysing the translocation behaviour of the RxLR-leaders from Phytophthora infestans avirulence protein 3a (AVR3a) and Phytophthora sojae avirulence protein 1b (Avr1b) we were unable to obtain conclusive evidence for specific RxLR-mediated translocation. Importantly, we confirm that the reported phospholipid binding properties of AVR3a and Avr1b are not mediated by their RxLR-leaders. In addition, we will present data showing that the observed phospholipid interaction of the AVR3a effector domain is attributable to a weak association with denatured protein molecules, and is therefore most likely physiologically irrelevant.

533. Identifying essential effectors from the soybean pathogen Phytophthora sojae. Hua Z. Wise1,2, Ryan G. Anderson3, John M. McDowell4, Brett M. Tyler2, 1) Center for Genome Research and Biocomputing and Department of Botany and Plant Pathology, Oregon State University; 2) Virginia Bioinformatics Institute, Virginia Tech; 3) Department of Plant Pathology, Physiology and Weed Science, Virginia Tech.
Breeding for resistance to plant pathogens is one of the most effective means of disease control. However, the ability of plant pathogens evolve new pathogenicity factors and evade host defense mechanisms drives the continual necessity to identify new resistance genes. We are exploiting genomic technologies in an effector-directed breeding approach that augments traditional breeding efforts against Phytophthora sojae, the causal agent of soybean root and seedling rot. This approach is founded on identifying monomorphic P. sojae effector genes that are essential for virulence, and using these genes as probes to identify new sources of resistance in soybean and related legumes. These essential effectors will make excellent candidates for screening for new, durable resistance to P. sojae, as these genes presumably cannot be mutated or deleted without a significant fitness penalty. The majority of predicted P. sojae RXLR effector genes are polymorphic amongst sequenced isolates of P. sojae, however, a subset of P. sojae RXLR effectors displays little or no allelic diversity. We have established a workflow for transient gene silencing and quantitative virulence assays. To date, we have silenced and assessed the virulence contribution of 17 PsAvh genes. Silencing of 13 of these effectors produced reduced virulence. Among these effectors, Avh16, Avh180 and Avh240 showed substantially reduced pathogen growth at early stages of host colonization and reduced disease symptoms at later stages of infection. We are currently using these three effectors as candidates in a high throughput screen system utilizing Pseudomonas Type III secretion system to screen for new resistance genes against P. sojae.

534. The LysM effector, Ecp6, is a virulence factor in the interaction of the hemibiotroph, Setosphaeria turcica, but not the necrotroph, Cochliobolus heterostrophus, with their common host, maize. Dongliang Wu1,2, Qing Bi1, Gillian Turgeon2, 1) Department of Plant Pathology & Plant-Microbe Biology, Cornell University, Ithaca, NY 14853, USA; 2) State Key Program of Microbiology and Department of Microbiology, College of Life Sciences, Nankai University, Tianjin, China, 300071.
Fungal phytopathogens are characterized as biotrophs, which derive nutrients from living cells, and necrotrophs, which kill host cells and retrieve nutrients from dead tissue. Hemibiotrophs are intermediate in that they initially establish themselves in living host tissue, then undergo rapid killing of plant cells later on. Hemi- and bio-trophic fungi utilize specialized effectors to prevent host recognition triggered by pathogen associated molecular patterns (PAMPs), whereas necrotrophs often produce toxins that induce programmed cell death. Chitin, a signature component of fungal cell walls, is one type of PAMPs known to trigger the plant resistance response. Several fungal chitin-binding LysM effectors have been identified that broker counter-defense against chitin-triggered immunity, the first characterized one, Ecp6, from the tomato biotroph, Cladosporium fulvum, Mg3LysM, from the wheat hemibiotroph, Mycosphaerella graminicola, and SpJ1, from the rice hemibiotroph, Magnaporthe oryzae. In this study, Ecp6 homologs were identified and deleted from the genomes of two maize pathogens which differ in pathogenic lifestyle, Setosphaeria turcica (hemibiotroph), causal agent of Northern Leaf Blight and Cochliobolus heterostrophus (necrotroph), causal agent of Southern Corn Leaf Blight. Deletion of SteECP6 caused reduced virulence, whereas absence of CcECP6 did not alter virulence to the host. Real time RT-PCR demonstrated that expression of pathogenesis related maize gene, PR1 gene and a chitinase gene was increased in Stec6 mutants compared to wild type at 4 days post inoculation. Additional in planta gene expression analyses are underway to compare host responses to these two fungi differing in pathogenic lifestyle on the same host.

535. Nematode-trapping fungi eavesdrop on nematode pheromones. Yen-Ping Hsueh1, Parag Mahanti2, Frank Schroeder2, Paul Sternberg3, 1) Howard Hughes Medical Institute and Division of Biology, California Inst of Technology, Pasadena, CA; 2) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY.
The recognition of molecular patterns associated with specific pathogens or food sources is fundamental to ecology and plays a major role in the evolution of predator-prey relationships. Recent studies showed that nematodes produce an evolutionarily highly conserved family of small molecules, the
ascarosides, which serve essential functions in regulating nematode development and behavior. Here we show that nematophagous fungi, natural predators of soil-dwelling nematodes, can detect and respond to ascarosides. Nematophagous fungi use specialized trapping devices to catch and consume nematodes, and previous studies demonstrated that most fungal species do not produce traps constitutively but rather initiate trap-formation in response to their prey. We found that ascarosides, which are constitutively secreted by many species of soil-dwelling nematodes, represent a conserved molecular pattern used by nematophagous fungi to detect prey and trigger trap formation. Ascaroside-induced morphogenesis is conserved in several closely related species of nematophagous fungi and occurs only under nutrient-deprived condition. Our results demonstrate that microbial predators eavesdrop on chemical communication among their metazoan prey to regulate morphogenesis, providing a striking example of predator-prey co-evolution. We anticipate that these findings will have broader implications for understanding other inter-kingdom interactions involving nematodes, which are found in almost any ecological niche on Earth.

536. Molecular diagnosis to discriminate pathogen and apathogen species of the hybrid Verticillium longisporum on the oilseed crop Brassica napus. Van Tuan Tran, Susanna Braus-Stromeyer, Christian Timpner, Gerhard Braus. Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Grisebachstr. 8, 37077 Göttingen, Germany.

The cruciferous fungal pathogen Verticillium longisporum represents an alloploid hybrid with long spores and almost double the amount of nuclear DNA compared to other Verticillium species. V. longisporum evolved at least three times by hybridization. In Europe, virulent A1xD1 and avirulent A1xD3 hybrids were isolated from the oilseed crop Brassica napus. Parental A1 or D1 species are yet unknown whereas the D3 lineage represents Verticillium dahliae. The V. longisporum isolates from Europe or California corresponding to hybrids A1xD1 or A1xD3 were analyzed. Only one single characteristic type of ribosomal DNA (rDNA) could be assigned to each hybrid lineage. The avirulent A1xD3 isolates carried exclusively D3 rDNA, which corresponds to V. dahliae, whereas the rDNA of the virulent A1xD1 isolates originates from A1. Both hybrid lineages carry distinct isogene pairs of conserved regulatory genes corresponding to either A1 or D1/D3. D1 and D3 paralogues show high identities but differ in several single nucleotide polymorphisms. Distinct signatures of the VTA2 regulatory iso gene pair allow the identification of V. longisporum hybrids by a single PCR and the separation from haploid species as A1 or D1/D3. The combination between the VTA2 marker as a barcode marker and differentiation of the rDNA type represents an attractive diagnostic tool to discriminate alloploid from haploid Verticillia and to distinguish between A1xD1 and A1xD3 hybrids, which differ in their virulence towards B. napus. Furthermore, the VTA2 gene was demonstrated to be a virulence factor that is required for fungal morphogenesis and plant infection.

537. Investigating the Pathogenicity of Armillaria, Kathryn Ford1, Beatrice Henricot2, Kendra Baumgartner2, Gary D. Foster2, Andy M. Bailey1. 1) Molecular Plant Pathology, University of Bristol, Bristol, United Kingdom; 2) USDA-ARS, Plant Pathology, University of California, Davis, CA; 3) Royal Horticultural Society, Plant Pathology, Surrey, United Kingdom.

Armillaria sp., or ‘honey mushroom’, is a generalist pathogen of fruit, nut and timber trees in gardens, forests and agricultural systems worldwide, causing Armillaria root disease and resulting in significant yield losses and millions of dollars worth of damage annually. Several questions regarding the infection mechanisms used by basidiosporas, hyphae and rhizomorphs and their subsequent colonisation processes remain unanswered. We established a reproducible method of producing fruiting bodies in culture in order to generate basidiospores for use in Agrobacterium-mediated transformation to facilitate further exploration of Armillaria’s pathogenicity. Results will be presented on the construction and utilisation of various plasmids conferring hygromycin resistance and fluorescent protein expression that have been used to transform A. mellea in order to study the infection mechanisms in herbaceous plants.

538. Detoxification of nitric oxide by flavohemoglobin and the denitrification pathway in the maize pathogen Fusarium verticillioides. Thomas Baldwin1,2, Anthony Glenn1. 1) Plant Pathology Department, Univ of Georgia, Athens, GA; 2) USDA-ARS, R.B. Russell Research Center, Toxicology and Mycotoxin Research Unit, Athens, GA.

The ephemeral nitric oxide (NO) is a free radical, highly reactive, environmentally rare, and a potent signaling molecule in organisms across kingdoms of life. This gaseous small molecule can freely transverse membranes and has been implicated in aspects of pathogenicity both in animal and plant hosts. Fusarium verticillioides is a mycotoxigenic pathogen of maize, notable for its ability to persist as an asymptomatic endophyte. One potential determinant of this lifestyle conversion between overt pathogen and symptomless endophyte may be the regulation of NO. Detoxification of NO is a known pathogenicity factor for the fungal human pathogen Candida albicans and the bacterial plant pathogen Erwinia chrysanthemi. Both mediate detoxification by a flavohemoglobin protein (CaYHB1 and HmpX, respectively). BLASTP search of the F. verticillioides genome revealed two putative flavohemoglobin homologs, denoted FHB1 and FHB2. Microarray analysis revealed a significant induction of FHB2 (13-fold) when the fungus was exposed to exogenous NO. FHB1 had a 2-fold increase. Also noteworthy from the microarray data is the distinct induction of genes within the denitrification pathway, including dissimilatory nitrite reductase (dNaR, 16-fold increase), dissimilatory nitrate reductase (dNiR, 226-fold), and PAR50 nitric oxide reductase (P450nor, 27-fold). Flavohemoglobin has been noted as a component of the denitrification pathway, having a role in converting NO to nitrate. Thus, FHB2 is postulated to be the paralog involved in the F. verticillioides denitrification pathway. Nitrate deletion mutants are being created in dNiR, P450nor, FHB1, and FHB2 to further evaluate functions of these genes in F. verticillioides. Mutants will be assayed for their endogenous production and regulation of NO, response to exogenous NO, virulence against maize, and mycotoxin production. Elucidating the function of these genes will give insight into the role of NO in F. verticillioides development, maize-fungal interactions, and denitrification, which has previously only been assessed in relation to anaerobic growth.

539. Family disintegration: One Fusarium verticillioides beta-lactamase gene at a time. Scott E. Gold, Xiu Lin, Nicole J. Crenshaw, Anthony E. Glenn. Toxicology & Mycotoxin Research, USDA-ARS, Athens, GA.

Fusarium verticillioides is a mycotoxigenic fungus found commonly on maize, where it primarily exhibits asymptomatic endophytic growth. The F. verticillioides genome possesses approximately 30 regions that potentially encode beta-lactamase enzymatic domains. These enzymes are classically involved in bacterial resistance to beta-lactam antibiotics, for example penicillins. Our attention was drawn to this enzymatic function by the recent finding that the gene VFE3_08291 is essential for resistance to maize phytoanticipins such as 2-benzoxazolinone (BOA), which possesses a gamma-lactam moiety, the presumed enzymatic target (see poster by Glenn et al.). VFE3_08291 belongs to a subset of these enzymes known as metallo-beta-lactamas. Beta-lactamase enzyme function is not well studied in the fungi, so in order to further evaluate the roles of these enzymes in F. verticillioides, we are in the process of deleting the members of their encoding gene family. We assigned directed-research undergraduates each a specific gene, for which they produced deletion constructs by DelGate and/or OSCAR methodology and generated fungal transformants for analysis. Deletion mutants in one of the other metallo-beta-lactamase encoding genes (VFE3_12159) showed a dramatic defective growth phenotype. This observation raises the interesting hypothesis that perhaps this mutant is no longer resistant to a lactam moiety containing compound produced by F. verticillioides itself. Data will be presented on initial progress with this project.

Xenobiotic compounds such as phytochemicals, microbial metabolites, and agrochemicals can impact the diversity and frequency of fungal species occurring in agricultural environments. Resistance to xenobiotics may allow plant pathogenic fungi to dominate the overall fungal community, with potential negative impacts on crop yield and value. The mycotoxigenic Fusarium verticillioides is such a fungus commonly associated with maize worldwide, often contaminating maize kernels with the fumonisin mycotoxins. The dominance of F. verticillioides as an endophyte may be due in part to its ability to metabolize phytoprotectants produced by maize. The benzoazinoids and benzoxazolines are broad spectrum allelopathic, antimicrobial, and anti-herbivory compounds from maize, yet F. verticillioides can rapidly biotransform these phytochemicals into non-toxic metabolites. We have identified the genes responsible for the biotransformation process. Two gene clusters were identified that correspond to the previously characterized FDB1 and FDB2 loci, with both loci being necessary for metabolic tolerance to β-2benzoxazolone (BOA), one of the maize phytoprotectants. Analysis of the nine ORFs (FVEG_08287 to FVEG_08290) at the FDB1 locus indicated that one of the genes (FVEG_08291) encodes a protein having a metallo-beta-lactamase domain, and deletion of the gene in wild-type strain M3125 resulted in the fungus being unable to grow on BOA-amended agar due to an inability to metabolize the compound. Deletion mutants were complemented to wild-type phenotype by transformation with the native allele. Other ORFs were not found to be essential when deleted in M3125. Microarray analysis indicated the metallo-beta-lactamase (FVEG_08291) had a 13-fold induction in response to BOA (2-hr incubation), with other genes in the cluster ranging from 3-fold (FVEG_08287) to 42-fold induction (FVEG_08292). Beta-lactamases are well-known for conferring bacterial resistance to lactam-type antibiotics, but to our knowledge this is the first report of fungal enzymes of this type metabolizing lactam-like xenobiotics. We are investigating other beta-lactamase encoding genes in F. verticillioides (see poster by Gold et al.) to further evaluate their possible role in tolerance to both exogenous as well as endogenous metabolites having lactam-type moieties.


Root tips of pea (Pisum sativum L.) are protected from N. haematococca infection by an extracellular DNA (exDNA)-based trapping process similar to that occurring in mammalian defense responses to bacterial and fungal pathogens. N. haematococca spores germinate rapidly in response to root border cell populations programmed to export exDNA and antimicrobial proteins as they detach from the root cap. Within 24 h, hyphae and border cells together form a mantle which ensheaths the root tip and separates, leaving the root apex uninvaded: >98% of root tips escape infection. When the exDNA is degraded with DNase added to the root at the time of inoculation, resistance is abolished: 100% of root tips are invaded by proliferating hyphae and root growth ceases within 24 hours. In N. haematococca isolates harboring a conditionally dispensable (CD) chromosome, the process occurs more rapidly than in CD-minus isolates; a ten-fold increase in spores from CD-minus isolates results in comparable dynamics. Putative DNase-encoding sequences have been detected on two different CD chromosomes, and direct tests have revealed increased extracellular DNase activity from CD-plus isolates compared with CD-minus isolates. The goal of this study is to examine predictions of the hypothesis that CD chromosome encoded DNase activity plays a role in pea root infection.

542. FvSNF1, a protein kinase of Fusarium virguliforme that affects SDS development in Soybean. K.T. Islam, Ahmad Fakhoury. Plant, Soil and Ag Systems, Southern Illinois University, Carbondale, IL.

Fusarium virguliforme is a soil-borne pathogen that causes Sudden Death Syndrome (SDS). SDS is one of the top four yield-robbing fungal diseases in soybean resulting in significant economic losses to producers. Despite the importance of SDS, a clear understanding of fungal genetic factors that affect the development of the disease is still lacking. The aggressiveness of F. virguliforme on infected soybean plants is believed to require the activity of cell wall-degrading enzymes (CWDE). The production of these CWDEs in phytopathogenic fungi is under catabolic repression. In Saccharomyces cerevisiae, catabolic repression is regulated by SNF1 (sucrose non-fermenting 1). To investigate the role of cell wall-degrading enzymes as determinants of F. virguliforme aggressiveness, the F. virguliforme SNF1 homologue FvSNF1 was targeted for disruption. The resulting FvDsnf1 transformant failed to grow on galactose and grew poorly when arabinose or sucrose where the main carbon source. The mutation did not seem to affect the ability of the fungus to grow with glucose, fructose, maltose, or xylose as the main source of carbon. More importantly, in greenhouse experiments, the FvDsnf1 transformant was severely impaired in its ability to cause SDS on challenged soybean plants.

543. Functional and molecular analysis of AstA sulfate transporter in pathogenic Fusarium sambucinum with respect to its virulence and ability to infect potato. Sebastian Pilsky1, Hanna Gawinska-Urbanowicz2, Renata Natorff2, Marzena Sienko2, Joanna S. Kruszewska2. 1) Laboratory of Fungal Glycobiology, Institute of Biochemistry and Biophysics, Warsaw, Poland; 2) The Plant Breeding and Acclimatization Institute (IHAR), Bonin, Poland; 3) Department of Genetics, Institute of Biochemistry and Biophysics, Warsaw, Poland.

AstA protein (alternative sulfate transporter) represents a little known type of sulfate trasporter, belonging to an extensive family of allantoate permeas Dal5. In Aspergillus nidulans the astA gene is under the control of Sulfur Metabolite Repression (SMR). The closest homologs of astA are frequent in evolutionarily distant fungi belonging to the Pezizomycotina order cells together form a mantle which ensheaths the root tip and separates, leaving the root apex uninvaded: >98% of root tips escape infection. The exDNA is degraded with DNase added to the root at the time of inoculation, resistance is abolished: 100% of root tips are invaded by proliferating hyphae and root growth ceases within 24 hours. In N. haematococca isolates harboring a conditionally dispensable (CD) chromosome, the process occurs more rapidly than in CD-minus isolates; a ten-fold increase in spores from CD-minus isolates results in comparable dynamics. Putative DNase-encoding sequences have been detected on two different CD chromosomes, and direct tests have revealed increased extracellular DNase activity from CD-plus isolates compared with CD-minus isolates. The goal of this study is to examine predictions of the hypothesis that CD chromosome encoded DNase activity plays a role in pea root infection.

544. WITHDRAWN

545. The adenylyl cyclase of the cereal pathogen Fusarium graminearum controls infection structure development, mycotoxin production and virulence to wheat. Jörg Sormann, Marike Johanne Boensich, Elena Brückner, Demet Firtat, Cathrin Kröger, Birgit Hadeler, Wilhelm Schäfer. Molecular Phytopathology, University Hamburg, Hamburg, Germany.

Fusarium graminearum is one of the most devastating pathogens of cereals. Mycotoxins accumulating in infected grains are a serious threat to food and feed worldwide. Knowledge about the molecular basis of infection and mycotoxin production is still limited. Cyclic 3′,5′-adenosine monophosphate (cAMP)
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is a nucleotide derived from adenosine triphosphate that acts as a second messenger throughout all kingdoms. Intracellular cAMP levels are subject to a large membrane-bound protein, the adenylate cyclase. In order to analyze the function of this gene and the importance of cAMP in the life cycle of F. graminearum, the adenylate cyclase gene (FSGS_01234) was deleted from the genome (Dfgac1). Dfgac1 displayed a drastically reduced growth on complete medium. This reduction in growth could partially be complemented by addition of a cAMP analog. Furthermore, the mutant was unable to produce perithecia on detached wheat grains but more artificial conditions like carrot agar allowed perithecia development. Possibly, this points to a sensing problem of Dfgac1. Although growth on agar was reduced, conidia production was increased. Pathogenicity towards wheat was drastically reduced in Dfgac1 compared to the wild type. Point-inoculated spikelets showed only small lesions even after 21 days post inoculation. No deeper infection occurred and mycelial growth was never detectable near the rachis. Thus, fungal hyphae never grew from the inoculated spikelet to the adjacent one. Fluorescence microscopy using a Dfgac1-strain expressing dsRed constitutively in the cytosol revealed that FgAC1 controls the development of infection structures like lobate appressoria and infection cushions. Removal of hyphae superficially colonizing flower leaves and subsequent analysis by scanning electron microscopy demonstrated the lack of any fungal penetration holes. Instead, hyphae on flower leaves produced massively new conidia, thereby circumventing the infection cycle, something never observed in the wild type. Dfgac1-strains are unable to produce the mycotoxin deoxynivalenol both in vitro and during wheat infection. In this study, for the first time, we implicate the cAMP signaling pathway to important processes in F. graminearum like development of infection structures, pathogenicity, secondary metabolite production and sexual reproduction.

546. The ATF/CREB transcription factor Atf1 is essential for full virulence, deoxynivalenol production and stress tolerance in the plant pathogen Fusarium graminearum. Thuat Van Nguyen, Birgit Hadeler, Cathrin Kröger, Wilhelm Schäfer, Jörg Bormann. Molecular Phytopathology, University Hamburg, Hamburg, Germany.

The filamentous ascomycete Fusarium graminearum is a highly organ specific pathogen that resides on small grain cereals like rice, wheat, barley, and maize. Grains infected with F. graminearum accumulate high amounts of mycotoxins, most prominent of which are deoxynivalenol (DON) and zearealenone (ZEA). The stress-activated MAP-kinase FgOS-2 (Saccharomyces cerevisiae Hog1) is a central regulator in the life cycle of F. graminearum (Nguyen et al., 2012. MPMI 25:1142-1156). FgOS-2 regulates, among others, virulence to wheat and maize, and DON- and ZEA-production. Here, we present data on the functional characterization of a putative downstream regulator, the ATF/CREB activating transcription factor FgAtf1. We created deletion and overexpression mutants of Fgatf1, the latter one also in an FgOS-2 deletion mutant. Like FgOS-2, FgAtf1 is mainly involved in osmotic stress response. Bimolecular fluorescence complementation demonstrates an interaction of both proteins under osmotic stress conditions. Deletion mutants in Fgatf1 (Dfagt1) are more sensitive to osmotic stress (e.g. mediated by NaCl) and less sensitive to oxidative stress mediated by H₂O₂ compared to the wild type. Furthermore, sexual reproduction is delayed: perithecia develop much slower and some remain immature even after prolonged incubation. Dfagt1 strains show an increased DON-production under in-vitro induction conditions compared to the wild type. However, during wheat infection, DON-production is strongly reduced. Expression of genes encoding for key enzymes in the DON-biosynthesis pathway is regulated accordingly. In infection assays on wheat and maize, the Dfagt1 strains show a reduced virulence compared to the wild type. Interestingly, constitutive expression of Fgatf1 leads to hypervirulence on wheat, maize and Brachyodium distachyon. Moreover, constitutive expression of Fgatf1 in a Dfgos2 mutant background partially complements Dfgos2-phenotypes regarding growth on osmotic-stress medium, sexual reproduction, and virulence towards wheat and maize.

Furthermore, FgAtf1 is involved in the regulation of light-responsive genes. Taken together, these results provide new insights in the stress response signaling cascades of F. graminearum and assign the transcription factor FgAtf1 a central role in pathogenic development and secondary metabolism.


Fusarium graminearum is one of the most destructive pathogens of cereals and a threat to food and feed production worldwide. It is an ascomycetous plant pathogen and the causal agent of Fusarium head blight disease in small grain cereals and of cob rot disease in maize. Infection with F. graminearum leads to yield losses and mycotoxin contamination. Zearealenone (ZEA) and deoxynivalenol (DON) are hazardous mycotoxins; the latter is necessary for virulence towards wheat. Deletion mutants of the F. graminearum orthologue of Saccharomyces cerevisiae Hog1 stress-activated protein kinase, Fgos-2 (Dfgos-2), showed drastically reduced in planta DON and ZEA production. However, Dfgos-2 produce even more DON than the wild type under in vitro conditions, whereas ZEA production is similar to that of the wild type. These deletion strains are dramatically reduced in pathogenicity towards maize and wheat. We constitutively expressed the fluorescent protein dsRed in the deletion strains and the wild type. Microscopic analysis revealed that Dfgos-2 is unable to reach the rachis node at the base of wheat spikelets. During vegetative growth, Dfgos-2 strains exhibit increased resistance against the phytophyloric fluodixoin. Growth of mutant colonies on agar plates supplemented conidia is reduced but conidia formation remained unchanged. Moreover, germination of mutant conidia on osmotic media is severely impaired. Gern tubes are swollen and contain multiple nuclei. The deletion mutants completely fail to produce perithecia and ascospores. Furthermore, Fgos-2 also plays a role in reactive oxygen species (ROS)-related signalling: Fgos-2 deletion mutants are more resistant against oxidative stress mediated by H₂O₂. We found that the transcription and activity of fungal catalases is modulated by Fgos-2. Among the genes regulated by Fgos-2 we found a putative calcium-dependent NADPH-oxidase (noxC) and the transcriptional regulator of ROS metabolism, Fgatf1. The present study describes new aspects of stress-activated protein kinase signalling in F. graminearum.

548. Innate Immunity in Fusarium graminearum. Yong shian Simon Ip Cho1,2, Gite Erbs3, Thomas Sundelin4, Peter Busk5, Mari-Anne Newman6, Stefan Olsson7. 1) Genetics and Microbiology, University of Copenhagen, Copenhagen, Denmark; 2) USDA-ARS Cereal Disease Laboratory, University of Minnesota, Saint Paul, MN, USA; 3) Transport Biology, University of Copenhagen, Copenhagen, Denmark; 4) Dept. Biotechnology, Aalborg University, Copenhagen, Denmark.

Fungi are now mostly recognized as plant pathogens that cause harm to important economical plants. In nature however, fungi are frequently victims of bacterial parasitism but little is known about fungal defense mechanisms. The potential existence of fungal innate immunity was studied using Fusarium graminearum as model organism and bacterial flagellin to mimic the presence of bacteria in an in vitro environment. The presence of flagellin triggered an initial mitochondrial and cell membrane hyperpolarization which was detected using the fluorescent dye DiOC(3). This was followed by the production of the secondary signalling molecule Nitric Oxide (NO), common to innate immunity signalling in other eukaryotes. NO was monitored using the fluorescent dye DAF-FM. NO appears to be produced by an inducible enzyme that is regulated by complex mechanisms but centrally modulated by Calcium/Calmodulin. Inhibition studies suggest the presence of a Nitric Oxide Synthase (NOS), but no typical arginine utilizing NOS was identified within the F. graminearum’s genome by homology search. Various genes bearing resemblance to the archetypal NOS, as well as argininosuccinate lyase were deleted. However, the mutants still produced NO. The presence of alternative pathways contributing towards the production of NO was investigated by adding a variety of potential substrates to challenged cultures. Various reactions were observed suggesting that several pathways are present. In conclusion, F. graminearum reacts strongly to the presence of the bacterial Microbial Associated Molecular Pattern (MAMP) flagellin with an up-regulation.
549. Balanced posttranslational activation of eukaryotic translation initiation factor SA is required for pathogenesis in *Fusarium graminearum*. Ana Lilia Martínez-Rocha¹, Mayada Woiwied¹,², Jan Chemnitz¹, Peter Willingmann¹, Joachim Hauber¹, Wilhelm Schäfer¹. 1) Molecular Phytopathology, University of Hamburg, Hamburg, Germany; 2) Cell Biology and Plant Biochemistry, University of Regensburg, Germany; 3) Heinrich-Pette-Institute for Experimental Virology and Immunology, Martinistrasse 52, D- 20251 Hamburg, Germany.

Activation of the translation initiation factor SA (eIF5A) requires a posttranslational modification, changing a lysine into the unique amino acid hypusine. This activation is a two steps reaction mediated by deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). First DHS cleavage and transfers the 4-amino butyl moiety from the spermidine to an specific lysine in eIF5A to form the deoxyhypusine intermediate, during the second step DOHH hydroxylates the 4-amino butyl moiety to create the active form of eIF5A containing hypusine. The activated protein transports mRNAs from the nucleus to the ribosomes, where initiates protein biosynthesis. This system is conserved from Archea to humans and is involved in processes diverse as HIV infection, malaria, cancer, and diabetes. Until now, inhibition or silencing of DHS or DOHH has been tested to modify hypusination. For the first time, we evaluate its importance by over-expressing the enzymes that control hypusination of eIF5A. Over-expression of DOHH (oexDOHH) prevents virulence of *Fusarium graminearum* to wheat and maize. In contrast, over-expression of DHS (oexDHS) leads to an increase of virulence to wheat. Simultaneous over-expression of both enzymes results in virulence comparable to the wild type strain. GFP assisted histology revealed that oexDOHH mutant is unable to form infection structures on wheat flower leaves and barely grows in point inoculated wheat spikelets. OexDHS results in an increase of infection structures and accordingly in an increase of virulence. We determined general hypusine formation by incorporating radiolabeled spermidine in eIF5A during in culture growth. Wild type and oexDHS showed similar hypusinization intensity, whereas oexDOHH showed an increased incorporation. The differential hypusinization state of eIF5A was determined by 2D gels and western blot due to the difference in isoelectric point of the three states; inactive (lysine), intermediate (deoxyhypusine) or active (hypusine). Preliminary results show the wild type strain with all three hypusination states, oexDHS and double over-expressing mutants with an increased inactive and intermediate forms, and oexDOHH mutant lacked the inactive and intermediate form, only the activated form was detectable. We conclude that a balanced hypusination is required for proper function of eIF5A.

550. The Con7 transcription factor, essential for pathogenicity, regulates the expression of genes involved in glycolysis and virulence in *Fusarium oxysporum*. Carmen Ruiz-Roldán¹,², Yolanda Pareja-Jaime¹,², M. Isabel González Roncero¹,². 1) Department of Genetics, University of Cordoba, Spain; 2) Campus de Excelencia Agroalimentario (ceiA3).

Transcription factors (TF) regulating the different stages of infection like adhesion to the host surface, differentiation of infection structures and penetration represent potential molecular targets for fungicides with specific modes of action. Our studies on *Fusarium oxysporum* have demonstrated the essential role of morphogenetic regulation in pathogenesis, including processes such as cell-wall biogenesis, cell division and differentiation of infection structures. We identified the Con7 TF whose inactivation produces non-pathogenic mutants with altered morphogenesis, including abnormal polar growth and hyphal branching. To identify genes dependent on Con7 protein profiles of wild type and Dcon7 mutant were compared by 2D electrophoresis. Expression of 126 proteins varied quantitatively between both strains with statistical significance. Among the 80 proteins identified by MALDI-TOF/TOF-MS 15, 9, and 4 were associated with secondary metabolism, glycolysis/gluconeogenesis (Gly/Glu) and pentose and glucuronate interconversions, respectively. Additionally, 6 proteins were known virulence factors, including citochrome P450 monooxygenase, O-methyltransferase, peptidyl-prolyl cis-trans isomerase D, peroxidase/catalase, phospholipase C and superoxide dismutase. Expression of the responsible genes was confirmed by qRT-PCR. To verify the role of Con7 in Gly/Glu pathways, the intracellular ATP levels and alcohol dehydrogenase (ADH) activity in Dcon7 were compared with wt. We detected 2.4 fold increased ATP and 27.5% reduced ADH activity in Dcon7. Additionally, Dcon7 showed a dramatic growth reduction in the presence of glucose, glycine or polygalacturonic acid, indicating nutrient assimilation defects. No significant differences were detected in intracellular glucose accumulated by both strains, while extracellular glucose levels in Dcon7 were significantly higher, suggesting defective sugar transport. RT-PCR analyses in the wt revealed the existence of four con7 transcripts that differ in size and abundance, indicating alternative intron splicing. To determine the functionality of the different deduced proteins, Dcon7 was complemented with cDNA fragments from each version of the mature Con7 protein. Phenotypic and pathotypic characterization of the transformants should reveal their role in the different phenotypes observed in the Dcon7.

551. *Fusarium oxysporum* produces volatile organic compounds that enhance the growth and stress resistance of *Arabidopsis thaliana*. Vasileios Bitas¹, Michael Axtell¹, James Tomlinson¹, Seogchan Kang¹. 1) Department Plant Pathology and Environmental Microbiology, Pennsylvania State Univ, University Park, PA; 2) Department of Biology, Pennsylvania State Univ, University Park, PA; 3) Department of Entomology, The Pennsylvania State University, University Park, PA.

Production of volatile organic compounds (VOCs) as signal molecules is a widespread and efficient mode of communication utilized by all organisms. Microbial VOCs promoting plant growth and stress resistance present an environmentally and economically attractive alternative to fertilizers and pesticides. Fungi are some of the most predominant and yet, under-investigated organisms that employ VOCs in order to regulate and affect surrounding environments including neighboring organisms. Certain isolates of *F. oxysporum*, a well-known soilborne fungus, produce VOCs that promote plant growth, alter morphological and physiological properties, and enhance biotic and abiotic stress resistance in the model plant *Arabidopsis thaliana*. In order to investigate the mode of action by which those volatiles function, we have employed genetic and molecular resources and tools including *A. thaliana* mutants that are defective hormonal signaling pathways and gene expression analysis. We are also trying to identify those compounds that affect *A. thaliana* through the use of GC-MS. Identification of the fungal VOCs triggering these plant responses and elucidation of the physiological and molecular alterations occurring in plants will help us shed light into the mechanism underpinning complex VOC-mediated interactions.

552. Lipolytic system of the tomato pathogen *Fusarium oxysporum* f.sp. *lycopersici*. G. A. Bravo Ruiz¹,², C. Ruiz Roldán¹,², M. I. González Roncero¹,². 1) Departamento de Genética, Universidad de Córdoba; 2) Campus de Excelencia Agroalimentario (ceiA3), E-14071 Cordoba, Spain.

The lipolytic profile of *Fusarium oxysporum* f.sp. *lycopersici* has been determined on the basis of in silico analyses search and validated by biochemical enzyme activity determination of Wheat Germ Oil (WGO) induced cultures. Twenty five identified structural secreted lipases show the conserved pentapeptide -Gly-X-Ser-X-Gly- characteristic of fungal lipases and the signal sequence for extracellular secretion. On the other hand, two transcriptional regulatory predicted lipase genes have been identified showing nuclear localization signals (NLS) and the Zn2Cys6 zinc finger DNA binding motifs. The transcription profile of twelve structural genes, during tomato plant colonization in the wild type strain, shows variable expression levels (100 fold-range) being lip1, lip2, and lip22 the highest induced (20% relative to the actin gene). The maximal level of expression is observed in roots at 21-96 hours post-inoculation. Targeted replacement of four structural (lip1, lip2, lip3 and lip5), and two regulatory ctf1 (orthologue to *Fusarium solani* ctf1 and to *Aspergillus nidulans* far1) and ctf2 (orthologue to F. solani ctf2 and to A. nidulans far8), lipase predicted genes originated the corresponding single deletion mutants.
and the double deletion mutant Dctf1Dctf2. In vitro qRT-PCR expression analyses of twelve structural lipase genes in the regulatory mutants Dctf1, Dctf2 and Dctf1Dctf2, in comparison with the wild type strain, demonstrate the existence of a complex lipase regulation network in *F. oxysporum*. The reduction of total lipase activity (10-30%), besides the severe altered pathogenic behaviour on tomato plants shown by the single Dctf1, Dctf2, and the double Dctf1Dctf2 mutants suggest an important role of the lipolytic system of this fungus in pathogenicity.

553. Role of glycogen metabolism in the pathotypic behavior of *Fusarium oxysporum* f.sp. *lycopersici* on tomato plants. C. Ruiz Roldan1,2, M. I. González Roncero1,2. 1) Departamento de Genética, Universidad de Córdoba; 2) Campus de Excelencia Agroalimentario (ceiA3), E-14071 Córdoba, Spain.

Glycogen can play different roles in biological systems, such as storage carbohydrate for energy and/or carbon or control of glucose metabolism. In most eukaryotes, glycogen is built on a self-glucosylating initiator protein core, glycogenin (ggn), which acts as a primer for glycogen synthase (gls). The branches are created by a specific enzyme (gbe) which transfers a block of 6-7 residues from the end of a linear chain of glucose units to an internal glucose residue by an α-1,6 linkage. De-branching, which is essential for the degradation of glycogen, is catalyzed by a distinct enzyme (gdb) which acts on branches containing only four residues, transferring three of them to another branch, thus creating a new branch. Similar hyphal agglutination patterns were observed in the three single mutants Dgnn5, Dgs10 and Dgbe17 compared to the wild type, whereas those strains affected in glycogen catabolism, Dgdb2, Dgph8, and the double mutant Dgph8Dgdb2 showed a dramatic reduction in hyphal agglutination. This phenotype did not exactly correlate with conidial anastomosis tube (CAT) formation since all the mutants, except Dgs10, showed a 50-50% reduction in hyphal fusions. We are currently performing tomato plant infection assays what will help us to gain insight into the role of glycogen metabolism in the virulence of *F. oxysporum*.


*Fusarium oxysporum* is a soilborne pathogen that causes vascular wilt disease on a wide range of plant species, including tomato (*Solanum lycopersicum*). The host signals that trigger fungal infection are currently unknown. A chemotropic response of *F. oxysporum* towards tomato root exudate was observed using a plate assay that measures directed growth of fungal germ tubes towards chemoattractants. To purify the chemoattractant compound(s) from tomato root exudate, we applied a series of purification methods including extraction with organic and inorganic solvents, fractionation by size exclusion and ion exchange chromatography. The compound(s) showing chemoattractant activity were found in the hydrolipidic fraction, had a molecular weight between 30 and 50 kDa and were sensitive to boiling and treatment with protease K, suggesting that they correspond to one or several secreted tomato proteins. Polycrystalamid gel electrophoresis of the active fraction revealed multiple protein bands of the expected size, two of which displayed a chemoattractant activity when eluted from the gel. Identification of the active protein(s) by LC-ESI-MS is currently ongoing. Identification of the secreted chemoattractant(s) from tomato roots will advance our understanding of the molecular events that trigger fungus-root interactions.


Infectious growth of fungal pathogens is controlled by environmental cues, including nutrient status. The soilborne fungus *Fusarium oxysporum* produces vascular wilt disease in more than a hundred different crop species and can cause lethal systemic infections in immunodepressed humans. Previous work showed that the preferred nitrogen source ammonium causes repression of infection-related processes in *F. oxysporum* that could be reversed by rapamycin, a specific inhibitor of the conserved protein kinase TOR. Here we generated mutations in upstream components that should result in constitutive activation of TOR, including null mutants in tuberous sclerosis complex 2 (TSC2), a small GTPase that represses TOR activity, and a dominant activating allele of the small GTPase Rag (Q86L), an activator of TOR. The Dsc2 mutants and, to a minor extent, the ragA (Q86L) strains showed defects in hyphal growth and colony morphology on several amino acids, as well as decreased efficiency in cellophane penetration and vegetative hyphal fusion. These phenotypes were exacerbated in Dtscc2RagA double mutants and could be reversed by rapamycin, suggesting that they are caused by hyperactivation of TOR. The mutants caused significantly lower mortality on tomato plants and on larvae of the animal model host *Galleria mellonella*. These results suggest that TOR functions as a negative regulator of fungal virulence on plant and animal hosts.


In the soilborne pathogen *Fusarium oxysporum*, a mitogen-activated protein kinase (MAPK) cascade homologous to the yeast filamentous growth pathway controls invasive growth and virulence on tomato plants. Full phosphorylation of Fmk1 requires the transmembrane protein Msb2, a member of the family of signalling mucus that have emerged as novel virulence factors in fungal plant pathogens. A yeast two-hybrid screen for proteins interacting with the Msb2 cytoplasmic tail identified UreG, a component of the urease enzymatic complex. UreG belongs to a set of accessory proteins needed to activate Apo-urease, which converts urea to yield ammonia and carbon dioxide. The *F. oxysporum* genome contains two structural urease genes, ure1 and ure2. Mutants in ureG or ure1 showed reduced growth on urea as the sole carbon and nitrogen source. Lack of urease activity in the mutants resulted in failure to secrete ammonia and to increase the extracellular pH. The DureG mutants caused significantly reduced mortality on tomato plants and on the animal model host *Galleria mellonella*, while Dure1 mutants only showed reduced virulence on tomato plants. Real-time qPCR analysis of key genes involved in nitrogen uptake and assimilation, as well as in the urea cycle, during infectious growth of *F. oxysporum* in *G. mellonella* revealed increased transcript levels of arginase, which converts arginine to urea. Our results suggest a role for the urease accessory protein UreG in fungal virulence on plant and animal hosts.

In the tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici*, most known effector genes reside on a pathogenicity chromosome that can be exchanged between strains through horizontal transfer. Expression of these effector genes is induced upon infection, but the mechanism by which this is regulated is unknown. We noticed that targeted deletion of the effector genes on the pathogenicity chromosome has a particular low rate of success, when compared to genes on the core genome. Possibly, the pathogenicity chromosome has a more compact (less accessible) chromatin structure. It has been shown that release of chromatin condensation can be a way to regulate gene expression, for example of secondary metabolite gene clusters in *Fusarium* [Reyes-Dominguez et al., FGB 2012]. To test whether DNA methylation in *F. oxysporum* can influence effector expression, knock-outs of HP1 (heterochromatin protein) and DIM2 (DNA methylase) were tested for expression of the effector gene *SIX1*. No differences compared to wild-type were observed. Previously it was shown that expression of *SIX* requires 5′g1, a conserved transcription factor encoded in the core genome. Loss of DNA methylation did, however, also not bypass the requirement of 5′g1 for *SIX1* expression (in *Dhp1Dg1* and *Ddm2Dg1* double mutants). Both DIM2 and HP1 are not required for pathogenicity of *Fusarium oxysporum* f.sp. *lycopersici*, and DNA methylation in this strain in general seems to be very low. To obtain more insight in the regulation of effector gene expression we are currently focussing on the potential targets of the transcription factors encoded on the pathogenicity chromosome itself.

558. Mechanistic investigation of Trichoderma cf. harzianum SQR-T037 mycoparasitism against *Fusarium oxysporum* f. sp. *cubense* 4, (banana wilt disease). Jian Zhang1,2, Ruiyu Zhang1,2, Irina S. Druzhinnina1,4, Qirong Shen1,2. 1) Key Laboratory of Plant Nutrition and Fertilization in Low-Middle Reaches of the Yangtze River, Ministry of Agriculture. 210095, Nanjing, China; 2) Jiangsu Key Lab and Engineering Center for Solid Organic Waste Utilization, Nanjing Agricultural University, 210095, Nanjing, China; 3) Microbiology Group, Institute of Chemical Engineering, Vienna University of Technology Getreidemarkt 9/1665, A-1060 Vienna, Austria; 4) ACIB - Austrian Centre of Biotechnology, Petersgasse 14, 8010 Graz, Austria. 

Besides the effective stimulation of banana growth, the wild strain of *Trichoderma cf. harzianum* SQR-T037 (SQR-T037) is capable to control the soil-born pathogen *Fusarium oxysporum* f. sp. *cubense* 4 (Foc4), the causative agent of banana wilt disease. In this work we focussed on mechanisms involved in the mycoparasitic attack of SQR-T037 on Foc4. In vitro, in dual confrontation assays, SQR-T037 was able to cover (overgrow) the hyphae of Foc4 what resulted in damage of the cell wall of the pray fungus and its death. At early stages of the interactions, when SQR-T037 hyphae started to combat the colony of Foc4, the droplets of the yellowish exudate putatively secreted by SQR-T037 were observed. The GC-MS analysis identified that the exudate contained almitic and stearic acids, several hydrolytic enzymes (mainly chitinases and proteases) and essential amount of H2O2. It allowed us to assume that these compounds play the major role in killing the Foc4. We have detected that hyphae of SQR-T037 indeed accumulated H2O2 when it physically made contact with Foc4 and moreover that H2O2 suppressed Foc4 growth. The results of transcriptomics analysis of genes putatively involved in the mycoparasitic attack through H2O2 or its regulation will be presented.

559. Epichloënin A, a unique siderophore of Epichloë endophytes and its role in restricting fungal growth in planta. Natasha Forester1, Geoffrey A. Lane1, Iain Lamont1, Linda J. Johnson1. 1) Forage Improvement, AgResearch Limited, Palmerston North, Manawatu, New Zealand; 2) University of Otago, Dunedin, New Zealand.

We have previously shown, through characterisation of a non-ribosomal peptide synthetase gene (NRPS), sidN, that the biosynthesis of a novel extracellular ferric iron-chelating siderophore designated as epichloënin A is required for maintaining mutualism of *E. festucae* with its grass host, perennial ryegrass (*Lolium perenne*). We have extended our investigation of the role of fungal siderophores in iron homeostasis through the characterization of other siderophore biosynthetic genes, including sidA which encodes a putative L-ornithine N5-oxyngeenase that catalyses the first enzymatic step in siderophore biosynthesis and sidC, encoding a NRPS siderophore synthetase. Using *sidA*, *sidC* and *sidN* mutants we have discovered that *E. festucae* synthesises two siderophores, epichloënin A that requires SidN and an intracellular siderophore, ferricin (FC) that requires SidC; production of both siderophores is totally abolished in *sidA* mutants. In contrast to *sidN* mutants, *sidC*-infected plants showed very little phenotypic consequences due to loss of FC, and this is likely to be due to functional redundancy of epichloënin A. The levels of iron-bound epichloënin A inside the cell are significantly higher than FC, suggesting that epichloënin A acts both as an extracellular and intracellular siderophore. While investigating the influence of the siderophore mutants in *planta* with hydroponically supplied nutrients, we unexpectedly demonstrated that iron exacerbates rather than complements the *sidN* mutant phenotype. We observed increased in fungal proliferation, production of dense mycelial mats, and increased plant stunting. In infected plants, compared to wild-type (WT), there was also an increased uptake of iron in *sidN* cells by reductive iron assimilation. Analyses in culture indicated that in the *sidN* mutant more iron was stored (in FC and in vacuoles) than in WT fungi, suggesting that *sidN* has increased access to iron relative to WT. However, ultra-structural studies of *sidN* hyphae in stunted plants (that were not associated with the host vasculature) suggested that *sidN* hyphae were often non-viable, implicating resource exhaustion from inappropriate growth and misuse of available iron. We therefore propose that epichloënin A is a multiskating siderophore specialising in iron sequestration to moderate cellular iron supply.

560. Interaction between the saprotrophic fungus Serpula lacrymans and living pine roots. Nils OS Högborg1, Anna Rosling1, Annegret Kohler2, Martin Francis2, Stenlid Jan1. 1) Department of Forest Mycology, BioCenter, SLU, Uppsala, Sweden; 2) INRA, Nancy, France.

Recently it has been shown, with a Comparative genomic perspective, that brown rot and mycorrhiza fungi have evolved from white rot ancestors. Wood is a composite material composed of lignin, cellulose and hemicellulose. White rot fungi are able to degrade all of these components with a combination of carbohydrate active and oxidative enzymes. During the course of evolution brown rot and mycorrhiza have lost most of the genes in these gene families. Nevertheless, brown rot fungi are efficient wood decomposers that degrade cellulose and hemicellulose by means of hydroxyl radical production and remaining carbohydrate active enzymes. The family Boletales includes both brown rot fungi and mycorrhiza and it is tentative to speculate that there has been a parallel evolution of these ecological strategies. Here we test the effect of infecting pine roots with the brown rot fungus *Serpula lacrymans*. The interaction was neutral since plant growth was not stimulated but not reduced either. The fungus formed a mantle around the pine roots but not the Hartig net that is typical for ectomycorrhiza. Fungal gene expression was compared with the wood decay transcriptome. 1250 genes were more than two-fold upregulated compared to a glucose medium control. A large proportion of the upregulated genes (62 %) are unknown. Carbohydrate active genes represent only 3% of this gene set and genes with oxidoreductase activity, including monooxgenases represent 4% of the upregulated genes. This is considerably lower compared to saprotrophic growth on wood where carbohydrate active enzymes accounted for 26% and oxidative enzymes for 19% which dominated the gene expression on wood. Gene expression for genes involved in transportation was about the same, around 10% in this experiment and under wood decomposition. Several genes that indicate an interaction with a host were also upregulated. In conclusion, gene expression was markedly different between a glucose medium, wood decomposition and growth on pine roots. This may be a signal of symbiosis, the effect on pine seedling growth was neutral. Thus we cannot conclude if the interaction is beneficial or negative to the host.

561. You turn me on: *Pyrenophora tritici-repentis* genes differentially regulated early during infection of wheat. V. A. Manning1, I. Pandelova2, L. M.
FULL POSTER SESSION ABSTRACTS

Ciuffetti1,2, 1) Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331; 2) Center of Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331.

Pyrenophora tritic-repentis (Ptr) is a necrotrophic fungal pathogen of wheat causal to the disease tan spot and host-selective toxins (HSTs) produced by Ptr are the primary factors that contribute to virulence. One of these HSTs, Ptr ToxA, is a necrosis-inducing, proteinaceous HST that is also present in the wheat pathogen Stagonospora nodorum. Ptr ToxA is a chlorosis-inducing, proteinaceous HST produced by Ptr with active orthologues expressed in the brome grass pathogen, Pyrenophora bromi. Despite the presence of active, orthologous HSTs produced by other fungi, Ptr appears to have an advantage in some wheat growing regions in the world, surpassing in disease relevance the other necrotrophic pathogens that contain orthologous HSTs. To begin to understand the molecular mechanisms that underlie this advantage, we used RNA-seq-based transcriptome analysis to identify Ptr genes that are differentially regulated in planta early in the disease cycle (at thirty hours post-infection) as compared with those genes that are expressed in culture. Functional annotations of the differentially expressed-in-planta transcripts reflect the requirements of the pathogen for host penetration, cell wall degradation and the need to counteract the host response to infection; these include CAZymes, peptidases, transporters, and loci with predicted oxidoreductase activities including peroxidases. In addition, putative secondary metabolite clusters and Ptr-specific proteins are also differentially regulated. These findings provide the basis for understanding the roles of these proteins in virulence and the possibility of revealing common transcriptional regulatory elements activated by interactions with the host.


Heterobasidion annosum sensu lato (s.l.), is a devastating root rot pathogen on conifers present all over the northern hemisphere that causes losses of 500 of million Euro per year for forest owners. The H. annosum s.l. consists of five phylogenetic distinct species with different but overlapping host preferences. The genome sequence of one isolates from H. irregulare and H. occidentale is preferentially infecting pine and spruce species, respectively have been obtained. Analysis of the progeny of a genetic cross of the sequenced isolates resulted in a genetic linkage map of 15 groups representing almost the complete chromosome set-up. These groups have been aligned and anchored to the physical map of H. irregulare. Quantitative trait loci (QTL) for virulence on one-year-old Pinus sylvestris and two-year-old Picea abies seedlings were identified and positioned on the map allowing a straight forward identification of virulence candidate genes. Gene content and sequence divergence of the QTL regions will be presented. Detailed expression analysis of virulence candidate genes with Q-PCR and protein localisation with immunohistochemistry will deduce their role during infection.


In this study we present a new method to obtain the Trichoderma strains with enhanced antifungal and biocontrol activities. The method is based on the increase synthesis of the mevalonate pathway products. In this pathway some metabolites are produced such as terpenoids, trichodermin, harzianum A, mycotoxin T2, ligoren, ergokoin A and B and viridin which are known from their antifungal and antibacterial activities. All these compounds are synthesized from farnesyl pyrophosphate (FPP) which is itself synthesized from farnesyl pyrophosphate synthase encoded by ERG20 gene. FPP is also a substrate for dolichol and ergosterol production which are indirectly engaged in antimicrobial action. In this study we increased production of FPP in T. atroviride by overexpression of the yeast ERG20 gene. We based on the assumption that the increased activity of FPP synthase would stimulate production of all products of the mevalonate pathway. Five transformants showed higher activity of FPP synthase. Simple screening of the hydrolytic properties of the ERG20 transformants revealed that they grew faster on plates with polycarbohydrates as carbon sources. Detailed studies showed higher cellulolytic and chitinolytic activity of enzymes secreted to the cultivation medium by the transformed strains. Antifungal activity was examined by cultivation of plant pathogen Rhizoctonia solani in the atmosphere of volatiles liberated by the transformants and the control. We also analyzed growth of Pythium ultimum on plates which were previously overgrown by Trichoderma strains and were filled with their metabolites. Both experiment showed significantly stronger inhibition of growth of the pathogens by the transformed strains compared to the control. Since the above experiments revealed enhanced antifungal properties of the transformed strains we performed plant tests using the bean Phaseolus vulgaris L. Transformed strains increased both, the germination rate and the size of plants growing in soil infected by Pythium ultimum compared to the control strain. To conclude, an increased activity of the mevalonate pathway caused higher activity of hydrolytic enzymes and increased production of volatiles and secondary metabolites and that way boosted antifungal and biocontrol activities of the Trichoderma ERG20 transformants.

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564. The life history of Ramularia colly-cyni. Maciej Kaczmarek1,2,1) James Fountaine3, Adrian Newton3, Nick Read4, Neil Havis1. 1) Crop and Soil Research, Scotland’s Rural College, Edinburgh, United Kingdom; 2) Institute of Cell Biology, University of Edinburgh, Edinburgh, United Kingdom; 3) Cell and Molecular Sciences, The James Hutton Institute, Dundee, United Kingdom.

The filamentous fungus Ramularia colly-cyni causes the late season disease of spring and winter barley called Ramularia Leaf Spot (RLS). It has become an increasingly important problem for European farmers in the past decade and has recently been reclassified as a major disease of barley in the UK. The lack of apparent varietal resistance to the disease has led to significant amounts of fungicide being applied to crops in north western and central Europe in order to maintain green leaf area and prevent significant yield loss. These factors have contributed to an increasing focus on achieving a better understanding of the fundamental biology of this elusive pathogen in order to develop more successful strategies of RLS management. Therefore, disease development throughout the life cycle of the host barley plant has been analysed by the employment of transgenic R. colly-cyni isolate, expressing the GFP reporter molecule, and confocal microscopy. We have been able to examine the previously uncharacterised seed-borne stage and illustrate the mode of fungal transmission into barley seedlings. We have also analysed the potential sexual reproduction in the fungus by utilizing a range of correlative techniques, such as cryo-scanning electron microscopy, confocal microscopy and light microscopy. Here we describe for the first time the nature of speculated spermogronal stage called Asteromella and in addition, present preliminary evidence suggesting the existence of a perfect stage that, if functional, could resemble closely related Mycosphaerella species.

565. Mechanical stress sensing in Epichloë fungal symbionts during colonization of grasses. Kahandawa G.S. U Ariyawansa2, Rosie E. Bradshaw2, Neil A.R. Gow3, Nick D. Read4, Richard D. Johnson5, Duane P. Harland5, Christine R. Voisey1. 1) AgResearch, Grasslands Research Centre, Palmerston North, New Zealand; 2) BioProtection Centre, Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand; 3) School of Medical Sciences, University of Aberdeen, United Kingdom; 4) Institute of Cell Biology, University of Edinburgh, United Kingdom; 5) AgResearch, Lincoln Research Centre, Christchurch, New Zealand.

Epichloë festucae is an agronomically-important endophytic fungus that grows symbiotically within the intercellular spaces of temperate grass species
such as *Lolium perenne* and *L. arundinaceum*. Colonization of host seedlings by *E. festucae* occurs when hyphae in the shoot apex invade developing host leaves and extend via intercalary hyphal growth, a highly unusual mechanism of division and extension in non-apical compartments. We hypothesize that intercalary hyphal growth is stimulated by mechanical stretch imposed by attachment of hyphae to elongating host cells, and that this stress is sensed by mechano-sensors located on the hyphal membranes. Genome analysis revealed that homologues of known mechano-sensors in *Saccharomyces cerevisiae* such as Mid1 (a stretch activated calcium ion channel), Wsc1 and Mid2 (cell wall integrity sensors) are present in the *E. festucae* genome. Gene replacement studies of *mid1* and *wsc1* in *E. festucae* reduced radial growth rate in axenic culture confirming the role of both genes in hyphal growth. In axenic culture both *DsftA* and *Dmid1* mutants were sensitive to fungal cell wall modifiers such as Calcofluor White, supporting their role in cell wall integrity. Preliminary plant infection studies with *DsftA* and *Dmid1* mutants revealed a hyper-branched unsynchronized growth pattern within the host (*Lolium perenne*), and *Dsft1* also caused severe stunting in most plants suggesting a disruption in the symbiosis. A technique to stimulate intercalary growth under *in-vitro* conditions through mechanical stretch is being optimised to test the ability of Mid1, Wsc1 and Mid2 to sense mechanical stress and initiate intercalary growth.

566. *Aspergillus flavus* hypertrophy and hyphal entry by *Ralstonia solanacearum* is mediated by bacterial type three secretion system function. **Joe E Spraker**, Nancy P Keller. 1) Plant Pathology, University of Wisconsin Madison, Madison, WI; 2) Bacteriology, University of Wisconsin Madison, Madison, WI.

Fungi and bacteria are two of the primary pathogens of plants, often infecting the same crops, however shockingly little is known of how these organisms interact independently of plant hosts. In examining the interaction between two economically important pathogens of peanut, *Aspergillus flavus* and *Ralstonia solanacearum*, a bacterium and a fungus, respectively, we’ve shown that fungal hypertrophy is induced and that the bacterium is capable of entering these cells. The hypertrophic cells were imaged using calcofluor staining to show chitin cell wall structure. Bacterial invasion of these structures was demonstrated using confocal microscopy of GFP labeled bacteria. Further, we demonstrate that bacterial mutants deficient in type three secretion systems are incapable of eliciting the fungal hypertrophic response by culturing virtually isogenic bacterial type three secretion mutants. This is the first report of a well-known plant pathogenic bacterium eliciting fungal hypertrophy and invading hyphal cells. Current research is aimed at finding bacterial effectors that may be facilitating this interaction and elucidating their mode of action.


Vegetative hyphal fusion establishes the interconnection of individual hyphal strands into an integrated network of a fungal mycelium. It is suspected that vegetative hyphal fusion plays many important roles such as in nutrient translocation, intramycelial signaling, and emergence of genetic diversity via horizontal gene transfer or interspecific hybridization. However, experimental support for these suspected roles is still largely lacking. To investigate the role of hyphal fusion in fungal endophytes of *epichloae*, which form mutualistic symbiosis with grass hosts, we generated mutant strains lacking *sftA*, an ortholog of the hyphal fusion gene so in *Epichloë festucae*. The *E. festucae DsftA* mutant strains grew like the wild-type strain in culture but with reduced aerial hyphae, and completely lacked hyphal fusion. The most striking phenotype of the *E. festucae DsftA* strain was that it failed to establish a mutualistic symbiosis with the tall fescue plant host (*Lolium arundinaceum*), and instead, killed the host plant within two months after initial infection. This suggests that hyphal fusion may have an important role in the establishment/maintenance of fungal endophyte-host plant mutualistic symbiosis. To further investigate the importance of hyphal fusion in *epichloae*, frequency of hyphal fusion was quantified in different *epichloae* endophytes including sexual isolates, asexual interspecific hybrids and asexual non-hybrids. A majority of sexual *epichloae* underwent frequent hyphal fusion, whereas hyphal fusion was less frequently found in asexual *epichloae*. Moreover, hyphal fusion was less common in asexual non-hybrid *epichloae* compared to asexual hybrids. Thus, it appears that the ability to undergo hyphal fusion correlates with the presence of the sexual cycle, and the hybrid status of *epichloae* endophytes. Overall, our data provide evidence for the importance of hyphal fusion in establishment/maintenance of mutualistic symbiosis, and evolution of *epichloae* endophytes.

568. Oxygen and the stomatal cue: Dissecting stomatal tropism in *Cercospora zeae-maydis*. **R. Hirsch, B. Bluhm.** Department of Plant Pathology, University of Arkansas Division of Agriculture, Fayetteville, AR.

*Cercospora zeae-maydis* causes grey leaf spot of maize, one of the most widespread and destructive foliar diseases of maize in the world. Stomatal infection is a critical, yet poorly defined, component of pathogenesis in *C. zeae-maydis*. At the onset of infection, the fungus senses and grows towards maize stomata, and then breaches the leaf surface by producing appressoria over stomatal pores. Directed growth toward distant stomata during infection led us to hypothesize that *C. zeae-maydis* responded to an unknown chemical cue emanating from stomata. To elucidate mechanisms underlying infectious development in *C. zeae-maydis*, particularly stomatal tropism, a series of histological experiments were performed with epi-florescent and confocal microscopy. Upon sensing maize stomata, *C. zeae-maydis* either reoriented hyphal tip growth towards stomata, or initiated new hyphae originating from right-angle branches in close proximity to stomata. Hyphae exhibiting stomatal tropism were linear and lacked branches. On topographically accurate acrylic leaf replicas, *C. zeae-maydis* did not display stomatal tropism and failed to form appressoria upon encountering artificial stomata, which indicated that thigmotrophic cues were not sufficient to elicit pre-penetration infectious development. However, in non-host interactions, *C. zeae-maydis* exhibited stomatal tropism and retained the ability to form appressoria over stomata, which suggested that a chemical cue emanating from stomata elicited a chemo tropic response in the fungus. Stomatal tropism and appressoria formation in *C. zeae-maydis* were impaired when atmospheric oxygen levels were disturbed, implicating the role of oxygen sensing in pathogenicity. This study characterized stomatal tropism during infection of maize by *C. zeae-maydis*, directly implicated oxygen sensing as a component of pathogenicity, and provides a quantitative framework through which to study fungal pathogenesis and host/pathogen interactions in related systems.


The fungal symbiont, *Epichloë festucae*, colonises leaves of host grasses by ramifying through the shoot apical meristem (SAM) of the seedling, and then infecting the leaf primordia. Hyphal infection of the SAM is dependent on apical growth, however after primordia have formed, leaf tissues undergo a phase of intercalary expansion, which the fungus, attached to host cells, must recapitulate to remain intact. *E. festucae* hyphae entering the leaf expansion zone switch from apical to intercalary growth, and extend in synchrony with the host until the leaf tissues mature. How colonising symbiotic fungi accommodate the complexities of the plant developmental programme is currently unclear. Since CAMP signalling is often required for host colonisation by fungal pathogens, we disrupted the CAMP cascade by insertional mutagenesis of the *E. festucae* adenylyl cyclase gene (acyA). Consistent with reports
on other fungi, disruption mutants had a slow radial growth rate in culture, and colonies were highly compact relative to controls. Furthermore, the hyphae were convoluted and hyper-branching suggesting that apical dominance had been disrupted. Nitro blue tetrazolium staining of hyphae showed that cAMP disruption mutants were impaired in their ability to synthesise superoxide indicating that cAMP signalling is important for the production of ROS in culture in this species. This defect was reversed by re-insertion of a functional wild type acylA gene into mutant strains. Despite significant defects in hyphal growth and ROS production in culture, E. festucae DacylA mutants were infectious and capable of forming symbiotic associations with grasses, albeit at a lesser infection frequency than wild type. Plants infected with E. festucae DacylA mutants were indistinguishable from controls. However, as in culture, microscopic evidence showed that the mutant strains within the host were hyper-branching, and host tissues heavily colonised, indicating that the tight regulation over hyphal growth normally observed in developing and mature host tissues requires a functional cAMP signalling cascade. Further research is currently underway to understand how cAMP affects the hyphal growth transitions undertaken during host colonisation, particularly at the level of the cell cytokkeleton and hyphal cell wall synthesis.

570. Role of VCP1 and SCPI proteases in the mutlitrophic behaviour of the nematophagous fungus Pochonia chlamydosporia. Nuria Escudero1, Christopher R. Thornton2, Luis Vicente Lopez-Lorca3. 1) Laboratory of Plant Pathology, Multidisciplinary Institute for Environment Studies (MIES) Ramón Margalef. University of Alicante, Alicante, SPAIN; 2) Food Security and Sustainable Agriculture, Biosciences, College of Life & Environmental Sciences, University of Exeter, Exeter. UK. Pochonia chlamydosporia (Goddard) Zare and Gams is a fungal parasite of female nematodes and eggs, which has been widely studied as a biological control agent of cyst and root-knot nematode egg-shells. The nematode egg-shell is formed by several layers, including a chitinous layer composed of a protein matrix embedding chitin microfibrils. Extracellular enzymes, such as serine proteases (e.g. VCP1), secreted by egg-parasitic nematophagous fungi are known to play an important role in egg infection. SCPI, a recently reported serine carboxypeptidase from P. chlamydosporia was found during plant root endophytic colonisation by the fungus, its role in eggs parasitism is unknown. We have investigated the role of VCP1 and SCPI proteases in the mutlitrophic behaviour of the nematophagous fungus Pochonia chlamydosporia using immunological approaches using antiVCP1 and SCPI polyclonal antibodies, these were raised against synthetic peptides of both proteases. ELISA and immunofluorescence have confirmed the production of both proteases when Meloidogyne javanica eggs were used as inducer. P. chlamydosporia under starvation condition (water) also expressed both proteases. It seems that the signal of SCPI was more intense than of VCP1 under most conditions tested (eggs, protein substrate and starvation). Using proteomic, chitosan was previously found in our lab to induce VCP1 in P. chlamydosporia liquid cultures. Consequently, we have also evaluated the amount of VCP1 and SCPI in media with chitosan, to quantify the production of these proteases under multitrophic conditions. This study is casting light into the molecular aspects of the multitrophic behaviour of P. chlamydosporia. This will help to understand the biocontrol potential of the fungus and open new biotechnological applications.

571. Cellular development integrating primary and induced secondary metabolism in the filamentous fungus Fusarium graminearum. Jon Menke1, Jakob Weber2, Karen Broz3, H. Corby Kister2,3. 1) Department of Plant Pathology, University of Minnesota, St. Paul, USA; 2) Molekulare Phytopathologie, Universität Hamburg, Germany; 3) USDA ARS Cereal Disease Laboratory, St. Paul, MN, USA. Several species of the filamentous fungus Fusarium colonize plants and produce toxic small molecules that contaminate agricultural products, rendering them unsuitable for consumption. Among the most destructive of these species is F. graminearum, which causes disease in wheat and barley and often contaminates the grain with harmful trichothecene mycotoxins. Induction of these secondary metabolites occurs during plant infection or in culture in response to chemical signals. Here we report that trichothecene biosynthesis involves a complex developmental process that includes dynamic changes in cell morphology and the biogenesis of novel subcellular structures. Two cytochrome P-450 oxygenases (Tri4p and Tri1p) involved in early and late steps in trichothecene biosynthesis were tagged with fluorescent proteins and shown to co-localize to vesicles we call “toxosomes.” Toxosomes, the inferred site of trichothecene biosynthesis, dynamically interact with motile vesicles containing a predicted major facilitator superfamily protein (Tri12p) previously implicated in trichothecene export and tolerance. The immediate isoprenoid precursor of trichothecenes is the primary metabolite farnesyl pyrophosphate. When cultures are shifted from non-inducing to trichothecene inducing conditions, changes occur in the localization of the isoprenoid biosynthetic enzyme HMGC CoA reductase. Initially localized in the cellular endomembrane system, HMGC CoA reductase increasingly is targeted to toxosomes. Metabolic pathways of primary and secondary metabolism thus may be coordinate and co-localized under conditions when trichothecene synthesis occurs.

572. DNA double-strand breaks generated by yeast endonuclease I-Sce I induce ectopic homologous recombination and targeted gene replacement in Magnaportha oryzae. T. Arzato1,2, T. Younoumaru1, S. Ohsato1,2, T. Arie3, S. Kuwata1. 1) Meiji University, Kanagawa, Japan; 2) Tokyo University of Agriculture and Technology, Tokyo, Japan. The filamentous fungus Magnaportha oryzae causes the rice blast disease that is one of the most destructive fungal diseases of cultivated rice plants. To control this fungal disease, many resistant genes have been introduced into cultivated rice germplasm, however, breakdowns of the resistance often occur within several years by rapid evolution of the fungus. Therefore, studies on the evolutionary mechanisms of the fungus are important for elucidation of the rapid evolution. We set out a novel detection/selection system of DNA double-strand breaks (DSBs)-mediated ectopic homologous recombination (HR) that is one of the evolutionary mechanisms. The system consists of two nonfunctional yellow fluorescent protein (YFP)/blasticidin S deaminase (BSD) fusion genes as a donor and a recipient, and a yeast endonuclease I-Sce I gene as a DSB-inducer. In this system, ectopic HR can be detected and selected by restorations of YFP fluorescence and blasticidin S (BS)-resistance at a single cell level. These donor and recipient genes were simultaneously integrated into the M. oryzae genome and transformed lines were isolated. In the absence of the DSB-inducer, transformed lines showed relatively low frequencies of HR events (>2.1%). On the other hand, by integration of the DSB-inducer gene into transformed lines, we could observe the frequencies of DSB-mediated HR raising up to ~40%. This result clearly showed that DSB into a certain gene induce ectopic HR events between the gene and its homologs. Accordingly, we further applied I-Sce I mediated DSB for TGR in M. oryzae. To detect TGR, we constructed simple system using donor and recipient genes. The recipient gene was integrated into the M. oryzae genome and transformed lines were isolated. To recipient gene integrated lines, the donor gene was introduced and restorations of YFP fluorescence and BS-resistance were evaluated. As we expected, the TGR frequencies were increased at least 37-folds by I-Sce I co-transformation as compared with those obtained without I-Sce I. This result provides a new method using DSB for improving the TGR frequency in M. oryzae. Taken together, it is strongly suggested that DSBs can drive genomic rearrangement and accelerate pathogenic variability in M. oryzae through the ectopic HR between homologous sequences such as transposable elements and avirulence genes.

573. Investigation of the Magnaportha oryzae proteome and phosphoproteome during appressorium formation. William L. Franck1, Emine Gokce2, Yeonjee Oh2, David C. Muddiman2, Ralph A. Dean1. 1) Plant pathology, NC State University, Raleigh, NC; 2) W.M. Keck FT-ICR Mass Spectrometry
Magnaporthe oryzae, the causative agent of rice blast disease, infects plant leaves via formation of an appressorium which facilitates penetration of the leaf surface. In an effort to better understand the physiological changes accompanying the earliest stages of infection-related development, a nano-LC MS/MS-based global proteomics examination of conidial germination and cAMP-induced appressorium formation was undertaken at four distinct developmental time points resulting in the identification of 3200 proteins. Label free quantification by spectral counting identified 591 proteins whose relative abundance changed during germination. Furthermore, treatment of germinating conidia with cAMP to induce appressorium formation resulted in the identification of 493 proteins whose relative abundance changed compared to untreated samples. In developing appressorium, changes in cell wall modifying, transport, extracellular and plasma membrane localized proteins were observed. Proteomic analysis of a M. oryzae cAMP-dependent protein kinase A (cpkA) mutant defective in appressorium formation following treatment with cAMP identified a subset of proteins whose regulation is dependent on cAMP signaling. A comparison of proteome and transcriptome data revealed little correlation between protein and transcript regulation. Finally, to better define the role of protein phosphorylation and the CPKA protein kinase during the development of appressorium, the phosphoproteome of M. oryzae is being investigated. To date, a total of 980 phosphoproteins have been identified from conidia and experiments designed to identify changes in protein phosphorylation during appressorium formation are in progress.

574. Characterization of the binding site and downstream targets of the MST12 transcription factor in Magnaporthe oryzae. Guotian Li, Guanghui Wang, Jin-Rong Xu. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN.

Rice blast caused by Magnaporthe oryzae is one of the most devastating diseases on rice. In M. oryzae, appressorium formation is regulated by PMK1 mitogen-activated protein kinase (MAPK) pathway. Its orthologs are conserved a wide array of plant pathogenic fungi for regulating different plant infection processes. In M. oryzae, one of the transcription factors functioning downstream from Pmk1 is Mst12 that is essential for appressorium penetration and invasive growth. The MST12-GFP transformant showed strongest GFP signals in appressoria and invasive hyphae. Pmk1 weakly interacted with Mst12 in yeast two-hybrid assays. Overexpression of MST12 failed to complement the defects of the pmk1 mutant, suggesting that the activation of Mst12 by Pmk1 was required for its function. Site-directed mutagenesis analysis indicated that the MAPK-docking region and phosphorylation site were essential for the function of MST12. However, mutant alleles of MST12 with point mutations in any one of the two tandem zinc finger domains were still partially functional, indicating their overlapping or redundant functions. Expression of MST12 functionally rescued the invasive growth defects of the yeast ste12 mutant and EMSA analyses suggested that Mst12 had a binding site similar to the PRE sequence recognized by Ste12. During appressorium formation, transcription levels of 222 genes were found to be significantly altered in the mst12 mutant (P<0.005). Ten of these downstream targets of MST12 with putative PREs in their promoters were selected for functional characterization. Data on phenotype characterization of the resulting mutants and promoter element analysis will be presented.

575. CorA Magnesium transporters are key regulators of growth and pathogenicity in the rice blast fungus M. oryzae. Md Hashim Reza, J. Manjrekar, Bharat B. Chattoo. Microbiology and Biotechnology Centre, Genome Research Centre, MS University, Baroda, Vadodara, India.

Magnaporthe oryzae, the causative organism of rice blast disease, infects cereal crops and grasses at various stages of plant development. Thus, a comprehensive understanding of the fungal metabolism and its implications on pathogenesis is a must to counter this devastating crop disease. As a cofactor for functionally diverse enzymes, Mg2+ regulates a variety of biological processes. Magnesium transporters have been shown to be essential for viability of S. cerevisiae and some bacterial pathogens. We present the molecular identity, function and regulation of magnesium (CorA) transporters and their role in growth and pathogenicity of the filamentous fungal pathogen, M. oryzae. Complementation analysis in S. cerevisiae showed that magnesium transporters, MoALR2 and MoMNR2, have overlapping functions. Further, we have shown that reduction of Mg2+ transporters using a knock-down approach leads to decrease in intracellular magnesium levels, indicating that these transporters play a crucial role in Mg2+ homeostasis of M. oryzae. Functionally, the mutants display defects in surface hydrophobicity, cell wall stress tolerance, sporulation, appressorium (infective structure) formation and infection, making MoALR2 and MoMNR2 indispensable for growth and pathogenesis of the rice blast fungus M. oryzae. MoMPS1 (MAPK) and MoMPK1 (MAPK) driving signaling pathways have been shown to be essential for cell wall integrity, conidogenesis, appressorium formation and invasive growth. Expression analysis by qPCR of MoMPS1 (MAPK) and MoMPK1 (MAPK) signalling pathways, core to the above mentioned phenotypes, showed deviation from the wild type. We also detected corresponding differences in downstream MoMPS1 driven Cell Wall Integrity (CWI) signalling and the MoPMK1-regulated hydropophin, MoMPG1 expression.


The avirulence gene AVR-Pia, which induces hypersensitive reaction (HR) of rice cultivars with the resistance gene Pia was isolated from Magnaporthe oryzae strain Ina168 (Miki et al., 2009). The qRT-PCR analysis revealed that AVR-Pia expression was started from 24 hours post inoculation on the rice leaf. Furthermore, AVR-Pia protein localization to biotrophic interfacial complex (BIC: Kiang et al., 2010) was observed using AVR-Pia::eGFP fusion protein in compatible rice leaf sheath cells. On the other hand, the functional detail of AVR-Pia protein during infection was not understood. In order to analyze the function of AVR-Pia protein, the recombinant AVR-Pia protein was purified from E. coli. Recombinant AVR-Pia was revealed to induce HR-like browning spots when it was infiltrated into Pia rice leaf, suggesting the activity to trigger the host’s resistance reaction. An anti-AVR-Pia antibody was prepared with the recombinant protein, and its validity was investigated by Western blotting. Native AVR-Pia was detected from total soluble protein extracted from inoculated pia rice leaf sheath, and the MW of AVR-Pia was estimated as 7.4 kDa, corresponding to AVR-Pia w/o signal peptide. This result suggested that recombinant AVR-Pia has similar structure to the native AVR-Pia, and confirmed the affinity of antibody to native AVR-Pia. Quantification of secreted AVR-Pia by M. oryzae during infection was performed with anti-AVR-Pia. It was revealed that approx. 1.5 ppm (relative to total soluble protein of Ina168 inoculated rice sheath) of AVR-Pia was secreted during infection. These facts suggest that the Pia rice can recognize AVR-Pia at lower concentration than 1.5 ppm and trigger the immune response at the early stage of blast infection.


AVR-Pia, an avirulence gene in the genome of the rice blast fungus Magnaporthe oryzae, triggers a hypersensitive reaction in rice cultivars harboring the resistance gene Pia. The copy number of AVR-Pia was revealed to vary from one to three among M. oryzae isolates avirulent to Pia rice, and three copies of the gene were located on a single chromosome in strain Ina168, from which the gene was originally cloned. The spontaneous AVR-Pia mutant originated from Ina168m95-1, which lacks the AVR-Pia gene, and was therefore used to elucidate the molecular mechanism of the deletion of all three
copies of AVR-Pia. Screening and analysis of cosmid clones indicated that two copies of the DNA-type transposon Occan (Occan\textsuperscript{ns-1} and Occan\textsuperscript{ns-2}) were located on the same chromosome and three copies of AVR-Pia were located in between the two Occan elements. Ina168m95-1 contains a conserved Occan element, named Occan\textsuperscript{ns-1}, between sequences homologous to the 5'-flanking region of Occan\textsuperscript{ns-4} and the 3'-flanking region of Occan\textsuperscript{ns-1}. In addition, sequence polymorphisms indicated a homologous recombination between Occan\textsuperscript{ns-2} and Occan\textsuperscript{ns-2} which resulted in Occan\textsuperscript{ns-4}. Based on these observations, we propose the hypothesis that homologous recombination in the two Occan elements leads to the deletion of AVR-Pia in Ina168m95-1.

578. The interactome of pathogenicity factors in the rice blast fungus *Magnaporthe oryzae*. Xiaoying Zhou\textsuperscript{1
,} Yang Li\textsuperscript{1
,} Keerthi Jayasundera\textsuperscript{2
,} Anton Iluka\textsuperscript{3
,} Andy Tao\textsuperscript{3
,} Jinrong Xu\textsuperscript{1
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Rice blast is a disease of significant economic impact worldwide and a model system for studying fungal-plant interactions. To date, over 100 pathogenicity factors have been identified in *Magnaporthe oryzae*. However, there is only limited knowledge about their relationships. To better understand molecular mechanisms regulating plant infection processes, it is critical to identify plant-protein interaction networks important for pathogenesis. In this study, we characterized the interactome of selected pathogenicity-related proteins. The affinity purification and proteomics approaches were used to identify proteins that interact with over 60 known pathogenicity factors, including components of important signaling pathways. Protein-protein interaction maps were established for these pathogenicity factors based on affinity purification data and information about their orthologs in yeast. Co-immunoprecipitation, BifC, or yeast two hybrid assays were used to verify the interactions of selected genes. For a number of pathogenicity-factor-interacting genes, gene knock-out mutants were generated to determine their functions in pathogenesis. To our knowledge, results from this study represent the first study of protein-protein interaction networks of pathogenicity factors in plant pathogenic fungi in *M. oryzae*.

579. Interaction between phenolic and oxidant signaling in *Cochliobolus heterostrophus*. Benjamin A Horwitz\textsuperscript{1
,} Samer Shalaby\textsuperscript{1
,} Olga Larkov\textsuperscript{2
,} Mordechai Ronen\textsuperscript{2
,} Sophie Lev\textsuperscript{2
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The transcription factor ChAP1 is an ortholog of yeast YAP1 in the maize pathogen *Cochliobolus heterostrophus*. ChAP1 migrates to the nucleus upon exposure to oxidative stress, inducing antioxidant genes such as thioredoxin and glutathione reductase [1]. ChAP1 also localizes to nuclei on contact with the leaf and during invasive growth. Though reactive oxygen species are encountered on the host, ChAP1 nuclear retention can occur without oxidative stress. One of the signals responsible is provided by phenolic compounds [1-3]. Using a genetically-encoded ratiometric reporter of the redox state, we showed that leaf extract and phenolics, despite their antioxidant properties, promote nuclear accumulation of ChAP1. To study this dual role of ChAP1 we identified genes expressed in response to phenolics. Intradial oxidogenase CCHD1 is rapidly upregulated, independent of ChAP1 [2]. Coumaric acid caused rapid and simultaneous upregulation of most of the b-ketoadipate pathway genes. Deletion of CCHD1 provided genetic evidence that protocatechueic acid is an intermediate in catabolism of many aromatics [3]. The activity of a structure series showed complementary requirements for upregulation of CCHD1 and ChAP1 nuclear retention. The ability to metabolize a compound and ChAP1 nuclear retention are inversely correlated. To find additional genes induced by phenolics, microarrays designed from the predicted coding sequences of the *C. heterostrophus* genome [4] were hybridized to probes made from RNA of cultures exposed to coumaric acid, or controls. Expression of about 90 genes from different pathways primarily for metabolism, for example, the b-ketoadipate, quinic acid and shikimic acid pathways, as well as transporters from different families was altered in response to coumaric acid. The ability to respond to phenolics and detoxify or metabolize them via the b-ketoadipate pathway confers an advantage to plant pathogens, and explains the presence of at least two response pathways detecting these compounds. [1] Lev et al. (2005) Eukaryot. Cell 4:434-454; [2] Shanmugam et al. (2010) Cell. Microbiol. 12:1421-1434; [3] Shalaby et al. (2012) PMPM 25: 931-940; [4] Ohm et al. (2012) PLoS Pathog 8: e1003037. Supported in part by the Israel Science Foundation. We thank Michal Levin and Itai Yanai for help with microarray hybridization.

580. Mode of Action of Chitosan: Antifungal and Gene Modulator from Natural Origin. Luis V. Lopez-Lorca, Laboratory of Plant Pathology, Department of Marine Sciences and Applied Biology, Multidisciplinary Institute for Environmental Studies (MIES) Ramon Margalef. University of Alicante, E-03080 Alicante, Spain. email: lvlopez@ua.es.

Chitin is an abundant, easily obtained and renewable natural polymer, second only to cellulose. Chitin is a main structural component of barriers (cuticles and cell walls) of invertebrates (crustaceans, insects and nematodes) and fungi. Its deacetylated form, chitosan, has higher solubility and is known to have interesting biological properties. Chitosan, as a polycation, permeabilises the fungal membrane in an energy-dependent manner. Chitosan kills or compromises the growth of important plant and human fungal pathogens. Unlike these fungi, fungal parasites of invertebrates (FPI, mainly nematopathogenic and entomopathogenic fungi), widely used biological control agents in sustainable agriculture, are resistant to chitosan. Perhaps as a result of coevolution with their hosts, FPI have evolved chitosan-resistant low-fluidity membranes (high content of saturated FFA) and produce efficient chitosan degrading enzymes. Besides, chitosan activates fungus development (e.g. conidiation) and expression of FPI pathogenicity factors such as serine proteases involved in the degradation of host barriers. Using chemogenomic platforms with yeast (sensitive to chitosan) we have identified chitosan putative gene targets. One of them, ARL1, a member of the Ras superfamily that regulates membrane trafficking, confers chitosan sensitivity as a deletion mutant and resistance when overexpressed in yeast. Yeast ARL1 overexpression in the presence of chitosan mainly caused down-regulation of genes involved in cell energy generation (mitochondrial biology, ATP metabolism, energy storage metabolites) and associated by-products (oxidative stress, ROS) and up-regulation of cell cycle progression (mitosis/meiosis, chromatin dynamics and sporulation) genes. Neuraspora crassa conidia germination is particularly sensitive to chitosan. Low nutrient content of media increases chitosan driven membrane permeabilisation and N. crassa sensitivity. Using RNAseq we have found differential expression of genes involved in membrane permeability, cell energy/ROS generation and cell division as a response of *N. crassa* conidia to chitosan. Concluding, we are using cell and molecular approaches to fully understand the multimodal action of chitosan to fully exploit it in biotechnological and health applications.

581. Unraveling the metabolome: how zombie ant fungi heterogeneously control ant brains. Charissa de Bekker, David Hughes. Biology and Entomology, Center for Infectious Disease Dynamics, Pennsylvania State University, State College, PA.

Fungal entomopathogens rely on cellular heterogeneity during the different stages of insect host infection. Their pathogenicity is exhibited through the secretion of secondary metabolites. Infection strategies of this group of environmentally important fungi can thus be studied by analyzing their metabolome. Next to generalists such as *Beauveria bassiana* and *Metarhizium anisopliae*, specialist species exist that are able to control host behavior. One of the most dramatic examples is the death grip of ants infected by *Ophiocordyceps unilateralis*, where ants are being used as a vehicle and finally bite into vegetation before dying, aiding fungal spore dispersal after death. To establish this the fungus must not only overcome the immune system of the host, but also manipulate the brain and atrophy the muscles. To date, most work on manipulation of host behavior has described the ant’s behavior,
leaving the molecular processes from the fungal point of view unresolved. To start unraveling the mechanisms underlying this phenomenon we are combining metabolite profiling with an ex vivo insect tissue culturing system that allows us to study fungal metabolites secreted in different parts within the host. Using this technique we established that B. bassiana and M. anisopliae, and D. unilaterals heterogeneously react to brain and muscle tissue by secreting a significantly different array of metabolites. The combination of these approaches with a concrete understanding of the host-parasite interaction in nature is allowing us to understand both the diversity of secondary metabolites as well as make discoveries regarding the temporal dynamics these fungi employ when releasing metabolites that affect the host. This project is financed by the Marie Curie International Outgoing Fellowships and Penn State University.

582. Gene expression of fungal aldehyde dehydrogenases in ectomycorrhiza. Catarina Henke1,2, Karin Krause1, Erika Kothe1. 1) Friedrich Schiller University of Jena, Institute of Microbiology, Microbial Communication, Neugasse 25, D-07743 Jena, Germany; 2) Max Planck Institute for Chemical Ecology, International Max Planck Research School, Hans-Knöll-Strabe 8, D-07745 Jena, Germany, chenke@ice.mpg.

Ectomycorrhizal fungi form a mutual symbiosis with trees and perform important functions in the ecosystem, particularly improving plant growth, nutrient supply and plant protection against pathogens. The molecular level of the association between the basidiomycete fungus Tricholoma vaccinum and the specific host spruce (Picea abies) is studied to investigate the molecular mechanisms of interaction. Differential display analysis revealed a fungal aldehyde dehydrogenase encoding gene ald1 from the basidiomycete T. vaccinum specifically expressed in ectomycorrhiza during interaction with the compatible host. Ald1 has a key function in the detoxification of alcohols and aldehydes occurring in mycorrhizal biotopes and is involved in production of the phytohormone indole-3-acetic acid. Gene regulation of ald1 is monitored via quantitative RT-PCR analyses. Transcription level was increased in the mutualistic association and could be stimulated by different external supplements, namely alcohols and aldehydes. The ald1 overexpressing mutants generated by Agrobacterium tumefaciens mediated transformation showed increased ethanol stress tolerance. Linkage between gene transcription level and phenotypic characterization will elucidate deeper understanding of biological function, particularly of the possible pathways of auxin synthesis, and will allow better understanding of aldehyde dehydrogenases in ectomycorrhiza.

583. Interaction of ectomycorrhizal fungi with environment. Katrin Krause, Ines Schlunk, Erika Kothe. Institute of Microbiology, Friedrich Schiller University, Jena, Thuringia, Germany.

Ectomycorrhizal fungi play an important role in the biogeochemical cycle, as act as decomposers and environmental indicators. Therefore they interact with different compounds of the environment like secondary metabolites of plant and soil living microorganisms, heavy metals and xenobiotics. Different enzymes and transporters are involved in these processes. One of these transporters, multidrug and toxic compound extrusion (MATE) nme1 of the ectomycorrhizal fungus Tricholoma vaccinum, was upregulated during symbiosis. By heterologous expression of nme1 in Saccharomyces cerevisiae, different metals, xenobiotics and secondary metabolites were identified as substrates for the MATE transporter. Furthermore, a retrotransposon retro showed an upregulation during the symbiosis with spruce. After long-term cultivation of the fungus an additional copy of the retrotransposon was detectable by Southern blot analyses in the fungal genome, showing transposition during co-cultivation with the plant. Since the fungus is prone to experience some plant-derived defense, the induction of transposition might be caused by plant induced stress.

584. Genetic exchange in an arbuscular mycorrhizal fungus; Rhizophagus irregularis. Pawel Rosikiewicz1, Ian Sanders2. 1) Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland Pawel.Rosikiewicz@unil.ch; 2) Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland, Ian.Sanders@unil.ch.

Rhizophagus irregularis is a model species of an arbuscular mycorrhizal fungus (AMF). The AMF forms symbiotic relationship with roots of land plants, improving plant growth and protecting plants against parasites. R. irregularis is a particularly important species of AMF because it colonizes roots of most of crop plants such as rice, potato and wheat. However, different isolates of this fungus can affect plant phenotype differently. Moreover, it recently has been shown that two isolates of AMF can exchange genetic material, a process that can alter both, plant and fungal phenotypes. R. irregularis, is a colonizing fungus, which means that many nuclei coexist and can move in the common cytoplasm. The genetic exchange between two AMF isolates occurs via vegetative hyphal fusion. However, unlike in most fungi AMF produces multinucleate spores and it has been shown that each isolate of R. irregularis carries genetically different nuclei, which are maintained in successive AMF generations. What is unknown is the fate of parental nuclei after the genetic exchange, how many parental nuclei are exchanged and whether the mixture of nuclei is random. In order to answer these questions we performed an in vitro experiment where two isolates of R. irregularis were grown in pairs and allowed to fuse and exchange their cytoplasm. Subsequently, spores from all in vitro cultures were collected and used to establish 215 potentially crossed AMF lines. Each AMF line was established from a single spore. Fifty-seven of this newly produced AMF lines where genotyped, resulting in identification of 40 crossed AMF lines. All genotyped single spore lines carried only one mitochondrial haplotype. Moreover, all the progeny of a given pair of parental AMF isolates received the mitochondria from the same parent.


Hydrophobins are small-secreted proteins with low sequence homology. However, all proteins contain eight cysteines, which form disulfide bridges. There are two classes of hydrophobins, depending on their solubility, which have a broad range of functions such as cell wall integrity, covering conidiospores, adhesion in pathogenic and ectomycorrhiza interactions. Therefore, hydrophobins as well as other genes in a mutual symbiosis are differential expressed. We showed this for hydrophobin tthyd1, which is upregulated in the Hartig‘net during interaction of Tricholoma terreum with pine. We investigate hydrophobins in T. vaccinum, a widely spread basidiomycete (Agaricales - Tricholomataceae) which forms ectomycorrhiza with spruce. The establishment of a high compatible mycorrhiza needs in a co-culture system about one month, in comparison to a low compatible one, which needs about four months. We know which hydrophobin T. terreum regulates specifically in both interactions. Is this also the case for T. vaccinum and can we improve the low compatible interaction by heterologous or over expression? So far, we investigated five T. vaccinum hydrophobins and 17 in the Tricholoma genus in total. We also want to show via quantitative Real-Time PCR in which stage of the life cycle respectively symbiotic interaction T. vaccinum produces hydrophobins, what kind of role they play with respect to function in the symbiotic tissue and their regulation under heavy metal stress. Due to the fact that T. vaccinum is a k-strategist and the triggers for fruiting body development are unknown, over expression of hydrophobin protein are important to characterise their properties. Also in silico analyses are done such as protein-protein complexes with HADDOCK (High Ambiguity Driven protein-protein Docking) for rodlet layer formation and phylogenetic trees (ITS, hydrophobin) to understand how the evolution of the host, fungus and its hydrophobins took place. (Identification of a hydrophobin gene that is developmentally regulated in the ectomycorrhizal fungus Tricholoma terreum, Manke et al., App. and Environ. Microbiol., 2002 HADDOCK: a protein-protein docking approach based on biochemical and/or biophysical information. Bonvin et al., J. Am.

*Alternaria brassicicola* is the causative agent of Brassicaceae black spot disease. This necrotrophic fungus causes glucosinolates degradation by plant myrosinase during infection. Isothiocyanates (ITCs), the major breakdown compounds, have been shown to be exert some toxicity to various *A. brassicicola* isolates. We showed, by application of specific fluorescent probes, that ITCs cause ROS production, disrupt the mitochondrial membrane potential and trigger apoptosis in *A. brassicicola* cells. The generation of an oxidative stress following ITCs application was confirmed by the fact that KO mutants deficient for the transcription factors AP1 and SKN7 (both being involved in the oxidative-stress response) are hypersensitive to ITCs and have decreased aggressiveness on glucosinolates accumulating host plants. Despite this host defense system, *A. brassicicola* is still able to complete its infectious cycle, indicating the existence of strategies to cope with this oxidative stress. Hypersensitivity to Al-ITC, Bz-ITC or Ph-ITC was observed in KO mutants deficient for distinct glutathione-S-transferases. Some of them were differentially expressed following exposure to ITCs and exhibited high transferase activity with ITCs as substrate. The polyol mannitol has been proposed to act as an antioxidant agent and protect fungal cells by quenching ROS produced by hosts in response to attack. We isolated the genes encoding the MPD and MDH enzymes, two essential enzymes of the mannitol metabolism in *A. brassicicola*, and used targeted gene disruption to create single and double mutants for each gene. Only mutants unable to accumulate mannitol in hyphae and conidia were sensitive to ITCs. Our results supported the involvement of fungal GST and mannitol metabolism in ITC-derived oxidative stress. GST participates in ITCs detoxification mechanisms and mannitol accumulation in protection against ROS. They highlight their importance with respect to the ability of *A. brassicicola* to efficiently accomplish its pathogen life cycle despite exposure to plant-derived antifungal metabolites.


Reactive oxygen species (ROS) are emerging as important regulators required for the successful establishment and maintenance of the mutualistic association between the fungal endophyte *Epichloë festucae* and its grass host *Lolium perenne*. The generation of reactive oxygen species (ROS) by the fungal NADPH oxidase, NoxA has previously been shown to regulate hyphal growth of *E. festucae* in planta; a result that has led to the hypothesis that fungal-produced ROS are key second messengers in the symbiosis. However, the highly reactive nature of these molecules dictates that cells possess efficient sensing mechanisms to maintain ROS homeostasis and prevent oxidative damage to cellular components. The *Saccharomyces cerevisiae* Gpx3-Yap1 and *Schizosaccharomyces pombe* Tpx1-Pap1, two-component H₂O₂ sensors, serve as model redox relays for coordinating the cellular response to ROS. While proteins related to the Yap1 and Pap1 basic-leucine zipper (bZIP) transcription factors have been identified in a number of filamentous fungi, the components involved in the upstream regulation remain unclear. This study investigated the role of the *E. festucae* Yap1 homologue, YapA, and putative upstream activators GpxC and TpxA, homologues of Gpx3 and Tpx1, respectively, in responding to ROS. YapA is involved in responding to ROS generated at the wound site following inoculation into ryegrass seedlings. However, deletion of yapA did not impair host colonization indicating redundancy in systems used by *E. festucae* to sense and respond to plant-produced ROS. In culture, deletion of *E. festucae yapA*, renders the mutants sensitive to only a subset of ROS and this sensitivity is influenced by the stage of fungal development. In contrast to the H₂O₂-sensitive phenotype widely reported for fungi lacking the Yap1-like protein, the *E. festucae* yapA mutant maintains wild-type mycelial resistance to H₂O₂ but conidia of the yapA mutant are very sensitive to H₂O₂. Using a degron-tagged GFP-CL1 as a reporter, we found YapA is required for the expression of the spore specific catalase, *catA*. Moreover, YapA is activated by H₂O₂ independently of both GpxC and TpxA, suggesting a novel mechanism of regulation exists in *E. festucae*. This work provides a comprehensive analysis of the role and regulation of the AP-1 transcription factor pathway in a filamentous fungal species.

*Rhizoctonia solani* is a broad-host-range necrotizing fungal pathogen that is responsible for significant diseases to diverse crops. In Australia, *R. solani* most notably causes bare patch of cereals and costs $77 million pa in direct losses, while internationally it is a significant problem for global rice production. In the absence of strong host resistance, an understanding of fungus pathogenesis underpins alternative approaches to enhance resistance in crop plants. While the majority of phytopathogens sequenced to date belong to the Ascomycota, *R. solani* is a basidiomycete with the closest sequenced relatives being biotrophic rust and smut fungi and saprophytic mushrooms, each possessing a lifestyle vastly different from *R. solani*. We have over-come complications associated with the multinucleate, heterokaryotic nature of *R. solani* to assemble a high quality consensus haploid genome of an AG8 isolate. Transcriptomics assisted with genome annotation and identified putative pathogenesis genes. Several of these genes display host-specific expression, while others show consistent infection-related expression across different anastomosis groups on different hosts. LC-MS based proteogenomics identified proteins from three fractions; soluble hyphal proteins, membrane localised proteins and secreted proteins, from *R. solani* growing in-vitro or in wheat infection mimicking conditions. OPCR confirmed up-regulation of some of the corresponding genes in infected wheat roots compared to *R. solani* grown in vitro, providing further support for a pathogenesis-related role. Functional testing of the role of candidate pathogenesis genes is on-going. On the plant side of the interaction, large scale gene expression and mutant analyses revealed the high degree of resistance in Arabidopsis (unlike its’ susceptible relative, canola) was dependent on reactive oxygen species and not jasmonic acid, ethylene or salicylic acid. By contrast, moderate resistance in *Medicago truncatula* was dependent on ethylene mediated defences, which when over-activated, lead to enhanced resistance. These findings suggest that different plant species employ different defences with differing effectiveness against the same pathogen and a collective understanding of the interplay of host and fungal responses may facilitate novel strategies for enhancing resistance.

589. The Cpc1 (CpcA/Gcn4) regulator of the cross-pathway control of amino acid biosynthesis is required for plant infection of the vascular pathogen *Verticillium longisporum*. Susanna A. Braus-Strömeyer, Christian Timper, Van Tuan Tran, Gerhard H. Braus. Molecular Microbiology and Genetics, Georg-August-University, Goettingen, Germany.

The plant pathogenic fungus *Verticillium longisporum* is the causal agent of early senescence and ripening in *Brassica napus* (oilseed rape, Canola) and other cruciferous crops. *Verticillium* wilts have become serious agricultural threats during the last decades. *Verticillium* infect host-plants through the roots and colonize xylem vessels of the host-plant, which provide a barrier to growth and colonisation via xylem fluid to cope with an imbalanced amino acid supply.

The transcriptional activator gene *VlCPC1* (similar to CpcA/GCN4) was knock-downed via RNA-mediated gene silencing and the expression of the two *CPC1* isogenes (VlCPC1-1, VlCPC1-2) in *V. longisporum* could be reduced up to 85%. The resulting mutants were more sensitive to amino acid starvation induced by 5-methyltryptophane (5-MT). In plant infection assays, the silenced mutant showed significantly less symptoms such as stunting and early senescence. Knockouts of *CPC1* in the haploid *V. dahliae* were sensitive to amino acid starvation and strongly reduced in symptom formation in their host *Solanum lycopersicum* (tomato).

The hybrid *V. longisporum* and the haploid *V. dahliae* are the first phytopathogenic fungi, which were shown to require *CPC1* for infection and colonization of their respective host plants oilseed rape and tomato.

590. Fungal-Specific Transcription Factor *AbPf2* Activates Pathogenicity in *Alternaria brassicicola*. Yangrae Cho, Robin Ohm, Igor Grigoriev, Akhil Srivastava. 1) Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI; 2) United States Department of Energy Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598.

*Alternaria brassicicola* is a successful saprophyte and necrotrophic plant pathogen. Molecular determinants of its life style shift between saprophyte and pathogen, however, are unknown. To identify these determinants we studied nonpathogenic mutants of a transcription factor-coding gene, *AbPf2*. Frequency and timing of germination and appressorium formation on host plants were similar between the nonpathogenic *Dabpf2* mutants and wild-type *A. brassicicola*. The mutants were also similar in vitro to wild-type *A. brassicicola* in vegetative growth, conidium production, and responses to chemical stressors, such as a phytoalexin, reactive oxygen species, and osmolites. The mutants, however, did not penetrate host plant tissues, though their hyphae continued to grow on the plant surface. Transcripts of the *AbPf2* gene increased exponentially soon after wild-type conidia encountered their host plants. A small amount of *AbPf2* protein, monitored by fused green fluorescent protein, was located in both the cytoplasm and nuclei of young, mature conidia. The protein level decreased during saprophytic growth but increased several-fold during pathogenesis. Levels of both the proteins and transcripts sharply declined following colonization of host tissues beyond the initial infection site. When the transcription factor was expressed at an induced level in the wild type during early pathogenesis, the expression of 106 fungal genes was down-regulated in the *Dabpf2* mutants. Notably, 33 of the 106 genes encoded secreted proteins, including eight putative effector proteins. Plants inoculated with *Dabpf2* mutants expressed higher levels of genes associated with photosynthesis, the pentose phosphate pathway, and primary metabolism, but lower levels of defense-related genes. Our results suggest that conidia of *A. brassicicola* are programmed as saprophytes, but become parasites upon contact with their hosts. *AbPf2* coordinates this transformation by expressing pathogenesis-associated genes, including those coding for effectors.

591. WITHDRAWN

592. Host-to-pathogen gene transfer facilitated infection of insects by a pathogenic fungus. Weiguo Fang, Xiaoxuan Chen. College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, China.

Insipie being of great concern to human health and the management of plants and animals, the mechanisms facilitating host switching of eukaryotic pathogens remain largely unknown. The endophytic insect-pathogenic fungus *Metarhizium robertsi* evolved directly from endophytes and its entomopathogenicity is an evolutionarily acquired characteristic. We found that *M. robertsi* acquired a sterol carrier (*MnNPC2a*) from an insect by horizontal gene transfer (HGT). *MnNPC2a* increased the amount of ergosterol in hyphal bodies by capturing sterol from insect hemolymph, and thus maintained cell membrane integrity and improved fungal survival rate. On the other hand, the reduction in sterol (substrate for molting hormone synthesis) in insect hemolymph elongated larval stage, which allows the fungus to fully exploit host tissues and produce more conidia. This is first report of HGT from host to a eukaryotic pathogen, and the host gene ultimately improved the infectivity of the pathogen.
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Plant pathogens have co-evolved with their host plants which have evolved the defense system against their pathogens. It is generally accepted that plants express basal immunity by the recognition of the pathogen-associated molecular patterns, but compatible pathogens suppress the plant basal defense by secreting effector proteins. In our previous study, we have obtained several pathogenicity deficient insertional mutants in Colletotrichum orbiculare by Agrobacterium tumefaciens-mediated transformation (AtMT). Among them, in the mutant named YK4524 it was shown that a T-DNA insertion disrupted a gene which presumably encodes an extracellular protein with signal peptide sequence. And BLAST search of the predicted sequence found no significant homologous genes in published databases, suggesting that it is unique to C. orbiculare. So we named this gene CoPRF1 (Pathogenesis-related factor). Target gene disruption mutants of coprf1 obtained by AtMT showed significant reduction in virulence on the host leaves. However, characteristics such as germination, appressorium formation and penetration hyphal formation of coprf1 mutants in vitro were normal, indicating that CoPRF1 is not essential for infection related morphogenesis. On the other hand, penetration ability of mutants was attenuated on intact cucumber cotyledons, and the elongation of its invasive hyphae was slower compared with the wild type. To confirm the possibility that decreased virulence of coprf1 mutants was involved in plant defense responses, we inoculated coprf1 mutant on cucumber cotyledons of which defense responses was disturbed by transient heat-shock. As expected, the pathogenicity of coprf1 mutant was restored. Furthermore, expression analysis of CoPRF1 by RT-PCR showed that CoPRF1 expressed in planta, but not in vitro culture. From these results, it was suggested that CoPRF1 would engage in evasion of basal resistance at the host infection.

594. CPS1 mutants in Coccidioides are avirulent and act as an attenuated vaccine in the valley fever mouse model. Hema P. Narra1,2, Lisa F. Shubitzi2,3, M. Alejandro Mandel1,3, Leslie Gunati1ka, Hien Trinh1,2,3, Marc J. Orbach1,2,3, 1) School of Plant Sciences, Univ. of Arizona, Tucson, AZ; 2) Veterinary Sciences and Microbiology, Univ. of Arizona, Tucson, AZ; 3) Valley Fever Center for Excellence, Univ. of Arizona, Tucson, AZ; 4) Department of Pathology, Univ. of Texas, Medical Branch, Galveston, TX; 5) School of Natural Resources and the Environment, Univ. of Arizona, Tucson, AZ.

Coccidioides species are mammalian pathogens endemic to the Southwestern US as well as parts of Mexico, Central and South America. The disease they cause, coccidioidomycosis, or valley fever, is considered an emerging infectious disease due to increases in reported cases over the past 10 years. To identify virulence factors of this pathogen that may be targets for therapeutics, we have identified and disrupted genes that are important for pathogenicity in both plant pathogens and other animal pathogens. Based on the work of Liu et al. (2003), we disrupted the Coccidioides ortholog of C. heterostrophus CPS1 in C. posadassi strain Silveira. CPS1 was originally identified as a potential non-ribosomal peptide synthase component, because it encodes a polypeptide with two AMP binding domains related to the adenylation domains in bacterial non-ribosomal peptide synthases. However it also contains a putative N-terminal DMAP1b domain. In mammals, this domain binds the DMAP1 transcriptional co-repressor that has been shown to bind regulatory proteins and is proposed to act as a co-repressor of transcription. The C. posadassi cps1 deletion strain is non-pathogenic in susceptible mice but does initiate the formation of spherules, the infectious form of Coccidioides. The mutant also forms spherules in vitro. Whether Cps1 plays a role as a regulator of virulence via the DMAP1b domain, or via production of a potential toxin is not known. This is being explored via expression analysis of CoPRF1 by RT-PCR showed that CoPRF1 expressed in planta, but not in vitro culture. From these results, it was suggested that CoPRF1 will engage in evasion of basal resistance at the host infection.

595. Elucidating the response of wheat to the exposure of Stagonospora nodorum effectors. Lauren A. Du Fall, Peter S. Solomon. Research School of Biology, Australian National University, Canberra, ACT, Australia.

The dothideomycete Stagonospora nodorum is a necrotrophic fungal pathogen of wheat and is the causal agent of Stagonospora nodorum blotch (SNB). This disease is responsible for over $100 million of yield losses in Australia annually. Recent studies have shown that this fungus produces a number of effectors proteins that are internalized into host cells of susceptible wheat cultivars. The mechanism by which these effectors induce tissue necrosis in susceptible hosts is yet to be fully elucidated. We have applied a multi-omics approach to elucidate the cellular processes leading to disease and provide insight into the mode-of-action of these effectors. Gas chromatography-mass spectrometry analysis of primary polar metabolites has been undertaken on tissue extracts and apoplastic fluid from StToxA infiltrated wheat. Results illustrate widespread perturbations in primary metabolism and reveal the first direct evidence of an increase in energy production in engerstand the host response to StToxA at the secondary metabolism level, samples were also analysed using liquid chromatography-mass spectrometry. Our data indicate StToxA causes an increase in defence-related secondary metabolites. The effect of these effectors on Metabolites of Stagonospora nodorum growth and sporulation in vitro and in planta has identified several compounds with novel anti-fungal properties. These complementary approaches have provided a novel insight into the contribution of the StToxA effector protein to SNB in wheat.


The genome of the downy mildew pathogen Hyaloperonospora arabidopsis, an obligate biotrophic oomycete, encodes several necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLP). The NLPs of H. arabidopsis (HaNLPs) constitute a family of 12 genes and 15 pseudogenes, most of which form a species-specific clade separate from NLPs of related Phytophthora species, suggesting that the family has expanded. The secreted HaNLPs were found to be nontoxic when tested on Arabidopsis or tobacco, in contrast to known necrosis-inducing NLPs, e.g. the P. sojae PsoJIP protein that is cytolytic and induces a strong cell death response in dicot plant tissues. Even HaNLP3, which is most similar to necrosis-inducing NLP proteins of other oomycetes, and which contains all amino acids that are known to be important for necrosis-inducing activity, did not induce necrosis. Chimeras constructed between HaNLP3 and the necrosis-inducing PsoJIP protein demonstrated that most of the HaNLP3 protein is functionally equivalent to PsoJIP, except for an exposed domain that prevents necrosis induction. The HaNLP genes are mostly expressed early during infection, suggesting an alternative function of noncytolytic NLP proteins during biotrophic infection of plants. To investigate if HaNLP production in the host affects susceptibility to infection, transgenic Arabidopsis plants were generated. Surprisingly, overexpression of HaNLP3, 5, 6, 9, and 10 resulted in plants with severely reduced growth. To be able to monitor NLP-effects on pathogen infection, in the absence of growth reduction, an Arabidopsis line with an estradiol-inducible HaNLP3 construct was generated. DNA microarray analysis revealed that plant immune responses were strongly activated upon estradiol-induced HaNLP3 expression. Furthermore, resistance to H. arabidopsis infection was activated, suggesting that the plant is able to recognize the pathogen-associated
HaNLP3 protein and mount an effective immune response. Our research is now focused on determining how Arabidopsis is able to respond to the HaNLPS and how the downy mildew pathogen can suppress the host immune response triggered by non-toxic NLPS.


Pathogenic fungi are having an increasing global impact in the areas of health, agriculture and the environment. As such it is essential to understand the mechanisms that fungi employ to survive and grow within a host. The emergence of many new “opportunist” fungal pathogens” has to a great extent altered the traditional view that pathogenicity was solely reliant on the inherent properties of the pathogen. In fact, the ability of a pathogen to cause disease in some hosts but not in others suggests that pathogenic determinants are complex and dynamic, and are largely dependent on specific pathogen-host relationships. Despite this there are conserved aspects of the interactions between host and pathogen. For example, hosts employ innate immune responses as an almost immediate recognition and attack mechanism against invading pathogens. Penicillium marneffei is a temperature dependent dimorphic fungus, growing in a hyphal form producing conidia at 25°C and as a yeast form at 37°C. Despite its importance as an opportunistic pathogen, little is known about the biology and mechanism of infection of P. marneffei. The infectious agents (conidia) are believed to be inhaled, reaching the alveoli of the lungs, where they are phagocytosed by alveolar macrophages for elimination. At this point that P. marneffei switches growth to a pathogenic yeast cell form, and is able to withstand macrophage cytotoxic attacks to cause infection. In order to understand how pathogenicity is regulated, we have examined the gene expression of the fungal transcriptome of P. marneffei using RNAseq. We compared expression of genes in cultures grown at 25°C (hyphal form) and 37°C (yeast form) to that of the wild type strain on certain host plants, but varied depending on the plant species tested. The mutant had similar vegetative growth and promoted normal (black and hard) sclerotial formation of the mutants. Furthermore, the oxalate accumulation in culture filtrates of the mutants and detected by HPLC, and the compound was identified as fumaric acid using L-tartrate metabolism. Oxalic acid production has been shown in numerous studies to be a pathogenicity factor. Oxalate-inhibiting medium (PDA amended with 50 mg/L bromophenol blue). The pH values of 6-8 day PDB culture filtrates were 1.8-2.0 for the wild type and 2.8-3.1 for the mutants. No oxalic acid was detected using HPLC in culture filtrates or in the mycelium of the mutants, but another acid compound was accumulated in culture filtrates of the mutants and detected by HPLC, and the compound was identified as fumaric acid using LC-MS. The mutants showed reduced vegetative growth on PDA and produced sclerotia that are beige in color and soft in texture. Artificial acidic conditions (pH 3.4 and 4.2) enhanced vegetative growth and promoted normal (black and hard) sclerotial formation of the mutants. Furthermore, the oxalate-minus mutants retained pathogenicity on pea, green bean and faba bean in detached leaf assays and on intact plants of Arabidopsis thaliana, and their virulence levels were similar to that of the wild type strain on certain host plants, but varied depending on the plant species tested. The mutant had increased expression levels of cell wall-degrading enzymes such as polygalacturonases compared to the wild type strain during the process of infecting pea leaves. The results showed that a low pH condition is very important for growth and virulence of S. sclerotiorum on its wide range of host.

598. Oxalate-minus mutants of Sclerotinia sclerotiorum via T-DNA insertion accumulate fumarate in culture and retain pathogenicity on plants. Liangsheng Xu1, Meichun Xiang1, David White1, Weidong Chen1,2. 1) Plant Pathology, Washington State University, Pullman, WA; 2) USDA-ARS, Washington State University, Pullman, WA 99164.

Sclerotinia sclerotiorum is a ubiquitous necrotrophic pathogen capable of infecting over 400 plant species including many economically important crops. Oxalic acid production has been shown in numerous studies to be a pathogenicity factor for S. sclerotiorum through several mechanisms. During our random mutagenesis study of S. sclerotiorum using Agrobacterium-mediated transformation, we identified three mutants that had lost oxalate production. Southern hybridization blots showed the mutation was due to a single T-DNA insertion, and plasmid rescue and DNA sequencing confirmed that the T-DNA insertion site was located in the ORF of oxaloacetate acetylhydrolase (Ssoah, SS1G_08218) of S. sclerotiorum. The mutants did not change the color of a pH-indicating medium (PDA amended with 50 mg/L bromophenol blue). The pH values of 6-day PDB culture filtrates were 1.8-2.0 for the wild type and 2.8-3.1 for the mutants. No oxalic acid was detected using HPLC in culture filtrates or in the mycelium of the mutants, but another acid compound was accumulated in culture filtrates of the mutants and detected by HPLC, and the compound was identified as fumaric acid using LC-MS. The mutants showed reduced vegetative growth on PDA and produced sclerotia that are beige in color and soft in texture. Artificial acidic conditions (pH 3.4 and 4.2) enhanced vegetative growth and promoted normal (black and hard) sclerotial formation of the mutants. Furthermore, the oxalate-minus mutants retained pathogenicity on pea, green bean and faba bean in detached leaf assays and on intact plants of Arabidopsis thaliana, and their virulence levels were similar to that of the wild type strain on certain host plants, but varied depending on the plant species tested. The mutant had increased expression levels of cell wall-degrading enzymes such as polygalacturonases compared to the wild type strain during the process of infecting pea leaves. The results showed that a low pH condition is very important for growth and virulence of S. sclerotiorum on its wide range of host.

599. Molecular characterization of fungi associated with superficial blemishes of potato tubers in Al-Qasim region, Saudi Arabia. Rukaia M Gashgar1, Youssuf A. Gherbawy1, 1) Biology Dept, King Abdulaziz university, Jeddah, Saudi Arabia; 2) Biology Dept, Taif university, Taif, Saudi Arabia.

Potato (Solanum tuberosum) becoming a more and more important foodstuff in the world. Also, the visual quality of fresh potatoes became a dominant criterion and a significative economical issue in potato market. According the vegetative reproduction of this species, requirements for visual quality are also needed for potato tubers. As an organ for reserve and propagation, the tuber grows underground and is in contact with soil-borne microorganisms, making it potentially exposed to blemishes. Some blemishes are due to known pathogens and others whose causes are unknown are called atypical blemishes. Therefore, knowledge about the pathogens is needed to set up efficient control strategies and to help potato growers to better know the causes of these blemishes and find technical solutions for improving the potato quality. Therefore, the objective of this proposed research study is the possibility of using some modern methods of molecular diagnostics and rapid detection of the presence of fungal contaminants in potato blemishes in Al-Qasim (Saudi Arabia). Polygonal lesions was the most observed blemish type in the collected samples. One hundred and sixty isolates were collected from different types of blemishes identified the species level. All Fusarium strains collected during this study were used to detect their pathogenicity on potato tubers. The inoculated fungi were re-isolated from the diseased potato tubers to prove the Koch’s postulates. This is the first comprehensive report on identity of major pathogenic fungi causing potato dry rot isolated from potato tuber blemishes in Saudi Arabia.

600. Patterns of Distribution of Bacterial Endosymbionts in Lower Fungi. Oleg Lastovetsky1, Xiaotian Qin1, Stephen Mondo1, Teresa Pawlowska2, Andriy Grygansky1, 1) Microbiology Dept, Cornell University, Ithaca, NY; 2) Plant Pathology & Plant-Microbe Biology Dept, Cornell University, Ithaca, NY; 3) Biology Dept, Duke University, Durham, NC.

Fungi are not typically known to have endosymbionts. However, some members of Glomeromycota and Mucoromycotina have recently been found to harbor bacteria in their hypheae and spores. The newly discovered association between Rhizopus microsporus (Mucoromycotina) and Burkholderia bacterium (betaproteobacteria) prompted us to search for endobacteria in other members of Mucoromycotina fungi. We screened a broad range of Mucoromycotina isolates for the presence of bacterial endosymbions using PCR with universal and Burkholderia-specific primers that targeted the 16S and 23S rRNA bacterial genes. Endobacteria were only found in certain strains of R. microsporus but in no other Rhizopus or Mucoromycotina isolates. A 28S rRNA gene phylogeny of the screened fungal isolates revealed a clustering of bacterial is R. microsporus isolates away from bacterial R. microsporus isolates. To explore this putative divergence within the R. microsporus lineage we are working on a multi-gene phylogeny of Rizopus isolates, which is based on multiple coding and non-coding regions.

Nematode-trapping fungi have for a long time been seen as putative biological control agents against parasitic nematodes. A better knowledge on the infection process will facilitate the development of these fungi as biological control agents and may also lead to the discovery of new nematicidal drugs. 

Monacrosporium haptotylum is a nematode-trapping fungus that captures nematodes using an adhesive trap called knob. In this study, proteins were extracted from knobs and mycelium and analyzed using SDS-PAGE combined with LC/MS/MS. Peptides were matched against predicted gene models from the recently sequenced genome of M. haptotylum. Furthermore, the transcriptome in the knob during infection of nematodes was analyzed. 

The analysis showed that there was a large difference in the proteome of the knob compared to the mycelium. In total 336 proteins were identified. A quantitative analysis showed that 54 proteins were expressed at significantly higher levels in the knobs versus the mycelium. Proteins containing a predicted secretion signal were overrepresented in knobs (knobs 41 %; mycelium 11 %). Five of the secreted proteins upregulated in knob were small secreted proteins (SSPs). Three of the SSPs were orphan genes since they showed no homology to the NCBI database and lack Pfam domains. Interestingly, two of them are upregulated in the transcriptome during infection of nematodes. 

Among the upregulated proteins were several putative cell-surface adhesins containing the carbohydrate binding domain WSC and repetitive regions enriched in threonine/serine residues. Upregulated were also several a diverse array of peptidases including serine endopeptidase (subtilisin), aspartic endopeptidase, metalloendopeptidase, aminopeptidase and carboxypeptidase. Several proteins related to stress response and biosynthesis were also identified in the trap proteome. During infection of nematodes, genes with the domains peptidase_S8 (subtilisin), DUF1329 and WSC are highly upregulated in the knob. 

Taken together, our analysis shows that the trap cell has a unique proteome containing components that are involved in the early stages of infection including adhesion and penetration of the nematode.
which can have enormous impacts on human and ecosystem health. Despite their ubiquity and importance, very little is known about the molecular mechanisms underlying these interactions. To address this, we select to study the interactions between Pseudomonas aeruginosa and Aspergillus fumigatus, the ubiquitous opportunistic bacterial and fungal pathogens, respectively, via redox-active bacteria-secreted phenazines. We hypothesize that the functions of these molecules are multifactorial, dependent on genetic and environmental factors. By combining genetic, physiological, electrochemical, and metabolic profiling strategies, here we report that redox-active phenazines can mediate biofilm interactions between P. aeruginosa and A. fumigatus in multiple ways, ranging from antagonistic to synergistic. We find that phenazine production patterns are generally correlated with bacterial-fungal interaction phenotypes, in a genetically- and temporarily-dependent manner. Further, fungi can convert the precursor phenazine-1-carboxylate (PCA) produced by bacteria into several other phenazines. These structurally related phenazines come in with characteristic physical-chemical properties including redox properties. Our most striking finding is to be able to draw connections between a phenazine’s structure and its mode of action. Under one given condition, some phenazines such as phenazine-1-carboxamide (PCN) can facilitate bacterial biofilm development by inhibiting fungal development; some others such as pyocyanin (PYO) show no apparent effect on fungal development, and 5-methyl-phenazine-1-carboxylic acid (5-Me-PCA) can synergistically facilitate both bacterial and fungal biofilm developments. In addition, we find that changing the ambient redox and pH conditions can affect a phenazine’s mode of action, likely via influencing its redox activity. Taken together, our findings imply that phenazines-mediated bacterial-fungal interactions have profound and diverse effects on multicellular behavior in competitive and mixed-species biofilm environments.

606. Genomic analysis of Mortierella elongata and its endosymbiotic bacterium. Gregory Bonito1, Andrii Gryganskyi2, Christopher Schadt2, Dale Pelletier2, Amy Schafer2, Gerald Tuskan2, Jessy Labbé3, Sofia Robb2, Rebecca Ortega1, Francis Martin3, Mitchel Doktycz1, Kurt LaButti3, Matt Nolan4, Robin Ohm5, Igor Grigoriev6, Rytas Vilgalys1. 1) Duke University, Durham NC; 2) Oak Ridge National Laboratory, Oak Ridge TN; 3) University of Washington, Seattle WA; 4) University of California, Riverside CA; 5) Institut National de la Recherche Agronomique, Nancy France; 6) Joint Genome Institute, Walnut Creek CA.

Mortierella belong to a group of basid fungi (Mortierellomycotina) common to soils and the rhizosphere and endosphere of many plant species. Mortierella species are known for rapid growth and abundant lipid production. Mortierella elongata is one species commonly isolated from forest soils and healthy plant roots where it grows asymptotically as an endosymbiont. Mortierella elongata is a heterothallic species but can also reproduce asexually through chlamydospores and sporangiospores. Recent reports indicate that some isolates of M. elongata host endosymbiotic bacteria, which may be transmitted vertically via spores. However, it is still unclear whether all Mortierella species host endosymbionts or whether these are lineage-specific associations. Given the geographically widespread distribution of Mortierella elongata and its ubiquitous presence in forest soils and plants we chose to sequence its genome through the JGI Forest Metatranscriptome CSP. We also sought to assemble the genome of the bacterial endosymbiont to address whether there are genomic signatures of co-adaptation or co-evolution in the genomes of Mortierella and its endosymbiotic bacterium, which may impact the function and growth of Mortierella elongata. The 50 Mb genome of M. elongata was sequenced to 112x coverage. Of the 220,113 putative proteins identified in M. elongata, 109,093 appear to be unique (e.g. only ~50% have orthologs in other fungal species having sequenced genomes). The M. elongata genome appears to be enriched in genes related to trypton metabolism, siderophore group nonribosomal peptides, glucan 1,4-alpha glucosidases, and in lipid metabolism (e.g. sphingolipids, etherlipids, and glycerophospholipids) compared to genome sequences of other basal fungi. The endosymbiotic bacterium sequenced along with the M. elongata isolate is related to Glomeribacter (endosymbiont of Gigaspora, Scutellospora, and other Glomeromycota) within the Burkholderiales. The ~2.6 MB endosymbiont genome is larger than that of Glomeribacter but quite reduced compared to free-living isolates of Burkholderia. The reduced genome size of this bacterium, and the fact that it has thus far evaded pure culture isolation, supports the view that this is an ancient and obligate symbiosis.

607. Diversity and Content of Maize Leaf Endophytes are Correlated With Maize Genotype. Alice C. L. Churchill1,2, Santiago X. Mideros1, Peter Balint-Kurti3, Surya Saha3, Rebecca J. Nelson4. 1) Department of Plant Pathology & Plant-Microbe Biology, Cornell Univ, Ithaca, NY; 2) USDA-ARS Plant Science Research Institute, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

All plants contain endophytes that have the potential to provide fitness benefits to their hosts by increasing tolerance to environmental stressors, boosting plant nutrition and growth, and providing increased resistance or tolerance to insect pests and plant pathogens. We are characterizing endophytic populations inhabiting aboveground maize tissues with the goal of associating maize genetic variation with the diversity, structure and constitution of maize-associated microbial communities. Nine maize lines, representing a diverse subset of the founders of the NAM (Nested Association Mapping) population, were grown at a single North Carolina field site in 2012 and assayed for culturable endophytic bacteria and fungi. Two distinct seed sources for each maize line were planted in a randomized experimental design, and three replicates per seed source were assessed. Using standard endophyte isolation methodologies, and ground leaf extracts were cultured on four media that select for slow- and fast-growing fungi and copiotrophic, diazotrophic, and oligotrophic bacteria. Approximately 65% of the samples contained one or more phenotypically distinct, culturable bacteria, 28% contained one or more fungi, 22% contained both bacteria and fungi, and endophytes were undetectable in 28% of the samples. A greater number and diversity of fungi were cultured from tropical maize lines than from temperate lines. Bacteria were isolated from all maize lines, with some lines exhibiting significantly greater microbial community diversity than others. Several phenotypically similar bacteria and fungi were isolated from multiple maize lines. Microbial identity via 16S and ITS sequencing, as well as identification of unculturable endophytes via whole genome metagenomic sequencing, are in progress. We are particularly interested in identifying members of the microbiome that modulate disease symptoms caused by maize leaf and ear pathogens. Hence, future studies will focus on in vitro and in planta endophyte-pathogen interactions.


Epichloë endophytes infect grasses within the subfamily Pooidae including some within the tribe Triticaceae. There have been no accounts of modern domesticated Triticaceae hosting epichloë endophytes but there have been reports in Elymus, Hordeum and other grasses within the tribe. Our goal is to isolate epichloë endophytes endophytes from the wild relatives of modern cereals and inoculate these into modern cereal crops. We surveyed populations of Elymus and Hordeum, primarily from Asia, and selected 29 Elymus and 13 Hordeum infected plants. We used simple sequence repeats (SSR) and tubulin sequencing to determine genetic similarity, hybrid status and closest non-hybrid ancestor. SSR data indicates 26 genetically distinct strains that fall into 5 major clades. b-tubulin and 18S rRNA sequencing shows that the majority of our isolates had Epichloë bromicola ancestry, with both hybrid and non-hybrid strains identified. Within the non-hybrid E. bromicola two major clades were identified. Of the hybrids we identified examples of E. bromicola x E. typhina and E. bromicola x E. amarillans. Although E. bromicola has been observed in Asian grasses we believe that this is the first report of an E. bromicola x E. amarillans hybrid. Of the remaining isolates, we found examples of strains with E. yangii ancestry (all non-hybrids) and E. elymi ancestry (both non-hybrid and
609. The Interaction of Mycoplasma-related Endobacteria with their Arbuscular Mycorrhizal Fungal Host. Mizue Naito\textsuperscript{1,2}, Teresa Pawlowska\textsuperscript{1}. 1) Dept. of Microbiology, Cornell University, Ithaca, NY; 2) Dept. of Plant Pathology & Plant-Microbe Biology, Cornell University, Ithaca, NY.

Arbuscular mycorrhizal fungi (AMF), comprising the monophyletic phylum Glomeromycota, are obligate biotrophs, and form symbiotic associations with 80% of terrestrial plants. AMF associate symbiotically with the roots of plants, and are specialized in the transfer of nutrients from the soil to the plant host. In return for increased nutrient uptake, the plants supply AMF with up to 20% of their photosynthetically derived carbohydrates. Thus, AMF symbiosis contributes significantly to global nutrient cycling and terrestrial ecosystems. AMF have been known to harbour two types of bacteria in their cytoplasm: (i) the Burkholderia-related Candidatus Glomeribacter gigaspororum and (ii) a Mycoplasma-related bacteria, which we refer to as Mycoplasma-related endobacteria (MRE). MRE live freely in the AMF cytoplasm, and have been found associated with all lineages of AMF worldwide. Virtually nothing is known about the MRE, such as their evolution, biological capabilities, and whether they are mutualists or parasites of their AMF hosts. In order to understand the nature of this symbiosis, and determine the role that the MRE play in arbuscular mycorrhiza, next generation sequencing (Roche 454 and Illumina) was performed on MRE isolated from AMF hosts, Claroideoglomus etunicatum, Funneliformis mosseae, and Racocetra verrucosa. Phylogenetic reconstruction and divergence dating using 22 conserved genes have revealed that MRE form a novel monophyletic subclade of the Mycoplasmas and have diverged from their Mycoplasma relatives at least 400 million years ago, which may indicate the establishment of the MRE-AMF association to be quite ancient. Analysis of annotated genes have revealed novel proteins that are likely to play a role in interacting directly with the fungal host. Preliminary data suggest that MRE are important in enabling the completion of the life cycle of their AMF hosts.

610. The Velvet gene is required for mutualism between Epichloë festucae and perennial ryegrass. Mostafa Rahnama\textsuperscript{1,2}, Richard Gardner\textsuperscript{1}, Damien Fleetwood\textsuperscript{1}. 1) School of Biological Sciences, University of Auckland, Auckland, New Zealand; 2) Forage Improvement Group, AgResearch, Auckland, New Zealand.

The velvet gene (velA or velA) is a key factor in the regulation of fungal developmental, biosynthesis of secondary metabolites and hyphal growth. This study aimed to determine the role of velA regulation in Epichloë festucae and its mutualistic interaction with the agriculturally important forage perennial ryegrass (Lolium perenne). Infection of perennial ryegrass with an E. festucae mutant deleted in velA caused rapid seedling death in two thirds of infected plants while removing plants displayed a normal interaction phenotype, although after several weeks these plants also become stunted and died in an unusual delayed plant-interaction phenotype. No hypersensitive response was observed by microscopy, suggesting the response is not driven by pathogen-like effector proteins. Microscopic analysis showed different accumulation of polysaccharides between mutant and wild type strains. The mutant strain could grow in higher concentrations of calcium and also there was different colony hydrophobicity between wild type and mutant strains. These different cell wall properties suggest a possible microbe associated molecular pattern (MAMP) trigger response.

611. An examination of phosphate solubilization and hormone production by two Penicillium species growing in the rhizosphere. Tim Repas\textsuperscript{1,2}, David Greenshields\textsuperscript{2}, Susan Kaminsky\textsuperscript{1}. 1) Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada; 2) Novozymes BioAg Ltd. 3935 Thatcher Ave, Saskatoon, SK S7R1A3, Canada.

Some soil microorganisms, including fungi, can enhance plant growth in the natural environment, however the mechanisms(s) by which they promote plant growth (PGP) are only partly known, and at any rate these will likely vary between organisms. Possible mechanisms for PGP include enhanced nutrient uptake, hormone production, pathogen biocontrol, and increased water use efficiency. We are evaluating the potential of two rhizosphere fungi, Penicillium biliae (Pbil) and a novel isolate (Skj340) that is most closely related to Penicillium atramentosum. Both fungi can solubilize in liquid culture each of Fe-, Al-, and Ca-phosphates, which are commonly found in soil. Both strains were able to solubilize phosphate minerals equally well. However, Pbil produces abundant organic acids, whereas Skj340 does not produce organic acids, nor even change the pH of the spent medium. Pbil has been tagged with red fluorescent protein (RFP); Skj340 has been stained with lactofuscchin; both were imaged with confocal fluorescence. Both fungi are found on the root surface, and neither could be isolated from surface sterilized plants, thus it appears that these strains are not endophytic. We are currently evaluating production of gibberellins and auxins from Pbil and Skj340 by assessing their ability to complement the phenotype of Arabidopsis mutants deficient in these hormones. We will also assess whether either strain root enhanced hair counts, which is expected to be correlated with both nutrient uptake and hormone activity.


The fungal endophyte Epichloe festucae and its host Lolium perenne are an interesting model system to study signals and mechanisms involved in mutualistic symbiosis maintenance. Mutants defective in components of the ROS producing Nox complex show loss of synchronized growth of the fungus in the grass resulting in stunted, multi-tillered plants (Tanaka et al. 2006, Takemoto et al., 2006). The small GTPase RacA is crucial to activate the Nox complex in E. festucae and plays a crucial role in establishment and maintenance of polarized hyphal growth (Tanaka et al., 2008). The objectives of this study were to determine whether key regulators of RacA in mammalian systems, the guanine nucleotide dissociation inhibitor (RhoGDI) and p21-activated kinases (Paks), also regulate fungal RacA in order to control polarised growth in culture and Nox activity for maintenance of the symbiosis with perennial ryegrass. We showed by yeast two-hybrid analysis that PakA (Clad homolog), PakB (Ste20 homolog) and RhoGDI interact with RacA, whereas the RhoGDI interaction is compromised in a mutant of RacA (R73E) required for RhoGDI binding. Only partial complementation is achieved when RacA (R73E) is expressed in the RacA deletion strain, indicating RhoGDI is important for controlling RacA function. Deletion of pkab had a mild effect on polarized hyphal growth in culture and wild-type growth in planta. Deletion of pakA had a severe effect on polarized hyphal growth in culture, with a reduction of radial growth and hyper-branching, a phenotype similar to the racA mutant but surprisingly plants infected with the pakA mutant had a wild-type interaction phenotype. The in planta results may reflect the fact that E. festucae grows by intercalary rather than tip growth in the intercellular spaces of perennial ryegrass leaves.


The fungal ear rot pathogens Aspergillus flavus and Fusarium verticillioides contaminate corn with aflatoxins and fumonisins, which pose severe health
risks and significantly limit grain marketability. Despite extensive breeding efforts, adequate resistance to mycotoxin accumulation has not been developed. Additionally, tools currently available to control mycotoxin contamination are limited in number and efficacy. Recently, host-induced gene silencing (HIGS) has emerged as a way to manipulate gene expression in fungal pathogens via expression of pathogen-specific hairpin RNA (hpRNA) in plants. The goal of this research was to generate transgenic corn inhibiting mycotoxin accumulation via HIGS. To this end, a high-throughput workflow was developed to create and validate HIGS expression vectors. First, candidate fungal genes regulating mycotoxin biosynthesis were identified through various approaches, including expression profiling and functional genomics. Second, a one-step cloning process was developed to simultaneously create hpRNA-encoding constructs comprised of sense and antisense orientations of target fungal genes, separated by an intron from the Magnaporthe oryzae cutinase gene, flanked by the TrpC promoter and terminator. Constructs were validated by phenotypic assessment after transformation into either A. flavus or F. verticillioides. Finally, constructs encoding hpRNAs that significantly reduced mycotoxin accumulation were cloned into a plant expression vector and transformed into maize. Currently, silencing vectors have been created that target the α-amylase (AMY1) and hexokinase (HKX2) genes in F. verticillioides and the polyketide synthase (skx) and hexokinase (kki) genes in A. flavus, and transgenic corn plants have been created. Thus, this research will advance the current understanding of HIGS in maize and will ultimately provide tools to control mycotoxin contamination of corn.

614. CDIT1, a novel type of proteinaceous toxin secreted by the necrotrophic pathogen of the roots of tomato Pyrenochaeta lycopersici. Pierre-Henri Clergeot1, Herwig Schuler2, Ejvind Mærz3, Maja Brus1, Simina Vintila4, Sophia Ekengren1. 1) Vaxtfysiologi, Stockholms Universitet, Stockholm, Sweden; 2) Alphalyse A/S, Odense, Denmark.

During the 24th Fungal Genetics Conference in Asilomar in 2009, we reported the isolation by Fast Protein Liquid Chromatography of a putative proteinaceous toxin of 18 kDa, secreted in liquid medium by the coryck root rot pathogen of tomato, the filamentous ascomycete Pyrenochaeta lycopersici. This molecule, CDIT1, was thought to be the cause of cell death observed in tomato leaves after infiltration of culture filtrates into their apoplast. Further characterization of CDIT1 revealed that it is secreted as a dimer and encoded by a single gene, whose expression peaks during tomato root infection. Infiltration into leaves of various hosts of P. lycopersici of recombinant CDIT1 purified by affinity confirmed its phytotoxic, but different activity. Especially, current tomato (Solanum pimpinellifolium) proved to be tolerant to a higher concentration of recombinant CDIT1 than cultivated tomato (S. lycopersicum). This correlates with the observation that roots of current tomato are less prone to intracellular infection by a transformant of the fungus expressing a reporter gene than those of cultivated tomato. Affinity-purified recombinant CDIT1 was also used in cut root assays to confirm lethal activity of the toxin on tomato root cells. Finally, searches made by sequence similarity in the genomes of other putative pathogenic Pleosporales showed that CDIT1 has a putative orthologue in the cereals pathogens Stagonospora nodorum, Pyrenophora teres f.sp. teres and Pyrenophora triticirepens, species known for secreting proteinaceous toxins contributing to virulence as well. In conclusion, our data validate the experimental approach of characterizing molecules secreted by Pyrenochaeta lycopersici and inducing disease-related symptoms after infiltration into host leaves, this in order to highlight potential genetic resistance against corky root rot in related species or varieties (see also Clergeot et al. 2012, Phytopathology, 102:878-891).

615. Nonhost-specific phytotoxicity of the polyketide-derived toxin solanapyrone A produced by Ascochyta rabiei and Alternaria solani. W. Kim1, L. Tymon1, D. Johnson1, W. Chen1. 1) Plant Pathology, Washington State University, Pullman, WA; 2) USDA-ARS, Grain Legume Genetic and Physiology Research Unit, Pullman, WA.

Solanapyrone A is a polyketide-derived metabolite produced by Ascochyta rabiei and Alternaria solani, which are the most destructive necrotrophic pathogens of chickpea and potato/tomato, respectively. They belong to the Order Pleosporales within the Class Dothideomycetes, but are phylogenetically distantly-related. All isolates of the two fungi tested so far are capable of producing solanapyrone A in synthetic media, which may imply that it is indispensable for their life cycle. However, very little is known about the genetics of solanapyrone A production and its role in pathogenesis and their life cycle. Recently, solanapyrone biosynthesis gene cluster was identified in Al. solani. Six genes (SoI1 - SoI6) form the gene cluster, spanning about 20 kb of the genome. Among them, SoI5 gene encodes a Diel-Alderase which catalyzes the final step of solanapyrone biosynthesis pathway. Knockout of the SoI5 gene in both A. rabiei and Al.solani resulted in the production of three compounds (presumably solanapyrone precursors), instead of solanapyrone A. Colony of soI5 mutants showed expansive growth in agar medium covering the entire plates, in contrast to restricted growth of colony of their corresponding wild-type progenitors. The restricted growth of the wild type strains is likely due to solanapyrone toxicity. Phytotoxicity of solanapyrone A was examined with various plant species including their natural host plants. Solanapyrone A produced similar size of necrotic lesions on all plant species tested. On the other hand, one of the putative solanapyrone precursors with the same molecular weight of solanapyrone A caused much smaller lesion only around the wounds of application sites. These results indicate that solanapyrone A is a nonhost-specific phytotoxin because it caused similar degree of lesions on host and nonhost species.

616. Crosstalk of the unfolded protein response and regulatory pathways controlling pathogenic development in Ustilago maydis. Kai Heimel1, Johannes Freitag2, Martin Hampel3, Julia Ast2, Michael Böker1, Jörg Kämper2. 1) Department of Molecular Microbiology and Genetics, Georg-August-University, Göttingen, Germany; 2) Genetics Department, Philipps-University, Marburg, Germany; 3) Genetics Department, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany.

Development of eukaryotic pathogens is accompanied by dramatic changes in morphology, lifestyle, nutrient acquisition and growth behavior. Consequently, pathogens require robust control systems to adapt to changing host environments and to maintain cellular physiology and homeostasis. The unfolded protein response (UPR) is a conserved eukaryotic signaling pathway counteracting endoplasmic reticulum (ER) stress during situations of increased demands on the secretory pathway. We identified and characterized the homologs of the central UPR regulators, Hac1 and Ire1 in the biotrophic fungus U. maydis. The UPR is tightly interlinked with the b mating-type-dependent signaling pathway that regulates pathogenic development. Exact timing of UPR is required for virulence and premature activation interferes with the b-dependent switch from budding to filamentous growth. A smut-specific C-terminal extension of the U. maydis Hac1 homolog, CB1, mediates direct interaction with Clp1, an essential component of the b-mediated signaling cascade. This interaction leads to stabilization of Clp1, increased ER stress resistance and thus prevents deleterious hyperactivation of the UPR during biotrophic growth of U. maydis. Since Clp1 expression is decisive for cell cycle release and fungal proliferation in planta we suggest that UPR activation serves as a checkpoint to time developmental progression and secretion of effector molecules, which promote the establishment of the biotrophic interaction.

617. Transcriptional profiling of the APSES genes in Trichophyton rubrum during growth on human nail. Elza A. S. Lang1, Nalu T. A. Peres2, Maíra P. Martins1, Tiago R. Jacob2, Pablo R. Sanches1, Larissa G. Silva2, Antonio Rossi2, Nílce M. Martinez-Rossi2. 1) Department of Genetics, Ribeirao Preto School of Medicine, University of Sao Paulo, Brazil; 2) Department of Biochemistry and Immunology, School of Medicine of Ribeirao Preto, University of Sao Paulo, Brazil.
The dermatophyte Trichophyton rubrum is worldwide spread and is the most prevalent causative agent of clinical cases of skin and nail mycoses in humans. Adhesion, invasion and colonization of keratinized host tissues are crucial for the success of the infection process and depend on the modulation of genetic responses during host-pathogen interactions. The APSES transcription factors are exclusive of the fungi kingdom and have been reported to play important roles in cell growth, differentiation, pathogenicity and virulence in several fungal species. In this work, in silico analyses of T. rubrum genome revealed the presence of genes encoding distinct proteins containing the APSES domain, suggesting that these proteins may play different roles in the cell. A high number of genes potentially regulated by the APSES regulators were identified by in silico analyses of 1000nt upstream of the annotated ORFs of T. rubrum. Transcriptional profiles of the APSES genes were analyzed during growth of T. rubrum on human nail or keratin, as the sole source of nutrients. In vitro infection of human nail was also evaluated by light microscopy. The results revealed that the transcription levels of the APSES genes are modulated during the human nail infection process and keratin degradation. Taken together, our findings suggest that the APSES genes of T. rubrum may be implicated in host-pathogen interactions. Financial support: FAPESP, CAPES, CNPq, FAEP.

618. Carbohydrate binding proteins of two Leptosphaeria pathogens of Brassica napus. Rohan G T Lowe¹, Bethany Clark², Angela Van de Wouw³, Andrew Cassin³, Jonathan Grandaubert³, Thierry Rouxel¹, Barbara Howlett¹. 1) School of Botany, University of Melbourne, Melbourne, Victoria, Australia; 2) INRA-Bioger, Campus AgroParisTech, Thiverval-Grignon, France.

Effectors include small secreted proteins (SSPs) produced by pathogens to modify or subvert defence responses of the host organism. Leptosphaeria maculans “brassicae”, the foremost pathogen of Brassica napus (canola), has 651 genes predicted to encode SSPs. The related species L. biglobosa “canadensis” more aggressively infects Brassica cotyledons, but causes fewer stem cankers. This difference in symptomology may be due to a different response from the host innate immune system. Compared to the L. maculans “brassicae” v23.1.3 reference genome, L. biglobosa has a relatively compact genome (30 Mbp) lacking the characteristic AT-rich, gene-poor repeats of L. maculans. We are using comparative transcriptomics to identify genes involved in early stages of infection. RNAseq analysis revealed that >300 L. maculans “brassicae” genes are highly upregulated (>100-fold) 7 days after infection compared to in vitro growth. These genes are enriched for SSPs, which comprise 25 of the top 100, but only 5% of the total gene complement. A major class of SSPs are carbohydrate active enzymes (CAZY), some of which play a role in evasion of chitin-triggered immunity in plants. L. maculans “brassicae” and L. biglobosa “canadensis” have different complements of the chitin-associated CAZY domains, CBM18 and CBM50 (aka lysM). Both of these domains bind chitin or peptidoglycan and may be found with chitinase domains in the same protein. The genomes of both Leptosphaeria species encode a similar number of CBM18 domains (27 and 29, respectively). L. maculans “brassicae” has predominantly multi-domain CBM18 proteins that are not highly upregulated 7 days after infection, whilst L. biglobosa “canadensis” has more abundant single domain proteins, two of which are highly upregulated (>100-fold) 7 days post inoculation. Homologs of the well-characterised CBM50/LysM protein, ECP6, from Cladosporium fulvum are present in both species, and a triplication of the N terminal LysM domain has occurred in L. maculans “brassicae”. The composition and regulation of CBM18 and CBM50-containing genes in Leptosphaeria may be involved in determining the degree of chitin-triggered immunity in the host canola, and concomitantly, the success of the pathogen. Silencing of key CAZY genes in L. maculans “brassicae” is underway, with a focus on members of the CBM50/LysM family.


The genome of the corn smut fungus Ustilago maydis codes for a large repertoire of secreted effectors. Some of them play crucial roles for virulence and establishment of the biotrophic phase. The chorate mutase Cmu1 is one such secreted translocated effector of U. maydis. cmu1 deletion strains are attenuated in virulence that is attributed to higher salicylic acid levels in plants infected with the mutant strain, most likely through alterations in the chelating of cholate from the plastids to the cytosol. Here we identify the motif in Cmu1 that is necessary for the translocation of the protein across the plant plasma membrane and present a mutational analysis of this region. To test for uptake we assayed the ability of mutant proteins to complement a cmu1 mutant strain as well as the retained ability to complement the growth defect of a Daro7 strain of S. cerevisiae in minimal medium. By deletion analysis a region of 20 amino acids adjacent to the signal peptide was shown to be essential for the translocation. Microscopic analysis of maize tissue infected with U. maydis strains expressing Cmu1-mcherry fusion proteins with or without the probable uptake motif revealed that the 20 amino acid motif allows binding of the protein to an as yet unknown plant plasma membrane component. We hypothesize that the translocation of Cmu1 across the plant plasma membrane is a two step process; initiated by bonding followed by translocation across the membrane. In addition, we present results where the 20 amino acid motif is substituted by motifs from other effectors.


Plant tissues and surfaces are a source of lipids which act as a potential carbon source and signals for the pathogenic development of the biotrophic smut fungus Ustilago maydis. This pathogen is an excellent model for the molecular genetic analysis of lipid use during disease and responds to lipids with a morphologic transition from budding to filamentous growth. In addition, the fungus possesses both peroxisomal and mitochondrial b-oxidation pathways, and numerous putative phospholipases (PLs), to exploit lipid carbon sources for nutritional and signalling purposes. We have shown that both peroxisomal and mitochondrial b-oxidation is important for the utilization of fatty acids and the pathogenic development of U. maydis and may therefore represent a potential target to combat crop disease caused by fungal pathogens. In U. maydis, deletion of components of these pathways influenced mating, lead to a decrease in virulence, caused a defect in fatty acid metabolism, a loss of acetate metabolism and the accumulation of toxic intermediates. To further explore the role of the b-oxidation pathway during morphogenesis and pathogenic development, we have investigated the effects of several non-steroidal anti-inflammatory drugs (NSAIDs), which are known to interfere with b-oxidation functions at various stages. Diclofenac inhibited the usage of fatty acids of different chain length and saturation state as sole carbon sources and had an influence on the filamentation efficiency of those fatty acids. Further it showed fungicidal activity by inducing apoptosis, and influenced mating and pathogenic development. In an attempt to further elucidate the role of lipid utilization during infection we are investigating the role of PLs as a potential mechanism by which host-derived lipid signals or fatty acids for subsequent b-oxidation are generated. We present an analysis of 17 candidate PL genes identified by genome mining, as well as a preliminary functional characterization of these genes during mating and infection. Overall, our work demonstrates the utilization of host-lipids by U. maydis as an important nutritional and signalling source that is required for pathogenic development. Furthermore, we have begun to elucidate the underlying mechanisms involved which represent a potential target to combat crop diseases caused by fungi.
leaf surface which is a prerequisite for the differentiation of infection structures. Consequently, msb2 mutants are attenuated in virulence (Lanver et al., 2010). The molecular mechanism leading to an activation of Msb2 and the downstream MAP kinase cascade is so far unknown. In yeast Msb2p is processed by the aspartyl protease Yps1p leading to an active cell-associated form and a secreted glycosylated part which has an inhibitory function in the full length protein. In U. maydis Msb2 is also processed, but so far there is no evidence that this leads to an activation of surface sensing. Using a yeast mutant lacking five aspartyl proteases we could demonstrate that yeast Yps1p is able to cleave U. maydis Msb2. In addition, by using this heterologous system, two U. maydis aspartyl proteases were identified that were weakly able to cleave Msb2. The respective genes were deleted in the solopathogenic strain SG200 and its Dmsb2 derivative expressing Msb2-HA-GFP. Possible phenotypic alterations in virulence as well as in Msb2 processing will be monitored. In addition, a synthetic codon-adapted YSP1 gene has been introduced in the above-mentioned U. maydis strains to analyze the effects of an increment in Msb2 cleavage on biological activity of the protein. Finally, the extracellular domain of Msb2 was subjected to a mutational analysis to identify regions with a presumed positive regulatory function. Lanver, D., Mendoza-Mendoza, A., Brachmann, A. and Kahmann, R. (2010). Sho1 and Msb2-Related Proteins Regulate Appressorium Development in the Smut Fungus Ustilago maydis. The Plant Cell 22, 2085-2101.

622. The U. maydis effector Pit2 inhibits maize cysteine proteases to suppress host defense. Andre Mueller1, Sebastian Ziemann2, Steffi Treitschke2, Daniela Abmann1, Gunther Doehlemann1. 1) MPI for Terrestrial Microbiology, Karl-von-Frisch-Strabe 10, 35043 Marburg, Germany; 2) Fraunhofer ITEM-R, Biopark 1, Josef-Engert-Straße 9, 93053 Regensburg, Germany.

The basidiomycete Ustilago maydis is the causal agent of smut disease in maize. Infected plants show tumor formation in all infected aerial parts as prominent symptoms. As a biotroph pathogen, U. maydis depends on living plant tissue and hence efficient suppression of plant immunity is required. Therefore, infectious hyphae secrete effector proteins that interfere with specific components of the plant immune system. One such secreted effector-protein is Pit2 (Protein Important for tumor-formation 2), which, in a previous study, was found to be essential for tumor formation in infected plants [1]. Instead of tumors, necroses can be observed at infection sites indicating that plant defense and cell death reactions are triggered in Dpit2 infections [1]. Using a combination of yeast-two-hybrid- and protease activity assays, we could show that Pit2 acts as an inhibitor of apoplastic plant cysteine proteases whose activity is directly linked with salicylic acid (SA)-associated plant defenses. Sequence comparisons with Pit2 orthologs from related smut fungi identified a conserved 14 amino acid motif. Mutation of this motif leads to a loss-of-function of Pit2 and consequently to avirulence of U. maydis, suggesting that the protease inhibition by Pit2 is essential for plant infection. Moreover, synthetic peptides of the conserved motif show full activity as protease inhibitor. Interestingly, expression of only this motif in U. maydis partially restores virulence of the Dpit2-mutant, substantiating the important role of this novel protease inhibitor in suppression of host immunity. [1] Doehlemann et al. 2011. Mol Micobiol 81: 751-766.

623. The Ustilago maydis MAP Kinase signaling pathway: Identification of direct MAP kinase targets by phospho-peptide enrichment. Vikram Naik1, Gerold J.M. Beckers2, Wolfgang Hoehnert3, Regina Kahmann1. 1) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany; 2) Plant Biochemistry and Molecular Biology Group, RWTH-Aachen University, Aachen; Germany; 3) Department for Molecular Systems Biology, Faculty of Life Sciences, University of Vienna, Vienna, Austria.

In the plant pathogenic fungus Ustilago maydis three MAP kinase modules have been identified mostly via their homology to genes in Saccharomyces cerevisiae. The module consisting of the MAP kinase kpp2, the MAP kinase kinase fuz7 and the MAP kinase kinase kpp4 controls pheromone signalling and plays an essential role in mating and pathogenicity. Kpp2 is involved in filamentation and appressorium development while the MAP kinase, Kpp6, which also acts downstream of Fuz7, is required for appressorial penetration of plant epidermal cells. Our goal is to identify crucial virulence factors which act directly downstream of the MAP kinase modules Kpp2 and Kpp6. For this we generated a strain in which MAP kinase signaling can be induced by expressing a constitutively active version of the MAPKK Fuz7 (Fuz7DD) under an inducible promoter in the presence or absence of the MAPKK Kpp6. For this we generated a strain in which MAP kinase signaling can be induced by expressing a constitutively active version of the MAPKK Fuz7 (Fuz7DD) under an inducible promoter in the presence or absence of Kpp2 and Kpp6. We then used a two-step chromatographic procedure combining phosphoprotein enrichment using Al(OH)3-based MOAC for phosphopeptide enrichment. This enabled detection of low abundant phosphorylated peptides using LC-MS/MS and allowed direct identification and site-specific quantification of phosphorylated peptides that differentially accumulated after MAP kinase activation in wild type and mutant cells. LC-MS/MS analysis of the phosphopeptide fraction obtained after the two-step MOAC yielded 111 putative substrates of Kpp2 and Kpp6 MAP kinases in three replicate experiments. Of these 20 differentially phosphorylated proteins were chosen for subsequent functional analyses. We are presently generating deletion mutants of these genes in compatible U. maydis strains that carry different a and b alleles and in a solopathogenic strain. In addition, we are analysing the expression pattern of the chosen genes during the different developmental stages of U. maydis. Results on the role of these U.maydis genes on signaling and pathogenicity will be present.

624. See1: A novel organ specific effector in the Ustilago maydis - maize interaction. Amey Redkar1, Christoph Hemetsberger2, Ziba Ajami-Rashidi3, Virginia Walbot4, Gunther Doehlemann1. 1) Max Planck Institute for Terrestrial Microbiology, Department of Organismic Interactions, Karl von Frisch Strasse 10, Marburg, 35043 Germany; 2) Department of Biology, Stanford University, Stanford, California. 94305-5020 USA.

Ustilago maydis is a biotrophic smut fungus which infects all aerial organs of its host plant maize. The disease progression and development of infection is by reprogramming of the plant tissue which ultimately results in formation of tumors. This tumor induction is likely being triggered by small secreted proteins by the fungus, so called effectors. Given the fundamental differences between the different maize organs that are colonized by U. maydis, we hypothesized that the fungus deploys organ specific effectors to manipulate physiology and development of specific host tissues (1). To further investigate the role of individual organ specific effectors in modulating biotrophy, we in the present study identified a novel secreted protein, termed See1 (Seedling efficient effector 1) that is strongly induced in seedling leaves but only weakly expressed in tassels and ears. U. maydis deletion mutants for see1 show a strong reduction of tumor formation in maize seedlings but not in floral tissues. Laser scanning confocal microscopy shows that the mutant hyphae successfully enter the leaf tissue but might be blocked during pre proliferation stages in the mesophyll tissue of the leaf. Moreover, by labeling replicating DNA by 5-ethyl-2'-deoxyuridine (EdU) we observed that maize seedling colonized by Dsee1 do not show mitotic activity during infection, while cell division in leaves is specifically induced in wildtype infected host cells. In contrast, the Dsee1 mutant induces normal tumor formation in tassels and also shows the stable cell division rate in colonized anthers. Overexpression of see1 causes a hypervirulent phenotype only in the vegetative parts of the tassel, which are not transformed to tumors in wild type infections. To localize See1 during the disease progression we are applying confocal microscopy with live cell imaging using mCherry-tagged See1 protein. Most importantly, we are aiming for the identification of see1 interaction partners to link the observed phenotypes with its molecular function to understand its organ-specific function for U. maydis virulence. (1) Skibbe D*, Doehlemann G*, Fernandes J, Walbot V. (2010) maize tumors caused by Ustilago maydis require organ-specific genes in host and pathogen. Science 328:89-92.

625. Investigation of unconvenditionally secreted proteins in Ustilago maydis. Stefanie Reissmann1, Sina Krombach1, Florian Bochen1, Till Ringel1, Saskia Kreibich1, Thomas Bifort1, Kerstin Schipper2, Matthias Mann1, Regine Kahmann1. 1) Organismic Interactions, Max Planck Institute for Terrestrial...
Secreted fungal proteins play a crucial role during the biotrophic interaction between the smut fungus *Ustilago maydis* and its host plant *Zea mays*. In the last decade it has been well established that proteins without a signal peptide can also be targeted to the outside of the cell in an ER/Golgi independent manner. We want to identify such unconventional secreted proteins in *U. maydis* and investigate their potential function as pathogenicity factors. Our approach is based on affinity purification of tagged candidate proteins, detected in the apoplastic fluid of infected maize leaves. Four of twelve candidate proteins tested so far could be detected in culture supernatants. One candidate protein, Um11938, displays 55 % amino acid similarity to the human sterol carrier protein 2 (SCP2) and we were able to demonstrate that it co-localizes intracellularly with peroxisomes. Mammalian SCP2 is detected in peroxisomes but also found in the cytoplasm. It interacts with a variety of phospholipids as well as cholesterol and has been implicated in non-vesicular cholesterol transport and in regulating lipid rafts (Schröder et al., 2007). um11938 deletion strains of *U. maydis* are severely compromised in virulence and peroxisomal localization of Um11938 is required to fulfill its function as a pathogenicity factor. um11938 deletion strains display no growth defect on minimal media supplemented with different fatty acids as sole carbon source, suggesting that the Um11938 protein is not involved in bona fide b-oxidation. The results of ongoing experiments aimed to differentiate whether the pathogenicity relevant function of Um11938 is performed extracellularly or within the fungal peroxisomes will also be presented.


### 626. Identification of a key regulator for the developmental switch leading to sporogenesis in *Ustilago maydis*.

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*Ustilago maydis* is a biotrophic pathogen of maize. Its life cycle begins with the mating of two compatible haploid sporidia to form a dikaryotic infectious filament. After penetrating the plant cuticle, the dikaryon spreads inside the plant tissues and induces the formation of tumors. At a defined time of development, the sporogenesis program begins in tumor tissue: the hyphae start to fragment into individual cells that eventually differentiate into mature teliospores. The isolation of several mutants affected in sporogenesis has shown that a tight regulation of the cAMP signaling pathway and the activity of two transcriptional regulators Hda1 and Rum1, potentially functioning in the same chromatin modifying complex, are required. We have identified a new regulator for the sporogenesis program of *U. maydis*. It belongs to a family of transcriptional regulators that are characterized by the presence of a WOPR DNA binding domain. The best studied member of this family is Wor1 from *Candida albicans* which plays a key role in the switch from the non-pathogenic to the pathogenic form of the fungus. The *U. maydis* Wor1 homologue *um05853*, was deleted in the compatible haploid strains FB1 and FB2. The deletion mutants were able to mate and to infect maize as efficiently as the wild-type. However, although the tumor rate was similar, no spores could be detected in plants infected by the deletion mutants. Confocal microscopy of the mutant dikaryon revealed that hyphal fragmentation and consequently spore maturation were not occurring. The hyphae were still spreading at a time when the wild type dikaryon had already formed mature spores. To further investigate the role of *um05853*, we expressed the gene in a haploid strain where the filamentous program can be induced in axenic culture via expression of a functional b-heterodimer. The cells failed to switch to filaments, started to enlarge and showed septation, with each section containing one nucleus. This result suggests that Um05853 is able to counteract the b function and trigger fragmentation. Preliminary results show that Um05853 might downregulate several b-dependent genes including the gene encoding the master regulator Rbf1. We are currently identifying targets of Um05853 using a microarray approach and expect that these results will highlight how Um05853 controls spore formation.

### 627. Genetic characterization of virulence in the *Pyrenophora teres* - barley pathosystem.

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*Pyrenophora teres* f. *teres* is a necrotrophic fungal pathogen that causes net blotch (NFBN) on barley throughout the world. Several resistance sources have been identified but few are effective against all *P. teres* f. *teres* pathotypes, indicating that the pathogen has an arsenal of effectors that are involved in disease induction. Genetic analysis identified two barley genotypes, Rika and Kombar, that each harbor unique dominant susceptibility genes located at the centromeric region of barley chromosome 6H. *P. teres* f. *teres* isolate 15A is virulent on Kombar but avirulent on Rika whereas *P. teres* f. *teres* isolate 6A is virulent on Rika but avirulent on Kombar. Based on the necrotrophic effector model, 15A and 6A each secrete unique effectors that are directly or indirectly interacting with genes on chromosome 6H in Kombar and Rika, respectively. A linkage map was generated using a mapping population developed from a sexual cross between 15A and 6A, and 118 progeny were phenotyped on barley genotypes Rika and Kombar. Two major virulence QTL derived from 15A contributed to virulence on Kombar, and two additional unique virulence QTL derived from 6A contributed to virulence on Rika. All susceptibility loci in the host mapped to the same region on barley chromosome 6H. Therefore, it is likely that at least four necrotrophic effectors present throughout the *P. teres* f. *teres* genome are interacting with host susceptibility genes located in one region of barley chromosome 6H. These results strongly indicate that the NFBN-barley compatibility is at least partially due to necrotrophic effector-host susceptibility gene interactions that result in disease induction. Currently, we are using a genotyping by sequencing (GBS) approach to generate a saturated map to identify candidate genes in the QTL regions in order to clone and characterize the effector genes involved in the NFBN interaction.
We have recently obtained and characterized the knock out strains of the three MEKs present in the Cryphonectria parasitica genome, Cpkk1, Cpkk2 and Cpkk3, homologues of yeast Mkk1p/Mkk2p, Ste7p and Pbs2p, respectively. We tried to infect each of the knock-out strain with Cryphonectria hypovirus 1 (CHV1), a mycovirus causing hypovirulence: Dcppk1 and Dcppk3 were easily infected by CHV1 through anastomosis, but we failed to infect Dcppk2. We then showed that hyphal fusion was prevented in such knock-out strain: for this reason we attempted at infecting the Dcppk2 strain with two alternative protocols that overcome the hyphal phusion impairment: stable transformation of protoplasts with a cDNA infectious clone and transfection of protoplasts with viral RNA transcripts obtained in vitro from a CDA infectious clone. We originated infected strains with both protocols using wild-type C. parasitica protoplasts, whereas no stable infected strain was obtained starting from Dcppk2 protoplasts, which, on the contrary, could be transformed with the empty vector carrying only the resistance gene for selection. Given the uniqueness of such result, we are now trying to show that is the specific molecular impairment that prevents CHV1 maintenance in Dcppk2 strain. A proteomic approach was undertaken using 2-DE MALDI-TOF MS/MS and shotgun coupled to LC-MS/MS to compare the WT and Dcppk2 strains. A number of metabolic pathways are heavily impacted in the mutant. Of interest, proteins involved in folding, transport and trafficking, are up-regulated suggesting an altered protein turnover. Defence machinery is also up-regulated, indicating that the fungus perceives a stress situation. Moreover, a strong down-regulation of proteins involved in energy production and conversion was detected, indicating a possible reduction of the energetic metabolism. Among them are some GAPDH isoforms. Given the recent discovery of the role of Ryp1 in viral replication complex of RNA viruses, we obtained anti-GAPDH antibodies in order to study its possible role in CHV1 viral replication.
632. A genomic analysis of the infection strategies employed by *Phoma medicaginis* a necrotrophic fungal pathogen of alfalfa and the model legume *Medicago truncatula*. Angela H. Williams1,2, James K. Hane3, Robert D. Trengove3, Karam B. Singh3, Richard P. Oliver2, Judith Lichtenzeig1. 1) Murdoch University, Perth, Australia; 2) CSIRO Plant Industry, Perth, Australia; 3) Separation Science and Metabolomics Laboratory, Murdoch University, Perth, Australia; 4) Department of Environment and Agriculture and the Australian Centre for Necrotrophic Fungal Pathogens, Curtin University, Perth, Australia.

*Phoma medicaginis* is a necrotrophic plant pathogen that causes black spot of alfalfa (*Medicago sativa*) and the closely related model legume *Medicago truncatula*. It is a member of the Didymellaceae family, a distinct clade within the order Pleosporales which includes some of the most important pathogens of legume crops. We present here the first genome assembly of *P. medicaginis* and the results of investigations into the host-pathogen interaction, focusing on identification of necrotrophic effectors (NEs) using a combination of proteogenomic and transcriptomic analyses. A draft genome assembly was constructed using Illumina paired-end reads, *de novo* assembled into 952 nuclear scaffolds totaling 31.4 Mbp, with ~27 x coverage and encoding ~10,500 predicted proteins (~50 amino acids). Of these, ~1,000 are predicted to be secreted. Peptide sequencing via mass spectrometry was conducted in order to validate the gene set and characterize the protein content of intracellular and necrosis-inducing secreted fractions. This enabled the confirmation of 554 predicted genes and identified 162 proteins in the necrosis-inducing secreted fraction. To further validate the predicted gene set and examine differences in gene expression, the transcriptome was sequenced via RNA-seq at four important lifestyle phases. These included: 1) 1-5 days post infection of *M. truncatula*; 2) vegetative growth *in vitro*; 3) sporulation *in vitro* and 4) during growth in media where the culture filtrate produces necrosis and chlorosis when infiltrated into the plant. Close to 10,000 genes were expressed under one or more of these conditions with ~3,000 showing differential expression between the *in planta* and *in vitro* samples. The combination of proteogenomic and transcriptomic analyses has enabled the validation and fine-tuning of the majority of *de novo* predicted gene models. Several novel genes were identified via manual annotation of RNA-seq data. We have previously demonstrated that the genome is manipulable via Agrobacterium-mediated transformation which means that the functions of potential effector genes can be readily investigated. Collectively these data form a valuable resource from which a short list of effector candidates was derived and genes involved in the pathogenicity mechanisms of Didymellaceae fungi against their legume hosts were predicted.


*Aspergillus flavus* is an opportunistic pathogen of several plant hosts, including maize. This interaction is mediated in part by oxygenated polysaturated acids, or oxylipins, that are produced by both the fungus and the plant host. Although much has been learned about the synthesis of these oxylipins, how the fungus perceives them remains unknown. We hypothesize that G protein-coupled receptors (GPCRs) are responsible for receiving and transducing oxylipin signals in *A. flavus*. We have deleted and overexpressed two GPCRs, *gpc* and *gpD*, and found that they are important in regulating density-dependent development, which is thought to involve oxylipin signaling. Specifically, depletion of both *gpc* and *gpD* blocks the fungus into a low-density state, even when grown at high density. Furthermore, this mutant is unable to respond to spent medium of a wild type high-density culture. Inoculation of these mutants on corn kernels will ask whether Gpc and GpD are important for pathogenicity, and heterologous expression of GpC and GpD in *Saccharomyces cerevisiae* is being used to address questions concerning direct ligand-receptor activation.


The repertoire of secreted proteins defines the nature of interactions between microbe and host at the molecular level. Thus, cataloging and characterizing the list of secreted proteins from a given pathogen is a pivotal step in understanding molecular mechanisms of pathogenesis. Unlike bacterial and Oomycete pathogens, however, only a limited number of secreted proteins has been identified and analyzed in plant pathogenic fungi. Here we set out to identify and characterize new secreted proteins in the rice blast fungus. SingaIP program predicted a total 1,885 genes encoding secreted proteins in *M. oryzae*. We prioritized 15 genes, *MoSPE1* to *MoSPE15*, with T-DNA mutants available for in-depth analysis. To reveal their roles in pathogenicity, gene deletion mutants were generated and characterized their functionality. Deletion of *MoSPE1* rendered the fungus non-pathogenic, while deletion of *MoSPE3*, *MoSPE6*, and *MoSPE15* resulted in reduced virulence. Rice sheath inoculation of *DMoSpe1* and *DMoSpe15* showed that defects in pathogenicity could be attributed to the inability to grow inside plant tissues, suggesting their implication in interaction with rice. In addition, the two genes were indeed up-regulated during invasive growth in rice. Proteins encoded by *MoSPE1*, *MoSPE6* and *MoSPE15* were capable of being secreted in yeast secretion trap system. We believe that our work would reveal novel function of secreted proteins, providing new insight into fungal pathogenesis.

635. The biosynthesis of oxalate is entirely dependent on oxaloacetate acetylhydrolase in *Sclerotinia sclerotiorum*. X. Liang1, D. Liberti2, M. Li1, Y.-T. Kim1, R. Wilson1, J. Rollins1. 1) Plant Pathology Department, University of Florida, 1453 Fifield Hall, Gainesville, FL, 32611-0608; 2) Nunhems Netherlands BV, PO Box 4005, Haelen 6080 AA, Netherlands; 3) Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; 4) Environmental Biotechnology Research Centre, 125 Gwahak-ro, Yuseong-gu, Daejeon 305-806, Republic of Korea.

*Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most devastating necrotrophic fungal plant pathogens worldwide and its successful infection involves the accumulation of oxalate (up to 10 mM) in plant tissues. Oxaloacetate acetylhydrolase (EC 3.7.1.1), catalyzing the hydrolytic cleavage of oxaloacetate to form acetate and oxalate, has been shown to be the key enzyme catalyzing oxalate biosynthesis in *Aspergillus niger, Botrytis cinerea* and *Cryphonectria parasitica*. To dissect the genetic regulation of oxalate biogenesis and pathogenesis of *S. sclerotiorum*, the *S. sclerotiorum* oxaloacetate acetylhydrolase gene Ss-oah1 was functionally characterized. Previously we demonstrated that oxalate accumulation in *S. sclerotiorum* is under strong alkaline induction. Strikingly, *Ss-oah1* gene expression is regulated in the same manner; neutral pH strongly induces the accumulation of *Ss-oah1* transcripts and this pH induction is completely suppressed in the *Ss-pac1* knock out mutant. *Ss-oah1* knock out mutants fail to accumulate oxalate in culture and during plant infection and these phenotypes are restored by complementation with the wild type gene. These data demonstrate that *Ss-oah1*-catalyzed oxaloacetate hydrolysis is solely responsible for oxalate production in *S. sclerotiorum*. On all tested host plants, *Ss-oah1* knock out mutants are dramatically reduced in virulence and induce a strong host defense response. On leaves, *Ss-oah1* knock out mutants produce limited dark brown-green lesions compared with the spreading, necrotic, light brown lesions produced by the wild type. Host tissue bordering the lesion is clearly defined with a thin, dark zone and while the uninected leaf tissue becomes yellow and senescent the colonized area often retains chlorophyll reminiscent of “green islands”. In sum, our experimental data establish the key function of oxaloacetate acetylhydrolase in oxalate biogenesis and pathogenesis in *S. sclerotiorum* and indicate that the oah1 oxalate minus mutant retains some aspects of virulence but cannot suppress host defense.
FULL POSTER SESSION ABSTRACTS

636. In vivo efficacy of antifungal treatment of Aspergillus terreus infections and the influence on host immune response in Galleria mellonella. Elisabeth Maurer¹, Neill Browne², Kevin Kavanagh², Cornelia Lass-Flörl³, Ulrike Binder¹. 1) Division of Hygiene and Medical Microbiology, Medical University Innsbruck, Innsbruck, Tirol, Austria; 2) Medical Mycology Unit, Department of Biology, National Institute for Cellular Biotechnology, NUI Maynooth, Ireland.

Background Infections with Aspergillus (A.) terreus are of major concern, due to its high likelihood of dissemination and its intrinsic resistance to amphotericin B (ambB). The reason for this resistance is not known yet and the exact mode of ambB action is still not fully understood. Recently, three clinical isolates have been found to be ambB susceptible in vitro. In order to investigate for differences in virulence of the respective isolates, and to test the ambB efficacy in vivo, we used Galleria (G.) mellonella as an alternative model. Methods Virulence of ambB resistant and ambB susceptible A. terreus isolates was compared in the invertebrate model G. mellonella. Further, we performed in vivo infection studies with combined antifungal therapy, and additionally we investigated the potential effect of A. terreus infection and antifungal treatment on the G. mellonella immune system. Proteomic analysis of larval haemolymph, haemocyte counts and post-treatment infection studies were performed according to Kelly & Kavanagh 2011. Results Larval survival rates differed for the various isolates tested, resulting in highest mortality rate for one ambB susceptible isolate. Increase in survival was seen for all tested strains, when larvae where treated with voriconazole. Treatment with ambB only showed success in the groups infected with ambB susceptible strains. Antifungal administration in larvae resulted in an increased number of circulating haemocytes. Proteomic studies showed different protein expression of a number of proteins which have immune function. Pre-treatment of larvae with different antifungals also increased their resistance to Staphylococcus (S.) aureus infection, indicating a general ability of antifungals to prime the insect’s immune system.


Recognition and response to non self, whether conspecific (between individuals from the same species) or heterospecific (individuals from another species) is essential to many aspects of life including development, symbiosis and protection against pathogens. However distinction between these modes of recognition and responses is somewhat blurred and can overlap. For instance in plants and animals Pathogen Recognition Receptors (PRRs) can occasionally lead to auto-immune diseases in absence of pathogens. The NLR and NBS-LRR STAND proteins (a class of signal transduction proteins) are major PRRs in plants and animals, but these receptors remain largely unidentified in fungi. In Podospora anserina vegetative incompatibility (VI), a conspecific non self recognition process, leads to cell death and autophagy. VI is determined by interaction of het-c, encoding a glycolipid transfer protein, with members of the hwnwd gene family encoding for STAND proteins. hwnwd gene family members display the hallmarks of PRR encoding genes, including fast evolution promoting production of a repertoire of receptors and ability to initiate a cell death reaction. het-c is also showing signs of fast evolution. We hypothesized that these genes are involved in pathogen recognition and recognition that heterospecific non self would initiate a response similar to the VI reaction. In this context, VI can be considered as an autoimmune disease. We undertook the task of deciphering the response of P. anserina to heterospecific non self, focusing our efforts on the description of the cellular response and the identification of fungal PRRs. We show that P. anserina’s responses to another fungal species (Epicoccum nigrum), or to bacteria such as Serratia entomophila or Pseudomonas putida largely overlap the VI response at all levels investigated so far, including cellular morphology and cytology, requirement of autophagy and induction of the expression of a set of genes. We also provide evidence that het-c encoding the GLTP contributes to the response to non self and argue that this protein may be targeted by pathogen’s effectors. We develop efforts to identify PRRs involved in the initiation of these responses.


Worldwide potato harvests are strongly diminished due the late blight disease caused by the oomycete Phytophthora infestans. The fungus-like eukaryote and its interaction with host plants has been extensively investigated during the last decades whereas diverse research projects focus on its infection processes. P. infestans colonizes potato as well as tomato plants and thereby differentiates haustoria. Those barriers between host cells and invading pathogens are capable for exchange of nutrients, minerals but also of macromolecule like RNA molecules as shown for haustoria of parasitic plants. In oomycetes the exchange of effector protein from the pathogen to its host has been demonstrated. The movement of RNAi signals was shown in the interaction of parasites with their host plants and can be used to target not only plant genes but also genes of plant invading organism in a mechanism called host-induced gene silencing (HIGS). This technique has been applied for gene silencing in plant parasites as well as in nematodes and fungi. In oomycetes the RNA silencing is used as a standard method to characterize genes either by transient or by stable gene silencing and enzymes of the RNAi machinery have been identified. However, no efficient HIGS of oomycetes could be observed so far. We defined a P. infestans gene expressed during diverse developmental and infection stages of the oomycete as a HIGS target and could show that in planta expression of a HIGS hairpin construct targeting this particular gene in transgenic potato lines can be employed for late blight control. Our results present the appropriate processing of transformed HIGS hairpin constructs to siRNAs, their efficient function to silence the specific target gene sequence as shown in the reporter gene assays and subsequently reduced infection levels and diminished disease spreading on those transgenic HIGS potato lines in the field.

639. WITHDRAWN

Population and Evolutionary Genetics

640. Fertility in Aspergillus fumigatus and the identification of an additional ‘supermater’ pair. Céline M. O’Gorman¹, Sameira S. Swilaiman¹, Janyce A. Sugui², Kyung J. Kwon², Paul S. Dyer¹. 1) School of Biology, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom; 2) Molecular Microbiology Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA.

Aspergillus fumigatus is an opportunistic human pathogen that causes a range of allergic and invasive diseases in severely immunocompromised individuals, with a very high mortality rate typically in excess of 50%. A functional sexual cycle was discovered in 2009 and a highly fertile ‘supermater’ pair, AF862 and AFIR028, was later identified from a collection of 50 isolates. Here we describe the results of a larger, worldwide fertility screen and present an additional ‘supermater’ pair. A set of 126 clinical and environmental A. fumigatus isolates were crossed against two Irish reference strains of each mating type. A subset of the eight most-fertile strains was then tested in all pairwise combinations. The pairing of isolates 47-169 x 47-154 had consistently high
mating efficiency and outcrossing ability after four weeks, therefore it was chosen as an additional ‘supermater’ pair for community use in mating projects. It is important to have alternative tester strains to allow for unexpected mating differences when crossing isolates of diverse genetic origins. This is because factors such as heterokaryon incompatibility (het) loci and single nucleotide polymorphisms, can considerably influence sexual compatibility. The worldwide fertility screen found that approximately 85% of isolates are sexually fertile, indicating that sexual reproduction should be possible in nature when suitable environments are present. Next, the plasticity of sexual crossing conditions was tested, to determine whether they could be manipulated to increase fertility in crosses involving low-fertility strains of interest. A range of environmental and growth conditions were examined, including incubation temperature, CO₂ level, and oatmeal agar type. Fertility levels were significantly affected by certain parameters. Work is ongoing to integrate these factors to further optimize fertility in the ‘supermater’ pairs.

641. Understanding the dynamic plant pathogen Ramularia collo-cygni at both the sequence and field level. James Fountaine, Peter Hobe, Maciej Kaczmarek, Marta Piotrowska, Neil Havis. Crop and Soils Systems Research, Scotland’s Rural College, Edinburgh, Scotland, United Kingdom.

The fungus Ramularia collo-cygni is the major biotic agent involved in Ramularia Leaf Spot (RLS). The fungus produces necrotic lesions on leaves, primarily after flowering takes place in the host plant. Despite being initially reported on crops in Italy in the late 19th Century RLS only became an economic pathogen of barley in the late 20th Century. The geographical spread of the disease now covers much of Europe, North and South America and New Zealand. Research in the last decade, using molecular tools, has helped elucidate the life cycle of the fungus and has indicated a seed borne stage. These tools have also allowed detailed testing of spring barley archive samples, which has revealed a significant increase in pathogen levels since the 1990’s. The pathogen appears to develop rapid resistance in the field to fungicides and mutations conferring resistance have also been detected in the archive samples. Ramularia collo-cygni is currently classified as a member of the Mycosphaerella genera and sequence data derived within our group suggests a genetic similarity between R. collo-cygni, Mycosphaerella graminicola and M. fijensis. These sequences focus primarily on the genes associated with the target sites for fungicides, such as Beta tubulin, Cytochrome b, Succinate dehydrogenase and eburicol 14a-demethylase (CYP51) genes. This presentation will demonstrate our current knowledge of this fungal pathogen and highlight the newly obtained genome and transcriptomic data generated by the combined approach of illumina/solexa and Roche/454 sequencing. This combined approach has enabled the assembly of a complete genome sequence. The finished assembled genome of R. collo-cygni is 30.2 Mb and is currently to be found in 35S contigs. The complete annotation of this genome is currently underway using the FGENESH 2.6 software to generate first consensus gene calls. This approach will allow for comparative genome analysis in related genomes which will help to address the biology of R. collo-cygni in areas such as pathogenicity, population genetics and fungicide resistance. These advances should enable a greater understanding of the complex relationship between the fungus and host plant and furthermore, assist in the development of environmentally sound strategies to control this increasingly important disease of barley production systems.

642. Comparing germination dynamics of conidia and ascospores from natural isolates of Neurospera crassa. Kolea C.K. Zimmerman¹, Dan Levits², Anne Pringle¹. 1) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; 2) Max Planck Institute for Demographic Research, Rostock, Germany.

Many organisms experience high mortality during the first stages of growth. This is especially relevant in fungi because of the large ratio between propagules produced and the number of those propagules that germinate and grow into a mature mycelium. Furthermore, in many organisms, asexually reproduced progeny have higher survival rates compared to sexually reproduced progeny. We have developed a high throughput pipeline using flow cytometry to analyze spore germination dynamics and have applied this pipeline to study the germination differences between asexual and sexual spores. Specifically, we applied this pipeline to study variation in germination of Neurospera crassa conidia and ascospores from 32 natural isolates and crosses among these isolates, respectively. Using publicly available sequence data, we computed all pairwise genetic distances between the 32 strains and crossed strains of varying genetic distance from each other to determine the effects of parent relatedness on ascospore viability. We found that viability of asexual spores is higher than viability of sexual spores in most cases and there is no clear linear relationship between genetic relatedness and ascospore viability. Future work will include experiments to purge deleterious mutations. The data from these experiments will help us quantify the relative costs and benefits of asexual vs. sexual reproduction in Neurospera crassa and inform evolutionary theories on the evolution of sex.

643. Cryptococcus gattii and the origins of outbreaks. Tien Bui¹, Anna Foley¹, Leona Campbell¹, Patrick Brunner², Bruce McDonald², Dee Carter¹. 1) School of Molecular Bioscience, University of Sydney, NSW 2006, Australia; 2) Institute of Integrative Biology, ETH, 8092 Zurich, Switzerland.

Cryptococcus gattii and its sibling species C. neoformans cause cryptococcosis in humans and a range of animal. Strains within these species fall into a number of distinct molecular genotypes, and these vary in their ecology, geographic distribution, and various virulence-associated phenotypes. In C. gattii, molecular type VG1 is found worldwide, usually in warmer regions, and causes sporadic infection in apparently healthy people and animals. VG1 is more restricted in distribution, and while it also causes sporadic infection it is responsible for significant outbreaks that have expanded its geographic range into temperate areas. VGIII infections occur predominantly in immunocompromised hosts in the southern California region, and cases of VGIII infection are so far restricted to southern Africa, with a single case from India. Our interests lie in understanding the ecology and evolution of C. gattii in the environment, and how these relate to its ability to cause infection and outbreaks of disease. We have found the level of sexual recombination varies by molecular type, and that while in general the C. gattii population structure is sexual, this varies by genotype and in VGIII is punctuated by periodic, clonal lineages. Here we refine our analysis using extended MLST data, haplotype networks and coalescence theory. We find the level of diversity among global VG1 and VGII genotypes is highly constrained and comparable to some recently evolved plant pathogens, while the more geographically restricted VGIV genotype is substantially more diverse. Outbreak VGIV clones are highly derived with an apparent history of expansion and extinction events. Evidence for purifying selection occurs at the master regulator of mating type for VGIII, suggesting recombination is important in the generation of outbreak lineages.

644. Evolution of the mating type locus in the species within and closely related to the pathogenic Cryptococcus species complex. Sheng Sun, Josh Granek, Joseph Heitman. Molecular Genetics & Microbiology, Duke University, Durham, NC.

C. amylolentus is the most closely related sister species to the pathogenic Cryptococcus species complex that includes the common human pathogenic fungi Cryptococcus neoformans and Cryptococcus gattii. We recently reported that C. amylolentus has a tetrapolar mating system, in which mating type is determined by two unlinked mating type (MAT) loci (A and B) that are located on different chromosomes. This is in stark contrast to the mating systems in the species within the pathogenic Cryptococcus species complex, where all the species are bipolar and have a contiguous large (>100 kb) MAT locus. Thus, analyzing the tetrapolar MAT loci of C. amylolentus could provide insights into how the derived bipolar MAT locus in the pathogenic Cryptococcus species complex evolved. In this study, we first provide a fully detailed characterization of both alleles for each of the two C. amylolentus MAT loci, illustrating expansion of the A MAT locus in C. amylolentus, as well as the chromosomal rearrangements between the alleles from the opposite
maturing types. Additionally, by analyzing meiotic progeny of *C. amylolentus*, we found evidences that both MAT loci are linked to their respective centromeres. Using both genetics and genomics techniques, we further narrowed down the candidate centromeric regions to be located within 150 and 100 kb from the A and B MAT loci, respectively. Furthermore, genome comparison between *C. neoformans* and *C. amylolentus* showed that the majority of centromeres in *C. neoformans* are flanked by sequences from different chromosomes in *C. amylolentus*, indicating that ectopic recombination within centromeric regions may frequently lead to chromosomal translocations. We propose a model in which the large bipolar MAT locus that is present in the pathogenic *Cryptococcus* species complex originated through ectopic recombination in the centromeres (possibly mediated by common repetitive sequences present in the centromeric regions). This process brought together the two MAT loci of the ancestral tetrapolar mating system onto the same chromosome, and subsequent chromosomal rearrangements (inversions and transpositions) resulted in the current state of the MAT locus seen in the derived bipolar pathogenic *Cryptococcus* species.

645. Evolutionary history and genetic diversity of *Exobasidium* sp., the cause of an emerging disease of blueberry. Marin Brewer, Ashley Turner. Department of Plant Pathology, University of Georgia, Athens, GA.

Emerging fungal diseases, usually the result of pathogen introductions, the evolution of virulent races, or adaptation to new niches, are an increasing threat. *Exobasidium* fruit and leaf spot of blueberry has rapidly increased in incidence in the southeastern USA over the past two years. We took a phylogenetic approach to understand the evolutionary history of this fungus. We sequenced the LSU-rDNA region from nine isolates collected from fruit or leaf spots of *Vaccinium* spp. from Georgia and North Carolina. Additionally, sequences from GenBank with high similarity to the emerging parasite and from *Exobasidium* spp. parasitizing other *Vaccinium* spp. in North America were obtained. The sequences were assembled, aligned and subjected to phylogenetic analyses. Results indicated that *Exobasidium* sp. from blueberry in the southeastern USA is unique and distinct from *Exobasidium* sp. that causes a leaf spot on lowbush blueberry in the northeastern USA and Canada. Both species, however, are genetically different from other *Exobasidium* spp. that cause diseases on cranberry and blueberry, and from *E. vannini* from *V. vitis-idea*. Results also suggested that while the Southeast the parasite is not genetically differentiated based on blueberry host species or cultivar, host tissue (fruit or leaf), or geographic region. To further investigate diversity and population structure of the parasite in the Southeast we sequenced ITS for 80 isolates from diverse host species, cultivars, and locations. We obtained 75 unique sequences, which is an extremely high level of diversity and is unexpected for any fungus let alone one causing an emerging disease. The high diversity indicates that the fungus causing *Exobasidium* fruit and leaf spot in the Southeast is not an evolutionarily young species and that the recent increase in incidence is not a result of increased aggressiveness or a recent host switch. Analyses of genetic differentiation of the ITS sequences confirm our findings with LSU-rDNA that isolates within the Southeast are not differentiated by host species or cultivar, host tissue, or geographic region, suggesting that a single population is causing this disease of blueberry across the Southeast. We hypothesize that an environmental change is responsible for the recent emergence of this disease.

646. Microsatellite markers reveal population structure and genetic diversity in the blueberry pathogen *Monilinia vaccini-corymbosi*. Kathleen M Burchhardt, Marc A Cubeta. Department of Plant Pathology, North Carolina State University, Raleigh, NC.

The ascomycete *Monilinia vaccini-corymbosi* (Mv) is a widespread fungal pathogen of blueberry (*Vaccinium* spp.) in North America. Both sexual and asexual sporulation are required within a season for the fungus to complete its life cycle. Overwintered infected fruit (mummies) produce apothecia that release aerially dispersed ascospores which infect newly emerging blueberry shoots, resulting in blighting of infected tissues following by production of conidia. Insect pollinators deposit conidia on flowers that infect the ovary through the gynoecial pathway, leading to fruit mummification. The primary objective of our research was to use population genetics-based approaches to examine genetic diversity, structure, and gene flow among populations of *Mv* throughout the United States. A total of 437 samples from 18 blueberry fields in 10 states (one field in GA, MA, ME, MI, MS, NJ, NY, OR, and WA and 9 fields in NC) were analyzed with 10 microsatellite markers. Population genetic analyses supported population structure and high intraspecific genetic diversity, with 203 unique multilocus haplotypes (MLHs) identified from the samples. However, there were differences in genetic diversity and population structure based on locality and host species. Low genetic diversity and selfing were suggested based on analysis of samples of infected shoots or fruit collected from rabbiteye (*V. virgatum*) varieties in MS, GA, and five fields in NC. Only three unique MLHs were identified from analyzing the 141 samples collected from the seven fields, with two of the unique MLHs detected within four and five of the fields, respectively. At least 12 unique MLHs were detected within all other fields except OR, with all MLHs being exclusive to their field of origin. Samples from the 10 fields were collected from either infected shoots of rabbiteye, northern highbush (*V. corymbosum*), or southern highbush (*V. corymbosum* × *V. darrowii*), or from infected fruit of northern highbush or lowbush (*V. angustifolium*). Analysis of molecular variance and the software STRUCTURE supported significant genetic differentiation among these isolates, indicating restricted gene flow. The majority of microsatellite markers were in linkage equilibrium within the fields, suggesting random mating. Future research will examine the potential for host specialization of isolates of *Mv*.


*Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat, is an economically significant necrotrophic fungal pathogen. In Australia, tan spot is the most damaging wheat disease, resulting in yield losses of $212 million per annum. The disease was first recorded in Australia in the 1950s, some ten years after it was initially reported on wheat in the USA. Here, we examine the genetic diversity of a collection of Australian *P. tritici-repentis* isolates using microsatellites. We discuss relatedness and structure, and consider the findings in the broader context of biogeography.

648. WITHDRAWN

649. Fungal community composition analysis by Internal Transcribed Spacer (ITS) sequencing using Illumina MiSeq. Robin A. Ohn1, Julien Tremblay2, Kanwar Singh1, Feng Chen1, Claude Murat1, Matthias Hess12,3, Francis Martin4, Susannah G. Tringe5, Igor V. Grigoriev6. 1) US DOE Joint Genome Institute, Walnut Creek, CA, USA; 2) Systems Biology & Applied Microbial Genomics Laboratory, Washington State University, USA; 3) Chemical and Biological Process Development Group, Pacific Northwest National Laboratory; 4) Lab of Excellence ARBRE, Tree-Microbes Interactions Department, INRA, Nancy, France.

Fungal species identification and community surveys relied for a long time on Internal Transcribed Spacer (ITS) sequencing using the Sanger platform. Later, 454 (Roche) pyrosequencing was used for the same purpose, capturing shorter ITS1 or ITS2 fragments, or more recently the entire ITS region using longer 454 XLR reads. The Illumina sequencing platform has now largely surpassed the 454 in terms of read quantity and quality (e.g., HiSeq2000 yields of up to 600 Gb in a single run) but the length of produced reads (up to 150 bp in HiSeq2000) is insufficient for ITS analysis. Illumina’s newly-introduced MiSeq sequencing platform can produce paired-end 250 base reads in a single day run, which, when combined, would cover most of either ITS1 or ITS2 regions.
651. Alkaloid genotype profiling of tall fescue endophytes to determine influence of ancestral progenitors. J.E. Takach, C.A. Young. Forage Improvement Division, The Samuel Roberts Noble Foundation, Ardmore, OK. 

Epichloë endophytes, comprised of Epichloë and asexual Neotyphodium species, associate with cool-season grasses such as the agronomically important forage tall fescue (Lolium arundinaceum syn Festuca arundinacea). This mutualistic symbiosis provides the plant host with protection from animal and insect herbivory through the production of multiple classes of bioactive alkaloids (ergot alkaloids, indole-diterpenes, loline, and peramine) by the endophyte partner. Many Neotyphodium species, including the endophytes present in tall fescue (Ne. coenophialum, Festuca arundinacea) taxonomic groups FaTG-2 and FaTG-3) arise from interspecific hybridization events and contain genomic information from multiple ancestral progenitor species. As such, hybrid Neotyphodium species are capable of producing multiple classes of alkaloids and can contain multiple copies of the loci from required for alkaloid production. Significant genetic and chemotypic diversity has been reported for tall fescue endophytes but few studies have assessed this diversity at a population level. The incidence and diversity of tall fescue endophytes present in tall fescue germplasm was evaluated using PCR-based genotype profiling of seed from a set of 97 tall fescue accessions obtained from the Germplasm Resource Information Network (GRIN). A total of 71 endophyte-infected accessions were identified from both Continental (summer-active) and Mediterranean (summer-dormant) tall fescue germplasm. Genotype profiles from the GRIN tall fescue collection were compared to previously characterized tall fescue endophytes in order to predict the species and probable chemotype. Variation based on presence and absence of genes within the loci required for each alkaloid indicated likely chemotypic diversity among and between species. The copy number of selected alkaloid genes was determined by sequence analysis of PCR amplicons. The ancestral progenitor origins of mating type and alkaloid genes were inferred from phylogenetic analyses of partial gene sequences. These results support prior evidence that multiple alkaloid gene copies are the result of inheritance, not post-hybridization gene duplication, and suggest that multiple independent hybridization events have occurred during the evolutionary life history of tall fescue endophytes.

652. Evolution of the pan-secretome among lineages of Magnaporthe oryzae attacking different host-plants. E. Fournier, E. Ortega-Abboud, L. Mallet, H. Chiapello, C. Guérin, F. Rodolphe, A. Gendrault, J. Amselem, M-H. Lebrun, T. Kroj, D. Tharreau. 1) INRA, BGPI lab, INRA, Montpellier, cedex 5, France; 2) CRAD, BGP lab, TA 54K, 34398 Montpellier; 3) INRA, MIG lab, 78352 Jouy-en-Josas, France; 4) INRA, URGI lab, 78026 Versailles, France; 5) INRA, BIA lab, 31326 Castanet-Tolosan, France; 6) INRA, BIOGER lab, 78850 Thiverval-Grignon, France.

Over the past decade, considerable advances have been made in the understanding of the role of fungal effectors, and especially small secreted proteins (SSPs), in the infectious process. NGS technologies offer powerful tools to study, at the genomic scale, how deep are SSPs involved in the adaptation of fungal populations to different hosts plants. We addressed this question in the plant pathogenic fungus Magnaporthe oryzae, the agent of blast on rice and other Poaceae. This species encompasses isolated genetic lineages specifically attacking different hosts. In the GEMO project, we sequenced eight strains of M. oryzae representing different genetic groups pathogenic of different species of Poaceae (5 strains attacking rice Oryza sativa, 1 attacking wheat Triticum sp., 1 attacking foxtail millet Setaria sp., 1 attacking finger millet Eleusine sp.), and one strain of the sister species M. grisea (attacking fonio millet Digitaria sp). The nine genomes have been sequenced using NGS technologies (454 and Solexa/Illumina) and assembled by the Genoscope (Ervy, France). We included the public reference strain of M. oryzae 70-15 in our analyses. Gene annotation and orthology predictions have been carried out. We also annotated transposable elements and assessed the amount of horizontal transfers. Here we present the characterization of the repertoire of SSPs in the nine genomes, established using classical predictors of peptide signals (SignalP), transmembrane domains (TMHMM), GPI anchors (PredGPI) and subcellular location assignment (TargetP). These lists were then curated using two complementary approaches: systematic tBlastn searches of the SSP predicted in each genome against the nine genomic databases of the project (including its own), and gene mining through the RNAseq analysis of the in planta transcriptome of one of the strain. We will compare these lists with orthology predictions to analyze the core-secretome and the dynamics of gains/losses/duplications of SSPs in the different lineages. We will also address the question of co-localization of SSPs with transposable elements. Finally we will search for signatures of adaptive evolution in SSPs.

653. Exploiting the high evolutionary potential of Leptosphaeria maculans minimises severity of blackleg disease of canola. Steve J. Marcroft, Angela P.
Van de Wouw1, Barbara J. Howlett2. 1) MacRostie Grains Pathology, Grains Innovation Park, Horsham, 3400, Vic., Australia; 2) School of Botany, the University of Melbourne, 2010, Vic., Australia.

Blackleg caused by *Leptosphaeria maculans*, is the most important disease of *Brassica napus* (canola) worldwide. Field populations of this sexually outcrossing fungus rapidly adapt to selection pressure from extensive sowing of varieties with major gene resistance and can ‘overcome’ resistance. This high evolutionary potential of the fungus is reflected in its genome structure. Effectors genes are embedded in AT-rich, gene-poor regions with transposable elements that have been degenerated by Repeat Induced Point (RIP) mutations. Thus effectors are easily gained, lost or inactivated. For the last decade we have monitored virulence of blackleg populations and disease severity of varieties in field trials across Australia. In 2003 after two seasons of extensive sowing, blackleg resistance of a set of varieties ‘broke down’ in the Eyre Peninsula, South Australia, causing 90% yield losses and withdrawal of these varieties from sale. By 2005, virulence of populations towards these varieties declined appreciably. Thus the blackleg-canola interaction behaves in a ‘Boom and Bust’ manner. Analysis of isolates collected before and after the resistance breakdown showed that deletions, RIP mutations and amino acid substitutions accounted for rapid evolution of four linked effectors, including the avirulence gene complementary to the resistance gene that had been overcome. After this resistance breakdown, Eyre Peninsula farmers sowed varieties with a different source of resistance. However in November 2011 significant levels of disease in trial sites and commercial paddocks were observed, which led to a warning in February this year that these varieties should not be sown. Growers heeded this advice and sowed varieties with different resistance sources. Our prediction of a resistance ‘breakdown’ was vindicated, as this variety had high disease levels in field trials on Eyre Peninsula, but not in other canola-growing regions. Commercial crops of other varieties on Eyre Peninsula had only low levels of disease. By sowing other varieties, not only have farmers have been saved $20 million (based on conservative estimates of area sown, predicted yield loss and current canola prices), but seed companies have been able to sell the ‘at risk’ varieties in other canola-growing regions, where resistance breakdown was not predicted.


Kin selection can favour cooperation between individuals. This requires assortment between genetically related individuals and genetic kin recognition is the predominant means to achieve this. However, Crozier realised that the diversity of kin-recognition alleles necessary for kin recognition, observed in many social organisms, poses a paradox: common alleles will receive more cooperation than rare alleles, and therefore increase in frequency, thus eroding genetic kin recognition diversity. We provide experimental evidence for Crozier’s theoretical prediction using somatic fusion between fungal individuals (mycelia) as a model for cooperation. Using fusion mutants and incompatible strains, we first show that fitness is strongly correlated with the degree of fusion, which demonstrates that fusion between mycelia is mutually beneficial. We then experimentally demonstrate Crozier’s prediction that positive frequency-dependent selection erodes kin-recognition diversity.

655. A completely unknown lifecycle in mushrooms: cyclical inbreeding and haplo-diploidy. Duur K. Aanen1, Tim Möhiman1, Eric Bastiaans1, Bart Nieuwenhuis1, Bertha Koopmanschap1, Thomas W. Kuyper1. 1) Plant Science Group, Wageningen University, Wageningen, Netherlands; 2) Department of Soil Quality, Wageningen University, Wageningen, The Netherlands.

*Mycena galericulata* (Basidiomycota, Agaricales) occurs in two forms, a clampless with two-spored basidia and a clamped with four-spored basidia. It is generally accepted that the two-spored form is haploid asexual (apomictic), and the four-spored form sexual (dikaryotic and heterothallic). In order to study the interrelationship between both forms, we performed mating tests and phylogenetic and genetic analyses of a sample of both forms. Surprisingly, our results are inconsistent with any currently known life-cycle. While the four-spored form is heterothallic indeed, we show that the two-spored form is diploid, and produces diploid spores via intratetrad selfing. However, the absence of genetic differentiation between both forms, and the high degree of heterozygosity in the two-spored form, indicate that the two-spored form frequently arises from the four-spored. We hypothesise that the two-spored form can again give rise to four-spored forms. Consistent with this, we discovered that a small percentage of fruiting bodies has both two-spored and four-spored basidia.

656. Diversity and evolution of ABC proteins in basidiomycetes. Andriy Kovalchuk1, Yong-Hwan Lee1,2, David Hibbet3, Fred O. Asiegbu4. 1) Department of Forest Sciences, University of Helsinki, Finland; 2) Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea; 3) Department of Biology, Clark University, Worcester MA 01610, USA.

ABC proteins constitute one of the largest families of proteins. They are implicated in a wide variety of cellular processes ranging from ribosome biogenesis to multidrug resistance. With the advance of fungal genomics, the number of known fungal ABC proteins increases rapidly, but the information on their biological functions remains scarce. In this work, we extended our previous analysis of fungal ABC proteins to include recently genome sequenced species of basidiomycetes. We performed an identification and initial cataloguing of ABC proteins from 23 new species representing 10 orders from within the class of Ascomycotina. To identify gene loci encoding ABC proteins in the fungal genomes, multiple tblastn and blastp searches against selected genomes were performed at the website of the Fungal Genomics Program of the Department of Energy Joint Genome Institute (JGI). Sequences of Coprinopsis cinerea ABC proteins representing all known subfamilies were used as queries. Phylogenetic analysis was performed with the program package MEGAS using neighbor-joining, minimum evolution and maximum likelihood algorithms and bootstrapping with 500 replicates. ABC proteins of each species were separated into subfamilies by their comparison with *S. cerevisiae*, *C. cinerea*, *C. neoformans* and *U. maydis* proteins. Set of ABC proteins identified in basidiomycetes and ascomycetes were compared, and their common features and principal differences are discussed. Two groups of ABC proteins specific for basidiomycetes were identified. Results of the survey should contribute to a better understanding of evolution of ABC proteins in fungi and support further experimental work on their characterization.

657. Co-evolution and life cycle specialization of plant cell wall degrading enzymes in a hemibiotrophic pathogen. Patrick C. Brunner1, Stefano F. F. Torriani1, Daniel Croll1, Eva H. Stukkenbrock2, Bruce A. McDonald1. 1) Integrative Biology, ETH Zurich, Zurich, Switzerland; 2) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany.

Co-evolution of species has long been recognized as a driving force in generating and maintaining biodiversity. Co-evolution is an ubiquitous phenomenon investigated in prey and predator, plant and herbivore, or mutualistic interrelationships. However, signatures of co-evolution are likely to be strongest in host-pathogen systems because of the strong selective pressures that each can exert directly on the other. While traditional studies mainly sought phenomenological evidence for co-evolution, more recent approaches look directly at the molecular/gene level. We hypothesized four main scenarios for host-pathogen co-evolution and predicted the corresponding genetic signatures. We combined comparative genomics, transcriptomics and selection analyses to investigate genes that are likely affected by co-evolution to assign them to one of these scenarios. *Zymoseptoria tritici* is an important fungal pathogen on wheat and has two closely related sister species *Z. pseudotritici* and *Z. ardeabiliae* that infect wild grasses. This recently
emerged host-pathogen system provides a rare opportunity to investigate the dynamics of gene evolution by natural selection within and between species and on different hosts. Here, we focused on evolution of plant cell wall degrading enzymes (PCWDEs) secreted by the fungus. We found widespread differential transcription among different members of the same gene family, challenging the idea of functional redundancy and suggesting instead that specialized enzymatic activity occurs during different stages of the pathogen life-cycle. We also found that natural selection has significantly affected at least 19 of the 48 identified PCWDEs. The majority of genes showed signatures of purifying selection, typical for the scenario of conserved substrate optimization. However, six genes showed diversifying selection that could be attributed to either host adaptation or host evasion. This information can be used to determine which genes are the most appropriate targets for subsequent wet lab experimentation to elucidate enzymatic function during relevant phases of the pathogen life cycle.

658. Recombination landscape of the plant pathogenic fungus Zymoseptoria tritici (syn. Mycosphaerella graminicola). D. Croll, M. Lendenmann, E. Stewart, M. Zala, B.A. McDonald. ETH Zurich, Zurich, Switzerland. Recombination is a fundamental process driving the evolution of genomes. The rate of recombination influences the level of genetic variation in populations and the efficacy of selection. Furthermore, heterogeneity in recombination rates along chromosomes shapes the genetic architecture of phenotypic traits. Hence, the evolution of virulence and other traits in pathogenic fungi critically depends on the rate of recombination. Despite the importance of recombination, the rate and homogeneity of recombination in fungal chromosomes is poorly understood. We analyzed 60 progeny from a controlled sexual cross between two isolates of the wheat pathogen Zymoseptoria tritici (syn. Mycosphaerella graminicola). We genotyped parental strains using whole-genome resequencing and we generated progeny genotypes by restriction site-associated DNA sequencing (RADseq). We obtained a total of 46’037 single nucleotide polymorphisms (SNP) segregating among the progeny. Based on the physical and genetic map locations of the markers, we showed that recombination rates were strongly heterogeneous among chromosomes and were influenced by gene density and GC content. We located multiple chromosomal hotspots of recombination that were interspersed by large segments of low recombination rates. Furthermore, we found that chromosomal regions that were enriched in SNP and indels showed lower recombination rates compared to less diverged regions. The local variation in recombination rates in Z. tritici may have significant effects on the evolutionary potential of different genomic compartments. Hence, heterogeneity in recombination rates may play an important role in the evolution of virulence.

659. The evolution of Sfp1 mediated, cell size control in Ascomycete fungi. Toni M. Delorey1, Jenna M. Pfiffner1, Sushmita Roy2, Jay Konieczka1, Dawn A. Thompson3, Aviv Regev4. 1) Broad Institute, 7 Cambridge Center, Cambridge, MA 02139; 2) Wisconsin Institute for Discovery (WID), 330 N. Orchard St, Madison, WI 53715.

Divergence in gene regulation can play a major role in evolution. We used a phylogenetic framework to measure mRNA profiles in 15 yeast species and reconstruct the evolution of their modular regulatory programs. We found that modules diverge with phylogenetic distance, with prominent regulatory changes accompanying changes in lifestyle and ploidy. Gene paralogs have significantly contributed to this regulatory divergence. To explore the role of trans regulator duplication, we examined Sfp1, as gain or loss of the Sfp1 binding site underlaid regulatory rewiring of carbon metabolism. In S. cerevisiae, Sfp1, a TOR target, activates transcription of “growth” genes. S. cerevisiae, sfp1Δ mutants have smaller cells and slower growth, suggesting that these phenotypes are intertwined. However, we show that duplication of SFP1 in other yeast species has resulted in sub- and neo-functionalization of regulatory programs controlling growth rate and cell size. In particular, in S. castellii, the two Sfp1 paralogs have subfunctionalized; one controls cell size while the other controls growth. Therefore, we hypothesize that Sfp1 regulation of ribosomal biogenesis underlies growth rate while cell size is mediated by a different, unidentified function. To better understand Sfp1-mediated cell size control, we used a two-tiered analysis system of comparing gene expression and ChIP Seq data to distinguish indirect or direct Sfp1 targets. Expression programs and phenotypes of sfp1Δ mutants were analyzed in S. cerevisiae, C. glabrata, S. castellii, K. lactis and S. pombe. To identify putative cell size regulators, we examined differentially expressed orthologs in species where sfp1Δ mutants had a small size phenotype (S. cerevisiae, C. glabrata and one paralog of S. castellii), and excluded genes involved in ribosomal biogenesis and those differentially expressed genes in species where sfp1 mutants grew slower but had normal cell size (K. lactis and S. pombe). We found 17 overlapping orthologs including a promising candidate for cell size regulation; the S. cerevisiae ortholog, Ard1, involved in telomeric silencing, and cell cycle control. Finally, we found that Sfp1 binds to the SCH9 promoter in S. cerevisiae and S. paradoxus. Sch9 is a kinase and mutants have reduced cell size. From these findings, we present a novel model for cell size regulation.


Identifying the genes for ecologically relevant traits is a central challenge in empirical population genetics. Species distributed across strong environmental gradients are excellent models to discover and identify the genetic targets of local selection as they are more likely to experience spatially heterogeneous selection pressures leading to local adaptation of ecologically important traits. We studied the origin of ecological differentiation in N. discreta phylogenetic species 4 (PS4), a species with a broad latitudinal distribution. We Illumina-sequenced the complete genomes of 52 individuals representing 8 collections sites in Alaska, New Mexico, Washington, California, and Western Europe (average sequencing depth: 52X). Reads were mapped to the N. discreta PS4 reference genomes, and analyses were based on a final set of ca. 1.2 million high-quality SNPs. Phylogenetic analyses identified four well-supported clades. Papua New-Guinea individuals formed the most basal clade. Individuals from Alaska and Europe on the one hand, and from New Mexico on the other hand grouped into sister clades, and individuals from California were basal to these two clades. Individuals from Washington, sampled within the same site, grouped with either the New Mexico individuals, or the California individuals, indicating the coexistence in sympatry of two divergent populations. The observed pattern of population subdivision is being used as a reference to identify genes departing from the genome-wide background, and showing increased divergence consistent with divergent selective pressures, or decreased divergence consistent with gene-flow. Our findings emphasize the need to continue exploration to uncover divergent populations of Neurospora, and place N. discreta, along with N. crassa, among the handful of species that have the attributes to serve as outstanding evolutionary and ecological model organisms.

661. WITHDRAWN

662. Evolutionary genomics of NRPS gene clusters in Beauveria and its allies. J-G. Han1, J. Oh1, M.-W. Hyun1, B. Shrestha1, G.-H. Sung1. 1) Mushroom Research Division, Rural Development Administration, Suwon 441-707, Republic of Korea; 2) College of Pharmacy, Chung-Ang University, Seoul 156-756, Republic of Korea; 3) Department of Microbiology and Institute of Basic Sciences, Dankook University, Cheonan 330-714, Republic of Korea.

Beauveria is an ascomycete asexual genus that comprises of 12 species of insect pathogens and linked to its teleomorphic stage of Cordyceps. Among species of Beauveria, Beauveria bassiana is economically important for its use as biological control agent and produces several secondary metabolites such as beauvericin and bassianolide, which are the causal metabolites of entomopathogenicity. Genes involved in these secondary metabolites are including
nonribosomal peptide synthetase (NRPS) that are often physically clustered. To more understand the origins and evolution of these genes, we performed genome-wide comparative analyses among B. bassiana and its allied species including B. pseudobassiana, B. sungeri, Cordyceps militaris, C. pruinosa, Isaria tenuipes, and I. farinosa after assembling these genomes. Initially, all the potential gene clusters for secondary metabolites were predicted using antiSMASH. In B. bassiana, 16 NRPSs and 3 NRPS-PKS hybrid modules were estimated, while in C. militaris, 5 NRPSs and 3 hybrids were found.

Interestingly, the presence/absence of NRPSs and PKSs provides a clue in generating their evolutionary hypotheses (e.g., horizontal gene transfer and gene fusion). For example, bissianolide synthetase were specifically found in the species referred to Beauveria with the syntenic conservation of genes encoding calreculin, cycliphilin, DNA replication complex, IdgA domain protein, and phenol 2-monoxygenase. The inferred phylogeny based on the acyltransferase (AT) domain showed that ATs of beauvericin synthetase gene belong to two independent lineages, suggesting that beauvericin synthetase is a fusion gene which is formed from two previously separated genes. It is also suggested that bissianolide synthetase in Beauveria spp. are possibly acquired by horizontal gene transfer (HGT) from distantly related fungi.


The study of plants and animals has yielded a host of theories for the evolution of outbreeding and selfing mating systems, yet these theories have yet to be extended to microbial organisms, where they might be more easily challenged. Within the genus Neurospora there is evidence of multiple shifts to selfing (homothallic) from outcrossing (heterothallic) ancestral states. However, the most well-studied clade, the members of which produce brightly colored macroconidia, contains no homothallic members, and there is only one known clade of aconidial heterothallic Neurospora from which homothallic Neurospora could have evolved (Nguyen et al. 2011, Glass et al. 1990). We hypothesized that sampling of heterothallic Neurospora has been biased toward members that produce brightly colored macroconidia or perithecia in isolation, thereby ignoring aconidial heterothallic species. Collections of cryptic heterothallic Neurospora would allow comparison of the evolutionary forces acting on closely related pairs of selfing and outcrossing species. To this end, we have isolated 1 new strain of heterothallic Neurospora and 9 new strains of homothallic Neurospora from soil from two locations in California. Based on partial sequences of nik-1 (Nguyen et al. 2011) we constructed a phylogeny of Neurospora including the new strains. Surprisingly, the new heterothallic Neurospora was found to be almost identical to the only other aconidial, heterothallic Neurospora individual (Glass et al. 1990), despite over 2,300 km of separation. Seven of the eight sequenced homothallic strains were found to be most closely related to Neurospora novoguineensis, but the remaining strain was more closely related to the aconidial, heterothallic species. These remaining results suggest that additional sampling to obtain sufficiently large populations of related, aconidial heterothallic and homothallic Neurospora would facilitate studies of the transition from outbreeding to selfing as well as studies of the genetic basis of the development of macroscopic, brightly colored conidia. References, Glass, N. L., Metzenberg, R. L. & Raju, N. B. Experimental Mycology 14, 274-289 (1990). Nguyen, K. et al. Molecular phylogenetics and evolution 59, 649-63 (2011).

664. Profiling conditionally dispensable chromosomes of the plant-pathogenic fungus Zymoseptoria tritici (syn. Mycosphaerella graminicola), Ronny Kellner¹, Veronika Schott¹, Stephan Poppe², Rachel Brem², Eva H. Stukkenbrock¹. 1) Max Planck Institute for Terrestrial Microbiology, Fungal Biodiversity, Karf-von-Frisch-Strasse 10, 35043, Marburg, Germany; 2) University of California, Department for Molecular Cell Biology, 176 Stanley Hall, Berkeley, CA 94720-3220, USA.

Conditionally dispensable chromosomes (cDCs) are common genomic features in many parasitic ascomycetes. The presence of cDCs entails a high amount of intraspecific genomic variation that is inherited in a non-Mendelian manner. Because genes located on cDCs have in some species been shown to play a role in pathogenicity cDCs may promote rapid adaptive evolution in response to host defenses. With up to eight cDCs the genome of the wheat pathogen Zymoseptoria tritici (syn. Mycosphaerella graminicola) contains by far the largest known proportion of dispensable elements among all ascomycetes. In comparison to the core chromosomes, cDCs of Z. tritici are smaller, have on average less and shorter genes with a lower GC content and a higher amount of paralogous sequences and repetitive elements. Hitherto, the functional relevance of cDCs for Z. tritici remains unclear.

In this study we elucidate the relevance of cDCs in Z. tritici by assessing expression profiles during in-planta and axenic growth. Because our RNAseq dataset covers both host and non-host interactions we broaden the perspective of our approach by insights into host-specific expression profiles. In order to verify the current gene annotation we mapped all RNAseq reads to the genome of Z. tritici and predicted gene transcripts. By combining our results with the latest gene annotation we set up a new transcription list, which we further analyses. We demonstrate an overall significantly lower transcription of genes located on cDCs relative to genes located on core chromosomes. In addition, cDCs encode several unique genes that are expressed under certain conditions. We identify duplicated genes using a blast approach and show differential gene expression between paralogs on cDCs and core chromosomes. To link the transcription of cDC genes to specific stages in the interaction of Z. tritici and wheat we focus on a gene family of three paralogs where two genes encode secreted proteins. We quantified their expression via qPCR at seven different time points of the interaction. In summary, our study suggests the functional relevance of single cDC genes and the relevance of cDCs for gene innovation and adaptive evolution in Z. tritici.

665. Take a walk on the wild side: evolutionary consequences of resistance to apple scab introgressed from a wild host. C. Lemaire¹, T. Leroy¹, M. de Gracia², C.-E. Durel¹, M. Templeton¹, J. Bowen¹, V. Caffier², B. Le Cam². 1) IRHS, University of Angers, Angers, France; 2) IRHS, INRA, Beaucouzé, France; 3) Plant & Food Research, Auckland, New Zealand.

Theories on emergence of pathogens are dominated by ecological hypotheses. For instance overcoming of a host resistance gene by invading resistant hosts from infected susceptible cultivars. However invasion of virulent populations from wild habitats are often neglected. Though taking into account the occurrence of a preexisting allopatic virulent population greatly impacts conclusions made about emergence of new virulence and the nature of barriers to reproduction with the avirulent population. Indeed under allopatic scenario, these barriers are not expected to be only adaptive. Here, we intended to decipher the evolutionary history of the European populations of the apple scab pathogen Venturia inaequalis enabled to overcome the Rvi6 resistance gene introgressed in Malus x domestica from the crabapple Malus floribunda. Using microsatellite and sequence data in an Approximate Bayesian Calculation (ABC) framework, we demonstrate that virulence corresponding to a new resistance gene introgressed in agrosystems was previously present in the wild population within a host population that has diverged since several thousands years. We show that deployment of the corresponding resistance gene in agrosystems can then act as gateways for virulent populations to invade orchards. At last we show that secondary contact followed by mating between invading and resident population can reveal genetic incompatibilities (Dobzhansky-Müller Incompatibilities) accumulated during divergence between the two populations. These incompatibilities induce hybrid depression by negative epistasis. This study based on state-of-the-art tools of population genomics, phenotyping and genetic mapping is the first to demonstrate the occurrence of intrinsic post-zygototic barriers in pathogens revealed by a secondary contact. Overall, it points out the risk of generating new “hybrid” populations harbouring new pathogenic traits by introgression of resistance genes in agrosystems by breeders.
666. Genomic footprint of adaptive divergence in *Ophiostoma montium*, a fungal symbiont associated with the mountain pine beetle. J. F. Mao, B. Dhillon, C. Tsui, K. Ritland, R. Hamelin. Faculty of Forest Sciences, University of British Columbia, Vancouver, BC, Canada.

*Ophiostoma montium* is the most common fungus associated with the mountain pine beetle, the insect responsible for the destruction of 18 million ha of pine forests in Canada. In order to determine the evolutionary histories of *O. montium* populations, high-throughput genome sequencing was used to uncover the genetic changes that accompany divergence as lineages colonize different conifer hosts in different climatic regions. 36 fungal isolates from different hosts and different environments were sequenced. *De novo* genome assembly from one isolate was used as reference to call variants (922,000 SNPs and 126,000 Indels). Multiple analyses including whole genome variants, genetic distance, population structure and Identity by descent (IBD) identified three population lineages, corresponding to the various hosts and three geographic groups: US, Rocky, and North Canada (NC). Faster linkage disequilibria (LD) decays were observed in both Rocky and NC groups, indicating an increase in genomic recombination and/or high effective population size. Presence of genomic regions with high LD and negative Tajima's D was evidence that population size expansion (after bottleneck or a selection sweep) and/or purifying selection occurred in the NC group. Loci in the genome contributing to both host shift and climatic transition were also identified by multiple lineage-specific genomic scans for selection. Additionally, genome recombination events were recovered for lineages experiencing different demographic histories. Our study highlights the value of whole genome sequences both in evolutionary dynamics and genetics of plant pathogens.

667. Ecological context in symbioses: when is your enemy also your friend? Georgiana May¹, Paul Nelson². 1) Dept Ecol, Evol, Behavior,#100, Univ Minnesota, St Paul, MN; 2) EEB graduate program University of Minnesota St. Paul MN.

Most plants are rife with fungal symbiotic partners with many of these having little apparent effect on the host's health and fitness. In this work, we explore the degree to which the outcome of interactions between an endophytic fungus, pathogen and plant host depend on ecological context. In particular, we ask whether interactions between the endophyte of maize, *Fusarium verticilliodes*, with the pathogen *Ustilago maydis*, depend on host resistance to the pathogen. In the case of a host susceptible to the pathogen, the two fungal species should meet frequently, and compete over host resources, potentially driving greater virulence to the host in one or the other fungal species. In the case of a host resistant to the pathogen, the endophyte might be a "bystander" to the pathogen, because the two meet too infrequently to drive their co-evolutionary interaction. We show evidence that the two fungal species have evolved stronger antagonistic interactions in maize susceptible to the pathogen, and further, that this might be associated with greater virulence by the pathogen. Results of modeling will also be presented from which we predict longer term evolutionary trajectories for this 3-way interaction.
668. Population shifts and mating-type heterokaryosis in Aspergillus flavus, Rodrigo A. Olarte¹, Bruce W. Horn², Carolyn J. Worthington², Rakhi Singh³, Ignazio Carbonè¹. 1) Department of Plant Pathology, North Carolina State University, Raleigh, NC; 2) National Peanut Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Dawson, GA.

Aspergillus flavus is a heterothallic fungal pathogen of many economically important crops worldwide. We sampled A. flavus strains from a cornfield in Rocky Mount, North Carolina, USA. Plots were inoculated at tasselling with either A. flavus AF36 or NRRL 21882 (=Afla-Guard) nonaflatoxigenic biocontrol strains, both of which are mating type MAT1-2. Subsequently, aflatoxicin strain NRRL 3357 (MAT1-1) was applied to all plots, including control plots not inoculated with biocontrol strains. Sclerotia were harvested from infected corn ears and ninety single-ascospore isolates were obtained from ascocarps originating from plots treated with AF36 and NRRL 21882. In addition, eighty A. flavus isolates were collected from soil one month after planting (before biocontrol application) and one year after biocontrol application, for a grand-total of 250 isolates. PCR amplification revealed grouping of isolates into three distinct mating-type classes: MAT1-1, MAT1-2 and MAT1-1/MAT1-2. An overwhelming majority (54%) of isolates sampled prior to biocontrol treatments were heterokaryotic for mating type (MAT1-1/MAT1-2), but was shifted to only 9% of isolates from soil after biocontrol treatments; 39% of isolates obtained from ascospores were heterokaryotic, with the remaining comprising either MAT1-1 or MAT1-2. Multilocus genotyping indicated that ascospores might have originated from Afla-Guard as a putative parent; there was no evidence of AF36 or NRRL 3357 in ascospores or in pre- or post-treatment soil samples, which may explain the genetic structure of the indigenous population. The vertical transmission of MAT1-1/MAT1-2 to progeny ascospore isolates suggests that heterokaryosis can be maintained in subsequent generations. Furthermore, matings were performed to determine functionality of these MAT1-1/MAT1-2 strains and all isolates tested were strictly functional as MAT1-2. Further characterization of heterokaryons and their frequency in A. flavus populations may be important in understanding the adaptation of these fungi to changing environmental conditions and could lead to better and more effective biocontrol strategies specific to a geographic region. Understanding population structure is the key to unlocking the secrets of a successful biocontrol strain.

669. Structural variation of trichothecene mycotoxins has resulted from multiple evolutionary processes in the fungal order Hypocreales. R. H. Proctor¹, A. M. Stanley¹, M. G. Malmierca¹, N. J. Alexander¹, S. Gutiérrez¹, S.P. McCormick¹. 1) Bacterial Foodborne Pathogens and Mycology, USDA ARS NCAUR, Peoria, IL; 2) University School of Agricultural Engineers, University of León, Ponferrada, Spain.

Trichothecenes are secondary metabolites produced by fungi in at least six genera of the order Hypocreales. These metabolites are of concern because they are toxic to humans and other animals and can accumulate in grain used for food and feed. They also contribute to plant pathogenesis of Fusarium and to biological control activity of Trichoderma. Although all trichothecene share the same molecular skeleton, a tricyclic structure with a12,13-epoxide, different genera produce trichothecenes that differ in patterns of oxygenation and acylation. To investigate how such structural variation has evolved, we examined 1) variation in gene function and content in homologs of the trichothecene biosynthetic gene (TRI) cluster and 2) phylogenetic relationships of TRI genes among trichothecene-producing genera. The results suggest that the ancestral hypocrealean TRI cluster consisted of at least seven genes, including the enzyme-encoding genes TRI4 and TRI5 responsible for synthesis of the trichothecene skeleton, the regulatory genes TRI6 and TRI10, and the transporter gene TRI12. Phylogenetic analyses indicate that oxygenation of carbon atom 4 (C-4), which occurs in all trichothecene-producing genera, likely arose when different genera acquired different C-4 hydroxylase genes: e.g. TRI11b in Trichoderma and Myrothecium and TRI13 in Fusarium. In contrast, C-3 oxygenation, which occurs in only one genus, likely arose by a change in function of TRI4, a gene that exists in all genera. These results, and those from studies of Fusarium and Trichoderma, indicate that structural variation of trichothecenes has arisen by recruitment, changes in function, and deletion of TRI genes during evolution of the Hypocreales.

670. Evidence for birth-and-death evolution and horizontal transfer of the fumonisin mycotoxin biosynthetic gene cluster in Fusarium. R.H Proctor², F. Van Hove¹, A. Susca¹, G. Stea³, T. van der Lee⁴, C. Waalwijk⁵, A. Moretti⁶, T.J.J. Ward⁷. 1) Bacterial Foodborne Pathogens and Mycology, USDA ARS NCAUR, Peoria, IL; 2) Earth and Life Science Institute, Université catholique de Louvain, Louvain, Belgium; 3) Institute of Sciences of Food Production, National Research Council, Bari, Italy; 4) Plant Research International B.V., Wageningen, The Netherlands.

In fungi, genes required for synthesis of secondary metabolites are often clustered. The FUM gene cluster is required for synthesis of fumonisins, a family of toxic secondary metabolites produced predominantly by species in the Fusarium (Gibberellera) fujikuroi species complex (FFSC). Fusonins are a health and agricultural concern because their consumption is epidemiologically associated with multiple diseases in humans and other animals. Among FFSC species, the FUM cluster is discontinuously distributed but uniform in gene order and orientation. In this study, we demonstrate that the FUM cluster exists in at least four different genomic contexts within the FFSC and that phylogenetic relationships derived from analyses of FUM cluster genes are correlated with genomic context, but are inconsistent with species relationships inferred from analyses of multi-gene metabolite genes. In addition, analyses of synonymous site divergence suggested that FUM cluster divergence predated divergence of the FFSC. These results are not consistent with trans-species evolution of ancestral cluster alleles or with interspecies hybridization, but suggest duplication of the cluster within an FFSC ancestor and subsequent loss and sorting of paralogous clusters in a manner consistent with the birth-and-death model of evolution previously described for multigene families. A model based on horizontal gene transfer (HGT) could also explain these observations, but seems unlikely because it requires independent transfer events from multiple unknown donors to multiple FFSC recipients. However, analyses of phylogenetic relationships and synonymous site divergence provided strong evidence that F. oxysporum strain FRC O-1890 acquired the FUM cluster via a relatively recent HGT event from F. bulbicola or a closely related species within the FFSC. These results indicate that, as with other secondary metabolite clusters, species phylogenies do not provide an adequate picture of the complex evolutionary history of the FUM cluster within Fusarium.

671. Chemotype predominance in Fusarium graminearum is not directly affected by the use of the fungicides trifloxystrobin and isopyrazam. Matias Pasqua³, P. Théodore Dubos, Friederike Pogoda, Lucien Hoffmann, Marco Beyer. Environment and Agro-biotechnologies Department, CRP GABRIEL LIPPMAAN, Belvaux, Luxembourg.

Mitochondrial respiration inhibitors are effective fungicides used to control wheat diseases in Europe. Since the beginning of the monitoring of chemotype diversity in wheat fields in Luxembourg, we are trying to identify factors involved in the shift of chemotype prevalence (nivalenol vs. 15ADON vs. 3ADON). In this study we investigated whether the use of fungicides belonging to the respiratory inhibitors complex II and III (that are used in wheat fields for treating other diseases) may play a role in selecting a specific F. graminearum chemotype. Strains from Luxembourg and from a world collection with isolation dates ranging from 1969 to 2011 were chemotypes by genetic means and then analysed for their sensitivity to trifloxystrobin (inhibitor of respiratory complex III) and isopyrazam (inhibitor of respiratory complex II) using a microplate in vitro test on conidia. The maximum level of inhibition which could be obtained by trifloxystrobin ranged from 14 to 65% for the 55 strains analyzed with no complete inhibition up to a concentration of 3mM. Fortyone isolates tested for their sensitivity towards isopyrazam were insensitive with the average rate of inhibition converging towards 28%. For both fungicides, EC50 values did not significantly depend on the chemotype, suggesting that these two fungicides do not exert a direct pressure on the selection
of chemotypes in *F. graminearum*. Our study also suggests that *F. graminearum* seems to be significantly insensitive to respiratory complex inhibitors (II and III). Molecular mechanisms involved in the insensitivity are under investigation.


Three physiological races (1, 2 and 3) of *Fusarium oxysporum* f.sp lycopersici (Fol) have been identified based on their inability to infect tomato cultivars carrying Fol resistance genes (I, I-2 or I-3, respectively). We wished to unravel the molecular mechanisms underlying the evolution of Fol races. It is generally assumed that race 2 evolved from race 1 by loss of AVR1 and that race 3 evolved from race 2 by a point mutation in AVR2, thus overcoming I and I-2 mediated resistance, respectively. We have sequenced a genomic region of approximately 100 kb containing AVR1 in race 1 isolate Fol004 and compared it to the sequenced genome of race 2 isolate Fol4287. A genomic fragment of 30.5 kb containing AVR1 was found to be missing in Fol4287.

Further analysis suggests that race 2 evolved from race 1 by deletion of this 30.5 kb fragment, most likely due to recombination between helitrons bordering the fragment. A worldwide collection of Fol isolates was subjected to PCR analysis of the AVR1 genomic region, including the two bordering helitrons. The results suggest that, based on the deletion event that led to loss of AVR1, Fol isolates can be divided into distinct lineages that coincide with their geographical origin. Our results also suggest that transposable elements played a major role in the evolution of races within f.sp lycopersici of *Fusarium oxysporum*.

673. Detection of Mitochondrial DNA Heteroplasmy in the progeny of crossed genetically divergent isolates of Arbucular Mycorrhizal Fungi. Maryam Nadimi, Ivan de la Providencia, Gabriela Rodriguez, Denis Beaudet, Moahmed Hijri. IRBV, Biological Sciences Dep., University of Montreal, Montreal, QC, Canada.

Nonself fusion and nuclear genetic exchange has been documented in arbuscular mycorrhizal fungi (AMF) particularly in *Glomus irregulare*, which is a common and widespread species. However, mitochondrial transmission accompanying nonself fusion of genetically divergent isolates remains unknown.

We developed a series of crossing experiments between different isolates of *G. irregulare*, harboring genetically divergent mitochondrial DNA (mtDNA) haplotypes. We tested the hypothesis that heteroplasmy (i.e. mixture of genetically different mtDNA in a common cytoplasm) occurs in the progenies of the crossed isolates. Three isolates of geographically distant locations were used to investigate nonself fusions and mtDNA transmission in the progeny. To be able to trace the mtDNA haplotypes, we sequenced two mtDNAs of two *G. irregulare* isolates (DAOM-240415 and DAOM-234328) additional to the current available isolate DAOM-197198. We developed isolate-specific markers in variable regions of intergenic mtDNAs (cox3-nr) of these isolates. Three crossing combinations in pre-symbiotic and symbiotic phases were performed. Interestingly, nonself fusion frequency was low and was usually associated with irregular shape and aborted spores, although normal spores were also observed. Ten progeny spores per crossing combination were genotyped using isolate-specific markers. We showed the evidence that nonself fusion occurs between isolates originated from different continents both in pre-symbiotic and symbiotic phases. Genotyping patterns of individual spores from the progenies clearly showed the presence of markers of the two parental mtDNA haplotypes. Our results demonstrated the occurrence of mtDNA heteroplasmy in the progeny of crossed isolates. This raises the questions whether mtDNA heteroplasmy is transient or persistent in AMF? What are their consequences in evolution of AMF? Are there any conflicts of the presence of mtDNA heteroplasmy within an individual? Further studies on vegetative compatibility and incompatibility and putative sex machinery in AMF will provide new information to explore and solve these questions and thereby advance our understanding of the evolution of AMF.

674. Evolution of mode of infection in the rice blast fungus and allied species. Ning Zhang1, Shuang Zhao1, Jing Luo2, Guohong Cai2, Debashish Bhattacharya2, Bradley Hillman1. 1) Plant Biology and Pathology, Rutgers Univ, New Brunswick, NJ; 2) Ecology, Evolution and Natural Resources, Rutgers Univ, New Brunswick, NJ.

The family Magnaporthaceae contains devastating fungal cereal and grass pathogens, such as *Pyricularia oryzae* (Magnaporthaceae, rice blast fungus), *Magnaporthiopsis poae* (Magnaporthaceae, summer patch pathogen of turf grasses), and *Gaumannomyces graminis* (take-all fungus of various cereals and grasses), which are popular model organisms in fungal biology and host-pathogen interaction studies. Despite their ecological and economic importance, the phylogenetic relationships among the constituent species remain ambiguous due to the lack of convincing morphological characters and paucity of molecular data for the majority of the non-model species in the family. In this study, our multilocus phylogeny suggests that both *Magnaporthaceae* and *Gaumannomyces* are polyphyletic genera. Therefore, a new genus, *Magnaporthiopsis*, is proposed based on phylogeny and morphology. The phylogeny also provides insights into fungal biology and pathogenesis. *Pyricularia oryzae* formed a basal clade, while *Magnaporthiopsis poae* and *Magnaporthiopsis rhizophila* formed another well-supported clade with *Magnaporthiopsis incrustans* (*G*. incrustans), *G*. graminis and *Nakatkoa sigmaidea* (*Magnaporthe salvinii*). The basal species infects both root and aerial parts of plant host, while the aerial infection capacity seems to be lost in the taxa of the latter clade. The study indicates that anamorphic and ecological features are more informative than the teleomorphic characters in defining monophyletic groups among these taxa. In addition, we performed genome sequencing for 6 species in Magnaporthaceae: *Magnaporthiopsis rhizophila*, *Magnaporthiopsis incrustans*, *Harpaphora maydis*, *Nakatkoa sigmaidea*, *Ophioceras dolichostomum*, and *Pseudohalonectria lignicola*, in order to conduct phylogenomic and comparative genome analyses for both pathogenic and non-pathogenic members of this family.

675. WITHDRAWN

676. Population genomic analysis reveals a complex evolutionary history of *Neurospora tetrasperma*. Padraic Corcoran1, Fen Chen2, Martin Lascoux1, Peixiang Ni1, Hanna Johnesson1. 1) Uppsala University, Uppsala, Sweden; 2) BGI, Hong Kong, Hong Kong.

Fungal population genomics as a field of inquiry has seen a rapid growth in the recent years, with ability to sequence the genomes of multiple strains of fungi sampled from many populations. These studies have aimed at utilizing a population genomic approach to understanding the evolutionary forces that have had the greatest effect in shaping the genomes of fungal species. In this study, we extend the use of population genomics to help understand the evolutionary history of Neurospora tetrasperma. Neurospora tetrasperma has been the focus of much research in recent years, with most effort devoted to the study of its predominantly non-recombining mating type chromosomes. Here we present results of analysis on the whole genome resequencing of 86 homokaryotic strains of *N. tetrasperma*, sampled from locations in England, New Zealand and Louisiana, USA, together with one strain each of the close heterothallic species *N. hispaniola* and *N. sitophila*. These genomes were sequenced to a mean depth of between 25 to 30X coverage. Phylogenetic and population structure analysis of the genomewide SNP data produced identified that the sequenced strains of *N. tetrasperma* belong to 5 previously recognised lineages of *N. tetrasperma*. Comparisons of the multiple *N. tetrasperma* genomes with the *N. sitophila*, *N. hispaniola* and *N. crassa* genomes confirmed previous observations on introgression, but also revealed signatures of introgression between the English population of *N. tetrasperma* and *N. hispaniola*. Furthermore, comparisons between the genomes of the two homokaryons isolated from each heterokaryon revealed a large number of

In animals and plants, sex-biased gene expression plays a major role in gene evolution. In particular, reproductive genes with male-biased expression tend to exhibit rapid protein evolution and reduced codon bias as compared to female-biased or unbiased genes. Minimal data are available for fungi. Here, we demonstrate that sex-biased expression is associated with gene evolution in the filamentous fungus Neurospora crassa, but in contrast to animals and plants, the rapid evolution occurs for female-biased genes. Based on analyses of >25,000 expressed sequence tags (ESTs) from male (conidial), female (protoperithecial) and vegetative (mycelial) tissues, we show that reproductive genes with female-biased expression exhibit faster protein evolution and reduced optimal codon usage than male-biased genes and vegetative genes. Furthermore, our data suggest that female-biased genes are also more apt to experience selective sweeps. The sex-biased expression effects are observable at the species and population level. We argue that the rapid molecular evolution of female-biased genes is best explained by sexual selection via female-female competition, but could also result from mate-choice and/or directional natural selection.

678. Fungal Community Dynamics During Biomass Degradation in the Cow Rumen Determined by ITS Sequencing. Haiyan Piao1,2, Julien Tremblay3, Robin Ohm4, Kanwar Singh5, Fernanda Hoffner6, Stefan Bauer7, David Culley8, Kenneth Bruno9, Kerrie Barry10, Feng Chen11, Scott Baker12, Roderick Mackie12, Susannah Tringe12, Igor Grigoriev13, Matthias Hess14,15. 1) Washington State University, Richland, WA; 2) DOE Pacific Northwest National Laboratory, Richland, WA; 3) DOE Joint Genome Institute, Walnut Creek, CA; 4) Energy Biosciences Institute, UC Berkeley, Berkeley, CA; 5) Environmental Molecular Science Laboratory, Richland, WA; 6) University of Illinois, Urbana-Champaign, IL.

The microbial community that inhabits the cow rumen is composed of Archaea, Bacteria and Eukarya and is well known for its biomass-degrading ability. In order to understand this ecosystem we sampled the whole-systems level is important to monitor the dynamics of the individual community members. Sequencing of the 16S rRNA gene has been used extensively to obtain insights into the ecology of the prokaryotic fraction of the microbial rumen community. To obtain insights into the ecology of the fungal fraction of the rumen community and its dynamics during biomass-degradation, we amplified the ITS2 region from fungi that colonized corn stover and switchgrass during rumen incubation. Amplicons were generated from rumen-incubated switchgrass and corn stover and rumen fluid at six different time points and from two different host animals. Sequencing on Illumina’s MiSeq platform resulted in a total of 10,675,384 sequences with an average read length of ~240bp amounting to a total of >2.6 Gbp of sequence information. Succeeding sequence analysis revealed a fungal community of low complexity, with two phyla as the dominant players. Members of the phylum Neocallimastigomycota were absent on the pre-incubated biomass and appeared to colonize both corn stover and switchgrass throughout the incubation process. Members of the phylum Ascomycota were less dominant (~1%), but seemed to play a role in the later phases of the biomass-degradation process.

In summary, results presented here demonstrate that rumen fungi consistently colonize lignocellulosic substrates and may play a role in the degradation of recalcitrant biomass.


In the order Onygenales there are many well-studied mammalian fungal pathogens, including both dermatophytes and systemic pathogens, such as Trichophyton rubrum, Coccidioides spp., and Histoplasma capsulatum. Recently, a new species in the Onygenales was described, C. ophiodiicola, an emerging pathogen of snakes. However, members of the genus Chrysosporium produce aleuriospores and arthrospores with limited characteristics making it difficult to define morphological boundaries for the genus and to distinguish it from other genera with similar spore type. As a result, the genus is polyphyletic containing species that are not that closely related. Here, we report the characterization of a fungus with a nearly identical ITS sequence to C. ophiodiicola from two diseased snakes in Pennsylvania and present a molecular and morphological revision of the phylogenetic placement of C. ophiodiicola. Molecular analysis reveals that this fungus belongs to the same clade as C. ophidiicola, but that their placement in the genus Chrysosporium is inaccurate. Thus, we propose the description of a new genus, Undulatus, to accommodate our fungus as well as C. ophidiicola. In addition, we will present recent results from our comparative analysis of the Undulatus ophidiicola genome and its pathogenic and non-pathogenic relatives within the Onygenales. Comparisons of genome statistics, gene family expansions and contractions, unique KEGG categories, and secondary metabolite clusters will be presented. To relate the molecular results with biochemical characteristics, the ability of U. ophidiicola and related fungi to grow on different substrates was assessed using Biolog plates. We compared both the U. ophidiicola genome and growth results to develop hypotheses on how variability in pathogenicity and host specialization evolved among these related taxa.

680. Pair-wise linkage disequilibrium decay among linked loci suggests meiotic recombination in natural populations of Sclerotinia sclerotion. Renuka Nilmini Attanayake, Fei Weiding Chen. 1) Washington State University, Johnson Hall 303, pullman, WA. 99164; 2) USDA ARS Grain Legume genetics and physiology research unit, Pullman WA 99164.

Both clonal and recombining population structures have been reported in Sclerotinia scleroticon populations around the world. Association of independent and putatively unlinked markers indicates clonal population structure, whereas random association of the markers suggests recombination and outcrossing. However, high mutation rates of markers used for inference, like certain microsatellite markers, could interfere and compromise the inferences of recombination and outcrossing. To test if the recombination is due to outcrossing or mutation, we used 12 microsatellite loci distributed over four chromosomes to genotype 230 isolates sampled from seven populations in the USA and China from a variety of crops. All the isolates produced single alleles for each of the loci tested, indicating all the isolates were homokaryotic for the microsatellite loci in consideration. Pair-wise linkage disequilibrium (LD) tests (Hedrick’s D, Fishers exact test and IA) between physically linked loci showed relationships ranging from linked to random association with increasing distance between loci on three chromosomes. For the three loci on chromosome four, LD decay with increasing physical distance between loci was found in six of the seven populations. Likewise, LD decay was found for the three loci on chromosome six in four of the seven populations, and also for
the loci on chromosome five in two of the populations. The reduced pair-wise linkage disequilibrium with increasing distance cannot be attributed to mutation alone, and thus the high intrachromosomal recombination is most likely due to meiotic recombination following outcrosso in these populations. Recombination hot spots and cold spots were detected. Mating type loci in 59 isolates of two populations were genotyped using PCR with allele-specific primers. About 40% of the isolates showed both MAT1-1 and MAT1-2 idiomorphs, as expected for a homothallic species. However, the remaining 60% of the isolates had only the MAT1-2 idiomorph as detected by the allele-specific PCR. Although the nature of the absence of the MAT1-1 idiomorph remains to be determined, the results showed variations in mating type alleles in natural populations, suggesting that some of the isolates may not be truly homothallic.


Environmental heterogeneity may result in divergent selection, which in turn may lead to local adaptation resulting in populations showing habitat-based discontinuous variation. Very little is known on the patterns of adaptive divergence and local adaptation or underlying selective forces in ectomycorrhizal fungal populations. We are studying the population genomics of Suillus brevipes (Peck)untze, an ectomycorrhizal fungus associated with pine trees. Our goals are 1) to test the existence of barriers to gene flow across populations, and 2) detect genomic regions exhibiting selection sweeps. We sequenced the whole genomes of 30 S. brevipes individuals from two populations, one from a coastal Bishop pine forest in Mendocino Co (CA) and another from a high altitude Lodgepole pine forest in Yosemite National Park (CA). One isolate was selected as the reference strain and its genome was assembled de novo through a collaboration with the Joint Genome Institute. All other individuals were aligned to this reference and single nucleotide polymorphisms detected across populations. These markers are being used to find regions with evidence of positive natural selection, including possible islands of introgression, increased rates of non-synonymous substitutions, accelerated rates of divergence, and gene duplications. Results from our study will make a significant contribution for understanding patterns of neutral and functional variation as well as gene flow and selection in ectomycorrhizal fungi.

682. Ugly, understudied and undertreated: population genomics of the most common human fungal pathogen - the dermatophyte Trichophyton rubrum. M. Gajdczak, W. Li, J. Heitman. Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Trichophyton rubrum causes athlete’s foot, nail infections and ringworm in about twenty percent of the general population. Despite an annotated genome and hundreds of clinical isolates, the global genomic diversity and population biology of T. rubrum are not well understood. Additionally, T. rubrum cannot be crossed, genetic manipulation is difficult, and animal infections with T. rubrum are acute and highly inflammatory (rather than chronic and relatively mild as in humans). These factors have made it difficult to study virulence in T. rubrum and have limited the development of effective chemotherapies. This study will test three hypotheses: (1) clonal reproduction and limited dispersal have resulted in undiscovered local sequence variation and linkage disequilibrium (LD); (2) observed variation supports an out-of-Africa isolation-by-distance migration model; and (3) patterns of sequence diversity in sex- and virulence-associated genomic regions support the significant evolutionary roles of reproductive clonality and close interaction with humans. Worldwide isolates appear reproductively clonal and remarkably genetically monomorphic. Five common MLST markers reveal no variation among 50 European, Asian and North American strains. We Sanger sequenced 7.5 kb of non-coding intergenic regions in ten T. rubrum isolates collected over a span of ten years. These sequences revealed no polymorphisms useful for differentiating strains. Lastly, VNTR genotyping by our lab and other groups revealed two broad geographic types, though these are based on a few highly variable loci subject to homoplasy. We are assembling whole genome sequences of 32 geographically and clinically diverse strains to characterize sequence diversity more completely. The genome obtained by the Broad Institute will be used as a reference. We will obtain measures of diversity, polymorphism, LD, population differentiation and divergence. These measures will be used to compare isolates genome-wide, determine population structure and infer evolutionary and demographic forces contributing to observed patterns of variation. Locus-specific markers will be developed based on standing variation to genotype additional strains. Furthermore, we will test for variants associated with hypothesized virulence mechanisms involved in chronic T. rubrum infection.

683. Poppr: an R package for genetic analysis of populations with mixed reproductive systems. Zhiyan N. Kamvar1, Niklaus J. Grünwald2, 1) Botany and Plant Pathology, Oregon State University, Corvallis, OR; 2) USDA-ARS Horticultural Research Laboratory, Corvallis, OR.

Analysis of populations with mixed reproductive systems, including a blend of sexual and clonal reproduction, remains a challenge. We developed an R package implementing existing approaches for analysis of mixed populations. R is a multi-platform, open source, statistical environment that has gained popularity over the past few years. While there are a plethora of packages in R that perform population genetics analyses, many standard analysis methods for populations with mixed modes of reproduction remain hard to accomplish. Poppr aims at providing functions to facilitate rapid analyses of this data, particularly including methods for analysis of recombination (index of association), clone-censored analysis of full datasets in a hierarchical manner over all levels of sampling, genotypic diversity analyses, and distance analyses. As implemented, poppr requires minimal commands with convenient summary functions while providing compelling graphics. Unlike many platform dependent, standalone programs, poppr can be used for batch processing of data including all kinds of population genetic data (dominant/codominant; microsatellites, AFLP, SNPs). Poppr is available as a beta release for testing upon request and continues to be improved.

684. The heterothallic fungus Cercospora beticola contains fragments of both mating type genes. Melvin D. Bolton1, Zhaohui Liu2. 1) USDA - ARS, Fargo, ND; 2) North Dakota State University, Department of Plant Pathology, Fargo, ND.

In most heterothallic Ascomycota, the ability to reproduce sexually is determined by a single mating type locus (MAT1) represented by two idiomorphs known as MAT1-1 and MAT1-2 that are required to control nonself-recognition and mating of compatible partners. To investigate the MAT1 locus in the heterothallic fungus Cercospora beticola, we performed Southern analyses of both mating types using each mating type gene (MAT1-1-1 and MAT1-2-1) as a probe. Surprisingly, several bands of similar size were observed in both mating types using either probe, suggesting that multiple loci contain sequences of both MAT genes in the C. beticola genome. We screened a BAC library derived from a MAT1-2 isolate using both MAT gene probes to identify clones containing MAT gene sequences. Sequence analysis of four BAC clones confirmed that one BAC contained the true MAT1-2 idiomorph, while the other contained fragments of both MAT genes in close proximity (8 to 1.075 bp) to each other. To investigate whether this holds true in the opposite mating type, we sequenced the identical regions in a MAT1-1 isolate. Sequence analysis confirmed that the MAT1-1 isolate contained one full MAT1-1 idiomorph as well as additional regions that contained fragments of both MAT genes. In all cases, introns normally present in MAT genes were not present in the MAT1-1 or MAT1-2 gene fragments in either mating type. Although heterothallism has been suggested to be the ancestral state in the Dothisdeomycetes, the identification of fragments of both mating types in a single isolate suggests that homothallism is the ancestral state in C. beticola.

685. Population structure from mountain to coast of two Lophodermium endophytes; a case study comparing rare and common fungal species in pine needles. Ryoko Oono1,2, Laurel Koch3, A. Betsy Arnold4, Georgiana May5, François Lutzoni6, Ignazio Carboni7. 1) Department of Biology, Duke University,
Our understanding of the mechanisms that govern azole resistance in filamentous fungi is limited. While some clinical resistance has been reported, the mechanisms are not well understood. Research is ongoing to elucidate the cellular basis of azole resistance in Aspergillus fumigatus.

Other Topics

Understanding the cellular basis of Azole resistance in Aspergillus fumigatus. Michael J. Bromley, Marcin Fraczek, Rebecca Collins, Emma Davies, Paul Bowyer. Translational Med, Univ Manchester, Manchester, United Kingdom.

Resistance of Aspergillus fumigatus to the azole class of antifungals is becoming a major problem in Europe and is being driven by two factors, the prolonged exposure (a few months to several years) of patients to azoles and the extensive use of agricultural azoles driving environmental resistance. Our understanding of the mechanisms that govern azole resistance in filamentous fungi is limited. While some clinical resistant isolates harbor mutations...
in the azole target, lanosterol 14 a-demethylase (cyp51A), more than 50% do not. We have used a combination of whole genome sequencing, transcriptomics, transposon based mutagenesis and high throughput directed mutagenesis to identify novel mechanisms that may explain the resistance observed in these strains. I will summarize and discuss our progress to date and present a worrying mechanism that results in both pan-azole and amphotericinB resistance.

690. Chemically Induced Haploinsufficiency Screens to Identify Drug Mechanism of Action in Aspergillus Fumigatus. D. A. Macdonald1, A. E. Johns1, M. Eberle2, P. Bowyer2, D. Denning2, M. J. Bromley1. 1) Institute of Inflammation and Repair, Respiratory & Allergy Centre, University of Manchester, Manchester, United Kingdom; 2) Applied Microbiology, Institute for Applied Life Sciences, University of Karlsruhe, Hertzstrasse 16, 76187 Karlsruhe, Germany.

Current drugs used to treat Aspergillus infections are limited and suffer from a variety of shortcomings including low efficacy, toxicity and increasing resistance. Despite the discovery of numerous promising drug targets, few lead compounds have been discovered by target based approaches. This can be explained, in part, by the ‘druggability’ of a target as some compounds which demonstrate promising activity against an enzyme are not active against the whole cell or are toxic to humans. Consequently most of the antimicrobials presently on the market were originally discovered by random screening of compounds against whole cell screens. A solution to this problem is to identify gene targets utilizing compounds that already show antifungal activity and have clean toxicity profiles.

Chemical genetic profiling aids identification of drug mechanism of action as a diploid strain lacking a single copy of a drug’s target is hypersensitive to that drug. Haplozygote S. cerevisiae and C. albicans libraries have been used to identify the mechanism of action of several promising compounds; however, this has been hindered in A. fumigatus by the complexity in generating an adequate set of heterozygous strains. A high-throughput targeted gene KO method for A. fumigatus has been established by employing fusion-PCR to generate targeted gene disruption cassettes, optimizing the common transformation protocol for A. fumigatus high-throughput gene disruption, and utilizing a diploid Ku80/Ku80 mutant to facilitate more reliable homologous recombination. Preliminary efforts have produced 46 heterozygous KO strains and subsequently, the feasibility of chemical genetic haploinsufficiency studies in filamentous fungi has been demonstrated with several compounds. High-throughput methods of chemical genetic profiling by pooling multiple heterozygous KO strains into a single culture is currently being validated and preliminary data is promising. This will enable high-throughput methods for surveying the genome of A. fumigatus for new drug targets and supports unveiling the mechanisms of action of antifungal drugs.

691. Antifungal Pismum sativum defensin 1 Induces a non-Apoptotic Death in Aspergillus nidulans. Caroline M. Fernandes1, Luciano N Medeiros1, Landi VG Cortiglia1, Hilda Pets-Silva1, Patricia A de Castro2, Gustavo H Goldman2, Eleonora Kurtenbach1. 1) Federal University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil; 2) Sao Paulo University, Ribeirao Preto, Sao Paulo, Brazil.

Psd1 is a basic, cysteine-rich plant defensin isolated from Pismum sativum seeds which inhibits the growth of a broad range fungi species. Defensins are also non-toxic to mammalian cells, highlighting their potential as antifungal drugs. We have shown that FITC-labelled Psd1 was internalized in F. solani hyphae, interacting with cyclin F and leading to fungal cell cycle arrest. This internalization seemed to be dependent of glucosylceramide (CMH, of ceramide monohexoside), once C. albicans cells lacking the ceramide synthase are 25 % less susceptible to Psd1 than the parental strain. Fungal and mammalian CMH are structurally divergent, as the former presents a C8-unsaturation and C9-methylation on the sphingoid base, which could possibly drive Psd1 selectivity. In this work, we investigated the cell death mechanisms triggered by Psd1 in Aspergillus nidulans and the contribution of CMH structure to Psd1-induced fungal death. We characterized, through fluorescence microscopy, several apoptotic events, such as intense formation of reactive oxygen species (ROS), metacaspase activation and DNA strand breaks. Although A. nidulans hyphae treated with 20 mM Psd1 for 24 hours exhibited severe cell injury, no apoptosis-phenotype was observed. We also investigated whether Psd1 incubation would lead to membrane permeabilization typical of a necrotic death. To this, A. nidulans cells were maintained in the presence or absence of the peptide and the membrane damage was evaluated through Propidium Iodide (PI) staining. We observed 15 % PI positive cells in the suspension treated with Psd1, in contrast to 2 % in control culture. To investigate the role of fungal CMH and its structural modifications to Psd1-induced cell death, we constructed strains lacking the glucosylceramide synthase (ANID_08806), sphingolipid D8-desaturase (ANID_04592) and sphingolipid C9-methylase (ANID_05688 and ANID_07375) genes. Phenotype analysis showed impaired growth of strains deficient in ANID_08806 and ANID_04592 in comparison to the parental strain. Further investigation will be conducted to characterize Psd1 antifungal activity and apoptosis or necrosis induction in mutant strains. Unraveling the mechanisms of cell death induced by antifungal peptides may lead to the identification of new targets that drive antymycotic selectivity.

692. Is fungal secondary metabolism regulated by competing insects? Annika Regulin1, Nancy Keller2, Frank Kemppen1. 1) Department of Botany, Christian-Albrechts University, Kiel, Germany; 2) Department Medical Microbiology and Immunology, Dept of Bacteriology, UW-Madison, USA.

Fungi synthesize an astonishing variety of secondary metabolites, some of which belong to the most toxic compounds in the living world. Even though little is known about the benefit of these metabolites, the ability to regulate the secondary metabolism might be seen as an evolutionary adaptation. Presumably fungi regulate secondary metabolites (e.g. mycotoxin) in response to confrontation with natural competitors like insects to guarantee efficient exploitation of environmental resources (1-3). Admittedly it should be mentioned that secondary metabolites are not the only defence mechanisms of fungi (4). In order to enlighten the biological function of these secondary metabolites with reference to chemical defence reactions of insect-fungal interactions, we utilized complementary approaches of experimental ecology and functional genomic techniques. The vinegar fly Drosophila melanogaster and its natural antagonist Aspergillus nidulans are used as an ecology model system. To analyse fungal up- or down regulated target genes in the interaction of A. nidulans with Drosophila larvae microarray analysis was performed. This led to the identification of secondary metabolite genes up- or down-regulated under these conditions. Quantitative RT-PCR was employed to analyze secondary metabolite gene expression at different time points. Fungal single, double and triple mutations of identified up-regulated genes are currently analyzed in confrontation assays to identify potential modifications in gene expression and the survival rate of larvae concerning to chemical defense reaction of fungus-insect interaction compared to wild type. This could reveal insights about the biological function of secondary metabolite genes and clusters such as stc and mdp.


693. Eisosome distribution and localization in the meiotic progenesis of Aspergillus nidulans. A. Athanasopoulos1, H. Boleti1, C. Scacozzochio1, V. Sophianopoulou1. 1) Institute of Biosciences and Applications, Microbial Molecular Genetics Laboratory, National Center for Scientific Research, Demokritos (NCSR&D), Athens, Greece; 2) Intracellular Parasitism Group, Molecular Parasitology Laboratory, Department of Microbiology and Light
Microscopy unit, Institut Pasteur Hellenique, Athens, Greece; 3) Department of Microbiology, Imperial College, London, United Kingdom.

In the model filamentous fungus *Aspergillus nidulans*, PiIA and PiIB, two homologues of the *Saccharomyces cerevisiae* eisosome proteins PiIL1/Lsp1, and SurG, a strict orthologue of Sur7, are assembled and form tightly packed complexes in conidiospores. *A. nidulans* differs from the *Saccharomyces* in that it has the ability to reproduce through two different types of spores, conidiospores and ascospores, cells which have a radically different morphology and are formed through completely different developmental pathways. Ascospores are formed only after the completion of meiosis inside asci, conidiospores arise from mitotic budding of specialized cells (phialides). We thus investigated eisosome composition and distribution in ascospores. Our results show that core eisosome proteins PiIA, PiIB and SurG are not expressed in phialide cells or early ascospores, but are expressed in mature ascospores. PiIA forms static punctate structures at the plasma membrane as does PiIB (with higher concentration at the areas where the two halves of ascospores are joined together), while SurG was localized both at the membrane and perinuclearly. In germlings originating from ascospores the punctate structures were shown to be composed only of PiIA. In germinated ascospores PiIA foci did not colocalise with the punctate structures of AbpA, a marker for sites of clathrin-mediated endocytosis. In the presence of myriocin - a specific inhibitor of sphingolipid biosynthesis - PiIA-GFP foci of ascospore germlings were less numerous and their distribution was significantly altered. In this study we also investigated one of the two *A. nidulans* orthologues of Nce102, a protein that determines the structure and function of membrane microdomains in *S. cerevisiae*. In quiescent conidia localization of the closest orthologue, AnNce102 is detected in PiIA plasma membrane associated foci as well as in 3-5 round-shaped intracellular structures. In early hyphae, a cytoplasmic fraction of Nce102 is additionally detected in highly dynamic structures that resemble Golgi equivalents. Deletion of core eisosomal genes causes mislocalization of Nce102 from the plasma membrane to these cytoplasmic structures. Ongoing experiments are investigating AnNce102 localization in response to sphingolipid biosynthesis and the nature of the intracellular compartments where it is located.

694. **Fungal-bacterial interactions: Bacillus subtilis forms biofilm on Aspergillus niger hyphae.** *Isabelle Benoit*1,2, Marielle H. van den Esker2, Miaomiao Zhou1, Oscars P. Kuipers1, Ronald P. de Vries1,2, Ákos T. Kovács1. 1) Fungal Physiology, CBS-KNAW, Utrecht, Utrecht, Netherlands; 2) Microbiology & Kluiver Centre for Genomics of Industrial Fermentation, Utrecht University, Utrecht, Utrecht, The Netherlands; 3) Molecular Genetics Group, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands; 4) Terrestrial Biofilms, Institute of Microbiology, Friedrich Schiller University, Jena, Germany.

Pure cultures of the filamentous fungus *Aspergillus niger* and *Aspergillus oryzae* and the Gram-positive bacterium *Bacillus subtilis* are widely used in industry for the production of pharmaceuticals, food ingredients and enzymes. However both by design and by accident, industrial fermentation can also involve mixed populations of micro-organisms. Moreover, in natural biotopes, these organisms live in mixed communities and have complex interactions ranging from competition to symbiosis. *B. subtilis*, in specific conditions, is capable of forming beneficial biofilms on surfaces and interfaces from plant roots to metal surfaces. In this study, co-cultivations of *A. niger* and *A. oryzae* together with *B. subtilis* were performed. *A. oryzae* inhibits *B. subtilis* growth while a bacterial coating was observed on *A. niger* hyphae. Microscopic and transcriptomic approaches were combined to study this fungal-bacterial interaction example.

695. **Co-cultivations of fungi: microscopic analysis and influence on protein production.** *Isabelle Benoit*1,2, Armans Vinck1, Jerre van Veluw1, Thijs Gruntjes1,2, Han A.B. Wösten1, Ronald P. de Vries1,2. 1) Fungal Physiology, CBS-KNAW, Utrecht, Utrecht, Netherlands; 2) Microbiology & Kluiver Centre for Genomics of Industrial Fermentation, Utrecht University, Utrecht, The Netherlands.

During their natural life cycle most fungi encounter other microorganisms and live in mixed communities with complex interactions, such as symbiosis or competition. Industrial fermentations, on purpose or by accident, can also result in mixed cultures. Fungal co-cultivations have been previously described for the production of specific enzymes, however, little is known about the interactions between two species that are grown together. *Aspergillus niger* and *Aspergillus oryzae* are two of the most important industrial fungi worldwide and both have a long history of strain improvement to optimize enzyme and metabolite production. We have co-cultivated the wild type strains of these two *Aspergilli* with each other as well as the *XlnR* knock out strains. *XlnR* is a transcription factor inducible by the presence of xylose and responsible for the regulation of a variety of genes encoding plant polysaccharide degrading enzymes. The morphology and mechanism of the interaction of these cultures on wheat bran is addressed using microscopy and proteomics.

696. **Improving heterologous protein production in Aspergillus vadasinis.** *Ourdia Bouzid*1,2, Ronald P. de Vries1,2. 1) Microbiology & Kluiver Centre for Genomics of Industrial Fermentation, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands; 2) CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT, Utrecht, The Netherlands.

*Aspergillus vadasinis* is a good candidate for heterologous protein production, because it produces very low levels of extracellular proteases and does not acidify the medium. To improve protein production in *A. vadasinis* two strategies were tested: (i) identification of new promoters for high gene expression, and (ii) overexpression of the xylanolytic regulator, *XlnR*. Six new *A. niger* constitutive promoters were selected and compared to the *gpdA* promoter using an arabinofuranosidase (*abf*) encoding gene from *Fusarium oxysporum* as a reporter. Several of the new promoters resulted in higher Abf activity than *gpdA*. For the second strategy, *A. vadasinis* was transformed with *xlnR*, *xlnD* (encoding beta-xylanase) and *faeA* (encoding feruloyl esterase) alone, and with combinations of *xlnD* and *xlnR*, and *faeA* and *xlnR*. Southern blot profiles confirmed the presence of multiple copies of the genes in the transformants. *XlnD* and *FaeA* activities were measured and were compared to the control strain. This demonstrated that increased copy numbers of *faeA* and *xlnD* had a much larger effect on the corresponding activities than increased copy numbers of *xlnR*. These data demonstrate that the new promoters in combination with high copy integration of the target genes can result in higher protein production by *A. vadasinis*. Highlights from this study will be presented.

697. **Production and characterisation of esterases from Chaetomium thermophilum and their applicability in biomass conversion.** Xiaoxue Tong, Peter Busk, Morten Grell, Lena Lange. Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University Copenhagen, Denmark.

Xylan is the dominating hemicellulose constituent of plants and the most abundant renewable polysaccharide in nature after cellulose. Xylan and its hydrolysis products are potential resources for nutraceuticals, cosmetics, foods, bioalcohol, and industrial fine chemical production. Feruloyl esterase and acetyl xylan esterase are required for complete enzymatic hydrolysis of xylan due to its highly heterogeneous nature. The aim of this study was to produce and characterize esterases from the therophilic fungus *Chaetomium thermophilum*. The esterase genes were identified by a novel bioinformatics tool PPR (Peptide pattern recognition, Busk & Lange, 2011). A Feruloyl esterase gene (*CtFaeA*) and a xylan esterase gene (*CtXaeA*) were successfully expressed in the yeast *Pichia pastoris*. They were purified to homogeneity from the culture supernatants. The effect of temperature and pH on the activity and stability of the esterases, as well as their substrate specificities, were studied. Both *CtFaeA* and *CtXaeA* displayed broad thermal stability and pH stability. Moreover, both esterases were active on hydrolysis of wheat arabinoxylan. These results show that *Chaetomium thermophilum* has a high capacity for degradation of xylan in addition to its already described cellulolytic potential. Furthermore, the robust esterases from *Chaetomium thermophilum* have
698. Antioxidant adaptation by Eugenol and its derivatives and their effect on the expression of virulence in candida species. Aijaz Ahmad, Nikhat Manzoor. Bioscience, Jamia Millia Islamia, New Delhi, Delhi, India.

Present work investigates the antioxidant activity and mode of action of eugenol (EUG), and its three derivatives- methyl eugenol, thymol and carvacrol. EUG and its derivatives were tested for antifungal activity by standard methods of CLSI. These varied in their mechanism of action depending upon the period of exposure. Short exposures of S-15 minutes resulted in reduced H+ efflux by the H+-pump. From our studies we conclude that EUG and its derivatives induce production of free radicals which stimulates the enzyme SOD. An increased SOD activity resulted in an increase in the concentration of H2O2 which further stimulates the peroxide eliminating enzyme, primarily GPx. It is noteworthy that the levels of GSH an essential substrate of Gpx were drastically reduced by the test compounds and this reduction gets even greater as increased levels of H2O2 decrease the activity of G6PDH which provides reducing equivalents to GR, an enzyme that recycles GSH from GSSG. Decreased G6PDH activity aids further in the reduction of GSH. Again, reduced availability of GSH explains decreased Gpx activity. Another enzyme to eliminate H2O2 is catalase, which triggers a cellular response leading to an increase in its activity. Hence increase in the activity of two important antioxidant enzymes SOD and catalase, clearly demonstrates an increase in the concentration of ROS when the Candida were exposed to the EUG and derivatives. These enzymatic responses were not enough to defend the cell completely against a high rise in ROS and therefore did not meet the required cellular antioxidant demand. Ultimately, the outburst of free radical production led to severe lipid peroxidation. Cell death on exposure to EUG and its derivatives hence may be due to (i) decrease in the rate of H+efflux (ii) reduced endogenous content (iii) Induction of oxidative stress in the cell (iv) These processes impair membrane structure and function which form lesions. Infection process of Candida is characterized by crucial pathogenicity markers. The process of germ tube induction followed by the secretion of hydrolytic enzymes help in the invasion of the host cells. The expression profile of selected genes associated with Candida virulence by RT-PCR showed a reduced expression of HWP1, SAP1 and PLB2 genes in Candida treated with EUG and its derivatives.

699. Elevation of chitin is linked with multiparallel mechanisms in response to C. albicans cell wall stress. F. Nogueira, L. Walker, C. Munro, N. Gow. Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom.

The role of the MAPK, Ca\(^{2+}\)/calcineurin and cAMP/PKA signal transduction pathways in regulating the Candida albicans cell wall stress response was investigated. A library of mutants lacking receptors, signalling elements and transcription factors were screened for alterations in the ability to respond to a range of cell wall stressing agents, including CaCl\(_2\), Calcofluor White and caspofungin. Pre-treatment of wild-type cells with CaCl\(_2\) and CFW, activates the Ca\(^{2+}\)/calcineurin and PKC pathways, leading to an increase in chitin content, and reduced susceptibility to caspofungin. Although elevation of cell wall chitin content often resulted in decreased sensitivity to caspofungin, we show here that some strains with increased chitin levels remained sensitive to caspofungin. The results show that elevation of chitin is a common property of a range of mutants that are affected in coordinating cell wall stress pathways, but that multiple mechanisms are likely to operate in maintaining the robustness of the C. albicans cell wall.

700. Prezygotic and postzygotic control of uniparental mitochondrial DNA inheritance in Cryptococcus neoformans. Rachana Gyawali, Xiaorong Lin. Biology, Texas A&M University, College Station, TX.

Uniparental inheritance of mitochondrial DNA is pervasive in non-isogamic higher eukaryotes during sexual reproduction and postzygotic and/or prezygotic factors are shown to be important in ensuring such inheritance pattern. Although the fungus Cryptococcus neoformans undergoes sexual production with isogamic partners of opposite mating types a and a, most progeny derived from such mating events inherit the mitochondrial DNA from the a parent. The homeodomain protein complex Sxi1a/Sxi2a, formed in the zygote after a-a cell fusion, was previously shown to play a role in this uniparental mtDNA inheritance. Here, we defined the timing of the establishment of the mtDNA inheritance pattern during the mating process and demonstrated a critical role in determining the mtDNA inheritance pattern by a prezygotic factor Mat2. Mat2 is the key transcription factor that governs the pheromone sensing and response pathway, and it is critical for the early mating events that lead to cell fusion and zygote formation. We show that Mat2 governs mtDNA inheritance independent of the postzygotic factors Sxi1a/Sxi2a, and the cooperation between these prezygotic and postzygotic factors help achieve stricter uniparental mitochondrial inheritance in this eukaryotic microbe.

701. SIS, a sex genome defense mechanism operating in Cryptococcus neoformans. Xuying Wang, Sabrina Darwiche, Joseph Heitman. Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Cryptococcus neoformans is a human fungal pathogen that undergoes a dimorphic transition from yeast to hyphae during a-a opposite-sex mating and a-a unisexual reproduction (same-sex mating). Infectious spores are generated during both processes. We previously identified a sex induced silenced (SIS) pathway in the C. neoformans serotype A var. gruberi lineage, in which tandem transgene arrays trigger RNAi-dependent gene silencing at a high frequency during a-a opposite-sex mating, but at an ~250-fold lower frequency during asexual mitotic vegetative growth. Here we report that SIS also operates during a-a unisexual reproduction. A self-fertile strain containing either SIS2a-URAS or NEO-URAS transgene arrays exhibited an elevated silencing frequency during solo and unisexual mating compared with mitotic vegetative growth. We also found that SIS operates at a similar efficiency on transgene arrays of the same copy number during either a-a unisexual reproduction or a-a opposite-sex mating. URAS-derived small RNAs were detected in the silenced progeny of a-a unisexual reproduction and RNAi core components were required, providing evidence that SIS induced by same-sex mating is also mediated by RNAi via sequence-specific small RNAs. This study, together with our previous finding of SIS in a-a opposite-sex mating of the C. neoformans serotype A var. gruberi lineage, demonstrates that SIS is a conserved process between the divergent C. neoformans serotype A and serotype D sibling species. In each case, our data show that the SIS RNAi pathway operates to defend the genome via squelching transposon activity during the sexual cycles. Thus, our discovery of SIS brings a fresh perspective to meiotic silencing involving the upregulation of RNAi pathways as a strategy to guard genomic integrity during sex. More importantly, the presence of SIS in both a-a unisexual reproduction and a-a opposite-sex mating indicate that SIS may be triggered by the shared pheromone sensing Cpk1 MAPK signal transduction cascade. Ongoing studies focus on defining at a mechanistic level how the SIS RNAi pathway is initiated, including identifying new components involved in SIS.

702. Effects of the use of biocontrol agent (Phlebiopsis gigantea) on fungal communities of Picea abies stumps. E. Terhonen, H. Sun, M. Bude, R. Kasanen, L. Paulin, F. Asiegbu. 1) University of Helsinki, Department of Forest Sciences, P.O.Box 27, FIN-00014, University of Helsinki, Finland; 2) INRA, UMR 1136 INRA/Nancy Université Interactions Arbres/Microorganismes, INRA-Nancy, 54280 Champenoux, France; 3) DNA Sequencing and Genomics Lab, Institute of Biotechnology, University of Helsinki, P.O. Box 56, FIN-00014 Helsinki, Finland.

The saprotrophic fungus Phlebiopsis gigantea has for several years been used as a biocontrol agent against pathogen Heterobasidion annosum. This pathogen is the major cause of root rot disease in conifers that results in economic losses estimated at 50 million euros to Finnish forestry. A major
problem is that, although the effectiveness of *P. gigantea* as a bio-control agent has empirically been shown, the long term biological effect of this fungus on conifer trees as well as on other soil micro-flora has not been empirically proven. We investigated the impact of *P. gigantea* treatment on stump mycobiota using metagenomic pyrosequencing approach as this has not been done before. Samples from forest sites pre-treated with *P. gigantea* for 1, 6 and 13 years ago were collected, DNA was isolated and pyrosequenced. Similarly samples were also collected from untreated stumps within the same forest sites. Sequences were quality trimmed using Mothur software. After trimming we had 26,398 sequences from 53,117. For the extraction of the full ITS1 of the nuclear ITS region FungiAllTestxtractor was used and these sequences were clustered at 97% similarity using cd-hit-454 with the most abundant sequence types serving as cluster seeds. The most frequent sequence type in each cluster was used for the BLAST searches against NCBI BLASTN and 4.97% similarity across the entire length of the pairwise alignment was taken to indicate conspecificity. Differences between control and treated stumps were tested statistically with Paired-Sample T-test (SPSS 19). Also diversity indexes and similarity indexes between controls and treated were calculated using Estimates 8.2.0. After one year of the clear-cut we found from *Phlebiaopsis gigantea*-treated stumps 107 different fungal OTUs and from non-treated stumps 119 fungal OTUs, from which they shared 102 OTUs. After 6 years we observed from treated stumps 118 fungal OTUs and from non-treated 134 fungal OTUs and they shared 99 OTUs. After 13 years we found from treated stumps 131 OTUs and from non-treated 139 OTUs and shared OTU number were 109. However there were no statistical differences between control and treated. Based on our results our primary conclusion is that stump treatment should continue as there is no obvious adverse effect on the other stump mycobiota.

**703. Diversity of yeast and mold species in different cheeses, Nabara Banjara1, Kenneth Nickerson2, Heather Hallen-Adams3. 1) Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE; 2) Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE.**

The yeast and mold diversity from different commercial cheeses collected from local markets (Lincoln, NE, USA) was studied using microbial counting and molecular biological approaches. Twenty one distinct types of cheese samples were investigated. Briefly, 10 grams of each cheese sample was homogenized in distilled water, serially diluted from 10 to 10⁵, grown in Yeast Extract Glucose Chloramphenicol Agar (YGC) and the population was counted from dilution plates. Yeasts and molds were identified by amplification and sequence analysis of the nuclear ribosomal RNA genes, using ITS1 and TW13 primers. *Debaryomyces hansenii* was the predominant fungal species in most of the cheeses (59% of samples at up to 5.7 x 10⁵ CFU/gram). Other fungi isolated included *D. fabryi, D. prosopisida, D. subglobosus, Penicillium roqueforti and Candida sake*. In our samples, farmstead cheese had the highest (2.1 x 10⁶ CFU/gram) fungal population.

**704. Heterologous expression and characterization of soil organic matter-specific proteases secreted by the ectomycorrhizal fungus Paxillus involutus, Morten N. Grell1, Ulas Pupelsis2, Tomas Johansson3, Firoz Shah4, Lene Lange5, Anders Tunlid6. 1) Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University Copenhagen, Denmark; 2) Microbial Ecology Group, Department of Biology, Lund University, Sweden.**

*Paxillus involutus* (Batsch) Fr. (Basidiomycetes; Boletales) is widely distributed in the Northern hemisphere, and is one of the best-studied ectomycorrhizal fungi, especially with respect to its ecology and physiology. In a study on the mechanisms by which *Paxillus involutus* degrade complex organic matter extracted from plant litter material, transcripts were sequenced using the 454 technology and NimbleGen microarrays produced. A number of genes encoding extracellular enzymes showed an increased transcript level during degradation of soil organic matter (SOM), as compared with growth on a defined medium (MMN). They were suggested to constitute a Fenton-like, radical-based biodegradation system that disrupts the organic matter-protein complexes thereby mobilizing embedded nitrogen (Rineau et al. 2012, Environm. Microbiol. 14, 1477-1487). Supporting this, a number of protease genes were found to have a significantly increased SOM/MMN expression value and to be upregulated during growth on protein-rich substrates. Most highly expressed were aspartic, metallo, and serine proteases. This is in agreement with biochemical analysis of SOM degradation. To study substrate specificity and regulation of selected proteases in detail these are expressed in the yeast *Pichia pastoris*.

**705. Effector proteins in fungal defense against fungivorous nematodes: Targets and functional significance, Therese Wohlschlager1, Stefanie Schrodi2, Alex Butch3, Paola Grassi1, Alexander Titz1, Stuart Haslam2, Michael Hengartner2, Markus Aebi1, Markus Künzler3. 1) Institute of Microbiology, ETH Zürich, Switzerland; 2) Institute of Molecular Life Sciences, University of Zürich, Switzerland; 3) Division of Molecular Biosciences, Imperial College, London, United Kingdom; 4) Department of Chemistry, University of Konstanz, Germany.**

The defense of fungi against fungivores is largely based on the production of intracellular toxins. A significant proportion of these toxins are peptides and proteins that are synthesized by the ribosome and stored in the cytoplasm. Protein toxins include lectins that target specific glycoepitopes in the intestine of the fungivore upon ingestion and kill the fungivore by a yet unknown mechanism. In our laboratory, we focus on the functional characterization of fungal protein toxins that are directed against nematodes. We use the model nematode *Caenorhabditis elegans* to identify the targets and to study the toxicity mechanism of these fungal defense effector proteins in the nematode. In addition, we employ the fungivorous nematodes *Aphelenchus avenae* and *Bursaphelenchus willibardi* to study the diversity, the functional significance and the transcriptional regulation of these proteins in the fungus. Recently, we identified a nematotoxic lectin from the mushroom *Laccaria bicolor* that is homologous to animal lectins involved in innate immunity against bacteria. We found that the nematotoxicity of the lectin is based on its specific binding to methylated fucose residues on nematode N-glycans. Among animals, this epitope is only present in mammals and molluscs but not in insects or vertebrates. We performed affinity chromatography of *C. elegans* whole worm protein extracts using the *L. bicolor* lectin and other nematotoxic fungal lectins recognizing protein-bound glycans. The results of this analysis suggest that these lectins target the same set of glycoproteins in the nematode intestine and may confer toxicity by a common mechanism. In order to address the functional significance of these proteins for fungal defense against fungivorous nematodes, we expressed some of the fungal proteins displaying toxicity towards *C. elegans*, in the filamentous ascomycete *Ashbya gossypii*. These transformants were fed to *A. avenae* and the propagation of the fungivorous nematodes on the various transformants was determined. Expression of some effector proteins significantly inhibited propagation of the nematode suggesting that these proteins have a role in fungal defense against these organisms. Experiments addressing the relative fitness of the various *A. gossypii* transformants upon selective pressure of feeding by *A. avenae* are under way.

**706. Microfluidic platforms for monitoring interactions between fungi and bacteria, Martina A. Stöckl1, Claire E. Stanley2, Pauli T. Kallio2, Markus Künzler3, Andrew I. deMello1, Markus Aebi1. 1) Microbiology and Immunology, ETH Zürich, Zurich, Switzerland; 2) Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland.**

Bacteria and fungi share many microhabitats where they interact with each other. These interactions are diverse and play important roles in certain human infections, as well as in the biological control of plant diseases. The basis of these interactions, however, is not well understood, as their investigation is technically challenging. Conventional microscopic approaches are limited, in that they can only monitor interactions at a particular time.
point and are not able to follow dynamic interactions. Herein, the development of a microfluidic platform, to study the interplay of individual hyphae with bacteria in a confined compartment, is detailed. A microfluidic device, comprised of single, interconnected microchannels, is filled with medium. The device is manufactured from the polymer, polydimethylsiloxane, which is bonded to a glass layer. Importantly, this polymer possesses some desirable characteristics, making it compatible with experiments of biological nature, for example it is oxygen permeable, and allows optical detection from 240 nm to 1100 nm. The fungus can easily grow into the microchannels from an agar plug that is placed next to a lateral opening. Subsequently, the device can be co-inoculated with bacteria through a separate inlet, allowing changes in morphology, growth rate, and interaction patterns of the same hyphae upon the addition of bacteria to be investigated over time. As an example, the interaction of the basidiomycetous model organism, Coprinopsis cinerea, and the gram-positive bacterium, Bacillus subtilis, which are both found in dung of herbivores, has been studied. Using these microfluidic platforms, it has been observed that B. subtilis cells attach to hyphae in an end-on manner. The frequency of attachment within hyphae and between different hyphae varies. Furthermore, the tips of the hyphae are not colonised by the bacterial cells. In summary, this approach can be used to study phenotypic changes of bacteria and fungi over specific time periods. In future, it is envisaged that this data will be combined with fungal and bacterial genetic approaches.

707. WITHDRAWN


Fusaria are responsible for prejudicial diseases on cereal crops worldwide, such as crown rot and Fusarium head blight. Beyond economic losses due to infection symptoms, these pathogens can produce several types of mycotoxins that are harmful to livestock and humans. They are extremely diverse at the intra-specific levels in terms of types as well as quantities of toxins that a strain can produce. Developing appropriate strategies to limit contamination with Fusarium mycotoxins requires a greater knowledge about this variability. We have collected a large number of toxigenic Fusarium strains. Our collection now includes about 800 strains, mostly from the species graminearum, culmorum, verticilloides, proliferatum, and temperatum. Species were identified based on morphology and real-time PCR. More than half of our strains were further characterized for toxin production using biochemical and/or real-time PCR-based tools. We isolated about 70 F. graminearum strains from either wheat or maize grains originating from different French cereal production areas. Our results show a high representation of 15-acetyldeoxynivalenol-producing strains in our French samples. Within the same chemotype, we observe a large variability in toxin production levels. The F. graminearum strains were characterized with microsatellite markers and show a large genetic diversity. Two groups were delineated according to their genetic background, roughly corresponding to strains isolated from Europe in one hand and America in the other hand. Our results are also in agreement with the fact that only F. graminearum sensu stricto strains seem to be detected in France so far. The demonstrated genetic and phenotypic diversity provides a sound ground for a growing number of long-term downstream studies such as genetic association and quantitative genetics to understand the determinism of toxin production. Such information should be doubtlessly considered in plant breeding efforts and other disease management strategies aimed at reducing the mycotoxin risk in food and feeds. Our collection is a valuable tool to improve our understanding of toxigenic diversity in Fusarium species. It is managed through a database gathering all information collected on each strain, already available upon request and soon publically available as a web-based interface.

709. Chemical genetics: Discovery of novel fungicides and their targets in the phytopathogen Fusarium graminearum. G. Subramaniam, C. Moggy. Agriculture Canada, Ottawa, ON, Canada.

Chemical genetics screen is based on the ability of small molecules to bind to biological molecules and alter their function. Screening of pharmaceutical libraries has revealed novel molecules effective against cancer and other diseases. We have adopted similar approach and identify bioactive compounds that will block the growth and development of F. graminearum. We have developed a 96-well format to monitor the growth of F. graminearum in liquid media. The fungus is tagged with a green fluorescent protein (GFP) and the growth is monitored by the measurement of fluorescence of the GFP. This format facilitates high throughput screening for small molecules that could potentially disrupt the growth of the fungus. As proof of concept, we screened ~560 compounds from the TimTec NDL and showed that several compounds with anti-Fusarium properties. One compound identified from our screen, “Antofine” was purified from Vincetoxicum rossicum and was used in subsequent studies, to identify targets in the fungus. We used the gene deletion library of the budding yeast Saccharomyces cerevisiae to identify targets for Antofine. GeneMANIA (http://www.genemania.org), an online multiple association network integration algorithm was used to uncover information pertaining to genetic and physical interactions of these targets. Our efforts to identify targets in Fusarium against Antofine will be discussed.


The filamentous fungus, Aspergillus flavus, produces the toxic and carcinogenic, polyketide-derived family of secondary metabolites termed aflatoxins (AFs). In addition to the AF biosynthetic gene cluster, analysis of the A. flavus genome has identified 55 gene clusters predicted to be associated with secondary metabolism. To date, very few of the metabolites produced by these clusters have been identified. Secondary metabolism is controlled by global regulators such as LaeA and VeA. In a veA knockout mutant we identified a significantly down-regulated polyketide synthase (PKS) gene belonging to cluster 27. Although the metabolite produced by this cluster was unknown, in silico cluster analysis predicted that cluster 27 would consist of the PKS gene and four other genes. qRT-PCR analysis confirmed that expression of the cluster 27 PKS (pks27) gene was down-regulated in the veA mutant. Inactivation of the pks27 gene resulted in loss of the dark pigment associated with A. flavus sclerotia. Sclerotia are survival structures produced by condensation of mycelia and function as propagules in the field. Conidial pigmentation did not appear to be affected in the pks27 knockout strain. TLC and HPLC analysis of sclerotial extracts identified the cluster 27 metabolite as asparasone A. Insect feeding studies using wild-type and mutant sclerotia indicated that the pigment may act as a feeding deterrent. To our knowledge this is the first report on the identification of a gene that encodes a sclerotium-specific pigment. The pigment likely plays a role in sclerotial resistance to insect feeding and possibly other environmental stresses.


Mn amendment to P. ostreatus cultures enhances degradation of recalcitrant aromatic compounds. Manganese peroxidase (MnP) isoenzymes are key players in these processes. The MnP gene family is comprised of five Mn -dependent peroxidases (mnp3, 6, 7, 8 and 9) and four versatile-peroxidases (mnp1, 2, 4 and 5; VPs). In liquid medium, Mn amendment resulted in a drastic up-regulation of the predominantly expressed mnp3 and mnp9, and down-regulation of mnp4. To obtain direct evidence for the role of these enzymes, we produced genetically-modified (knockout, knockdown and/or over-
expression) strains in mmps and studied their degradation capacity. The compounds studied were: azo-dyes such as orange II and reactive black, recalcitrant pharmaceutical compounds found in treated waste water such as Carbamazepine and lignocellulosic agricultural waste. We engineered a transformant, constitutively expressing mnp4 a VP naturally repressed by Mn (designated OEmnp4) under the control of the b-tubulin promoter. Now, despite the presence of Mn in the medium, OEmnp4 produced mnp4 transcript as well as VP activity as soon as four days after inoculation. OEmnp4 decolorized the azo-dyes two days earlier relative to the wild type in Mn amended medium. RNAi silencing targeting mnp3 resulted in a delay in the decolorization capacity which occurred concomitantly along with a marked reduction of the expression level of all mmps, particularly mnp3 and mnp9. This observation supported the conclusion that MnpPs are involved in the process but could not determine the specific contribution of the different genes to the outcome. Therefore we produced a Dku80 strain, exhibiting a 100% homologous DNA recombination rate, to enable specific gene replacement. Subsequently, homokaryon mnp2, 3, 4 and 9 knockout strains were produced. In Mn amended GP, orange II decolorization was not significantly inhibited by any of these strains, indicating on functional redundancy. In Mn deficient GP, inactivation of mnp4 proved that it encodes the key VP responsible for Mn dependent and Mn independent peroxidase activity, as well as resulted in reduction of the azo dye reactive black S decolorization capacity. The tools and protocols developed increase the amenability of P. ostreatus to genetic manipulations and expand options for gene function analyses.

712. Temperature- and pH characteristics of endo-cellulases in Rhizophyctis rosea. Bo Pilgaard1, Frank Gleason2, Peter Busk3, Lene Lange1. 1) Section for sustainable biotechnology, Aalborg University- Copenhagen, Denmark; 2) School of Biological Sciences, University of Sydney, New South Wales, Australia.

The zoosporic true fungi of the order Chytridiales are of special interest since they constitute a central root position of the entire fungal kingdom. Enzymes of the anaerobic zoosporic rumen fungi have been studied quite extensively, but only scarce information is available about enzymes from aerobic zoosporic fungi from soil. We have previously confirmed the presence of cellulases in Rhizophyctis rosea (AUS13), which was isolated from soils of the Sydney Basin. Other studies have shown that this fungus can survive and grow within a wide pH-range (Gleason et. al 2010) and high temperatures (Gleason et al. 2005). We investigate the cellulolytic potential of Rhizophyctis rosea in several cellulase assays and characterize the enzymatic properties with respect to temperature and pH stability of the enzymes. Moreover, we are sequencing the Rhizophyctis rosea genome in order to find the genes for cellulose-degrading enzymes (Gh6, Gh7, Gh45, Gh6) that are present in Chytrids as compared to the cellulases of these families found in other fungal groups.


The demand on the biotechnological production of proteins for pharmaceutical, medical and industrial applications is steadily growing. Not every protein can easily be produced by the existing platforms. For the production of such challenging proteins, we aim to establish a novel expression system in the well characterized eukaryotic microorganism Ustilago maydis. In this fungus, secretion of the endochitinase Cts1 depends on mRNA transport along microtubules, which is mediated by the key RNA-binding protein Rrm4. We recently demonstrated that Cts1 secretion occurs via a novel unconventional route. We used b-glucuronidase (Gus) as a reporter for unconventional secretion. This bacterial enzyme is inactivated by N-glycosylation during its passage through the conventional eukaryotic secretory pathway. By contrast, in our system Gus was exported in its active form by fusion to Cts1 confirming its secretion by an unconventional route. Furthermore, we showed that this secretory mechanism can be exploited for the export of active heterologous proteins. As a proof-of-principle for economically important biopharmaceuticals we expressed an active single-chain antibody. Importantly, the novel protein export pathway circumvents N-glycosylation which is advantageous in many applications, for example to avoid undesired immune reactions in humans. Currently, the system is optimized with respect to product yield by e.g., reducing the proteolytic activity in the culture supernatants. Thus, the unconventional Cts1 secretion machinery has a high potential for the production of biotechnologically relevant proteins.

714. A new method for fungal genetics: flow cytometry of microencapsulated filamentous microcolonies. L. Delgado-Ramos1, A. T. Marcos1, X. Peñate1, M. S. Ramos-Guelfo1, L. Sánchez-Barrionuevo1, F. Smet1, D. Canovas1, S. Chávez1. 1) Dept of Genetics, Univ of Sevilla, Spain; 2) Union Biometrica, Geel, Belgium.

Genetic analysis of non-filamentous microorganisms is facilitated by the isolation of consistent, well-defined colonies on solid media and the handling of individual cells by flow cytometry. In contrast, some filamentous fungi are hard to be analyzed using these procedures; in particular by flow cytometry. The combination of single spores microencapsulation and large particle flow cytometry is a possible alternative for the analysis of filamentous fungi. Microencapsulation allows the early detection of fungal growth by monitoring the development of hyphae from encapsulated individual spores. Mycelium proliferation inside the microcapsules can be detected using COPAS™ large particle flow cytometry. Here we show the successful application of the Flow Focusing® technology to the microencapsulation of filamentous fungi in monodisperse alginate microspheres, using Aspergillus and Trychoderma as model systems. Using a Cellena® Flow Focusing microencapsulator, we managed to produce monodisperse microparticles containing individual spores and to develop microcolonies of these fungi upon germination in the appropriate conditions. Proliferation inside the particles was monitored by microscopy and large particle flow cytometry without requiring fluorescent labeling. Sterility was preserved during the microencapsulation procedure, preventing undesired contaminations. Conditional mutants were utilized to demonstrate the feasibility of the method. This procedure allows for the handling, screening and analysis of clonal colonies in liquid culture. Examples of applications will be provided.

715. DNA methylation dynamics during development in the rice blast fungus. Junhyun Jeon1, Jaeyoung Choi1, Sook-Young Park2, Aram Huh2, Ralph Dean3, Yong-Hwan Lee4,5. 1) Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, South Korea; 2) Fungal Bioinformatics Laboratory, Seoul National University, Seoul 151-921, Korea; 3) Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea; 4) Center for Integrated Fungal Research, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27607, USA; 5) Center for Fungal Genetic Resources, Seoul National University, Seoul 151-921, Korea.

Cytosine methylation is an important epigenetic modification of DNA that is involved in genome defense and transcriptional regulation in eukaryotes. In mammals and plants, many roles of DNA methylation depend on dynamic changes of DNA methylation pattern. In fungi, DNA methylation is considered primarily as a stable mark for silencing transposable elements. Here we used genetic manipulations and high-throughput bisulphite sequencing on the model plant pathogenic fungus, Magnaporthe oryzae to elucidate the dynamics and mechanics of DNA methylation during pathogenic development. We found that genome-wide reprogramming of DNA methylation in and around genes occurs during progression of fungal development and that such reprogramming is important for normal development. RNA-seq analysis showed that DNA methylation is associated with transcript abundance of genes in context-dependent manner. Our study reveals that DNA methylation in fungi could be a dynamic epigenetic entity that has assumed new roles in developmental processes other than genome defense.
716. pH-enotype array: a novel phenomics platform for filamentous fungi. Jaejin Park, Yong-Hwan Lee. 1) Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea; 2) Center for Fungal Pathogenesis, Center for Fungal Genetic Resources, Plant Genomics and Breeding Institute, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea.

Rapid increase of genome information and application of high-throughput mutagenesis technology allowed large-scale gene characterization in filamentous fungi. However, phenotype screening is a bottleneck as it entails time-consuming and labor-intensive processes. Thus there is a demand for a novel platform for high-throughput phenotype screening. Although several microplate-based assays are currently available, their application on organisms growing in filamentous form has been met with considerable difficulty due to uneven distribution of cells. As a solution, we developed pH-enotype array - a new phenomics platform in filamentous fungi. This platform is based on the pH change in the culturing media, reflecting viability and physiological status of cells. The pH in culturing media is continuously measured by a microplate spectrophotometer using two pH indicators, bromocresol purple and phenol red. The validity of the system was comprehensively evaluated with Magnaporthe oryzae strains and we confirmed that the growth responses to various stresses or nutrient conditions can be characterized reliably within 24 hours using the optimized medium. This phenomics platform would provide a novel, high-throughput phenotype screening method in filamentous fungi and promote phenotype standardization for comparative functional genomic studies.


We have confirmed that, as expected for a mismatch repair gene with both vegetative and meiotic roles, the phenotype of a msh-2 deletion in Neurospora crassa is recessive. Chromatid data indicates that deletion of msh-2 increases allelic recombination at his-3 by a factor of 1.6 with no effect on crossing over in the his-4 to ad-3 interval in which lies both his-3 and the recombination hotspot cog. Although analysis of a small number of octads from an msh-2 deletion cross suggested that the only non-Mendelian segregation is post-meiotic, the ease by which octads can be scanned for recombination events under a fluorescent microscope when GFP is inserted close to cog allowed us to reveal the wide range of recombination outcomes normally hidden by the activity of Msh-2. We report that a degree of mismatch repair is retained in the absence of Msh-2 and that recombination initiated by cog exhibits a strong bias for repair in the direction of restoration rather than conversion. In contrast to recombination events in budding yeast, symmetric heteroduplex appears to be frequent in the his-3 region, suggesting variation between recombination pathways utilised in the two species.


We have previously shown that although most genomic regions of Neurospora spo11<sup>−/−</sup> mutants lack meiotic recombination, crossing over in the his-3 region persists at close to wild-type levels. However, this residual recombination could conceivably occur after meiosis, during a transient period of partial diploidy. Using crosses heteroallelic for his-3 mutations, we have shown that in spo11<sup>−/−</sup> homozygotes, as in wild type, stable His<sup>+</sup> progeny are generated at high frequency. We have utilised mutations in either end of a histone H1-GFP fusion gene, inserted between the recombination hotspot cog and his-3, to show that the frequency at which GFP<sup>+</sup> spores arise in a cross homozygous for spo11<sup>−/−</sup> is comparable to the frequency of His<sup>+</sup> spores, and that glowing nuclei first appear during pachytene, as expected for a product of meiotic recombination. In similar spo11 deletion homozygotes, GFP<sup>+</sup> spores also arise at high frequency and glowing nuclei are also first seen at pachytene. Thus, spo11 mutant homozygotes experience both crossing over and allelic recombination during meiosis, suggesting there is a spo11-independent mechanism for initiation of recombination at his-3.


Nanotechnology, the study of the controlling matter of an atomic and molecular scale, has emerged as an interesting and important scientific field and the controlled synthesis of nanostructures from different chemical composition as well as their shape, size, and dispersity are important areas of research. The so-called ‘green chemistry’ or ‘nanobiotechnology’ employs microorganisms to fabricate nanostructures and has the benefit of improving the biocompatibility of nanomaterial, however the control over average particle size and uniform morphology is required but still a challenge. This work bases on the use of Neurospora crassa, a non-pathogenic filamentous fungus with rapid growth rate, for the biosynthesis of gold nanoparticles of controlled size and shape. Briefly, the fungal extract was incubated with the gold precursor solution at different conditions of temperature, pH and time of reaction. The best results obtained were from incubations at 60°C, at pH 3, particles of different shapes (e.g. spheres, triangles, hexagons, pentagons, rhombs and bars) were formed while at pH 5.5 and pH 10 small quasi-spherical particles were formed with size ranges of 6 to 21 nm and 3 to 12 nm, respectively. High resolution transmission electron microscopy (HRTEM) using a FEI Tecnai F30 transmission electron microscope confirmed the crystalline and elemental character of the gold nanoparticles. The synthesized gold nanoparticles of different shapes were shown to possess excellent surface-enhanced Raman scattering (SERS) enhancement ability relative to quasi-spherical gold nanoparticles. Small quasi-spherical nanoparticles of 3 to 12 nm enhances the Raman signals of methylene blue about 2 times, those of 6 to 23 nm enhance the Raman signals about 25 times whereas nanoparticles of different shapes with a broad size range enhances the Raman signals of methylene blue about 40 times. Results are promising and show that these gold nanoparticles might have potential applications for biological sensing and labeling systems.


The filamentous fungus Neurospora crassa is a well-studied model organism that is frequently isolated from the environment in association with burned vegetation. Enzyme activities related to the metabolism of plant cell wall material have been observed in N. crassa, but the overall cellular response of the organism to such a recalcitrant carbon source remains poorly defined. In order to investigate the elements involved in fungal growth on cellulose, we utilized the near full genome collection of single gene knockout strains that has been generated under the auspices of the Neurospora Genome Project. Strains with deletions in loci thought likely to play a role in cellulase activity or production - including the known secretome of N. crassa when cultured on plant biomass, homologs of the Saccharomyces cerevisiae secretion apparatus, and proteins predicted to traverse the secretory pathway - were singled out for analysis. This subset of the N. crassa deletion collection was cultured on a cellulosic substrate and the levels of secreted protein and cellulase activity were compared to wild-type. A number of hyper- and hypo-secretion mutants have been identified. Sequence analysis suggests that while many of the loci encode fungal-specific proteins of undetermined functions, some of the deleted genes likely act in transcription, protein synthesis, and intracellular trafficking. Early work with the latter found that their hyper- or hypo-secretion phenotypes were a specific response to cellulose, but not
limited to the production of cellulytic enzymes. The continuing characterization of these mutants will enhance our understanding of the ability of N. crassa to utilize the complex carbon sources present in its natural environment.


Self/nonself recognition is a ubiquitous and essential function for many organisms. In filamentous fungi, self/nonself recognition is conferred by genetic differences at het (heterokaryon incompatibility) loci. The genes that mediate H1 (heterokaryon incompatibility) exhibit characteristic evolutionary signatures, including balancing selection and trans-species polymorphisms. Recent analyses show that genes containing a HET domain are involved in H1, making HET domain genes good candidates for identifying new het loci. In this study, we utilized RNA-seq data from a population of 110 Neurospora crassa strains to look for HET domain genes that were highly polymorphic, have multiple alleles, and show balancing selection, and trans-species polymorphisms. Using this approach, we identified 19 of the 62 HET domain genes in N. crassa that fit the criteria for a het locus. Further, we showed that one of these HET domain genes, NCU09037, functions as a het locus.

722. Combinatorial cationic and oxidative stresses promote the killing of Candida albicans cells by human neutrophils. Alistair J P Brown1, Despoina Kaloriti1, Mette Jacobsen1, Zhikang Yin1, Anna Tillmann1, Miranda Patterson1, Deborah A Smith1, Emily Cook1, Tao You1, Iryna Bohovych2, Celso Grebogi1, Neil A R Gow3, Janet Quinn5, Ken Haynes5. 1) School of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom; 2) Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle University, United Kingdom; 3) School of Biosciences, College of Life & Environmental Sciences, University of Exeter, United Kingdom; 4) Institute for Complex Systems and Mathematical Biology, School of Natural and Computing Sciences, University of Aberdeen, United Kingdom.

Candida albicans is an opportunistic pathogen of humans. It is thought to have evolved as a relatively harmless commensal. C. albicans is a frequent cause of mucosal and skin infections (thrush). However, our immune system normally blocks potentially lethal systemic infections. Neutropenic patients are prone to systemic candidiasis because they lack the effective defenses provided by circulating neutrophils. We have shown that the efficient killing of C. albicans by human neutrophils is mediated by the potentiating combinations of stresses they impose on the invading fungus, rather than specific individual stresses. In particular, exposure to the combination of reactive oxygen species and cation fluxes kills C. albicans synergistically. We have explored the mechanistic basis for this synergistic killing using genomic exploration and molecular dissection in C. albicans combined with dynamic mathematical modeling. Signalling via the Hog1 stress activated protein kinase and the Cap1 AP-1-like transcription factor is inhibited by combinatorial cationic and oxidative stresses, and as a result, their downstream gene targets are not induced. This prevents the activation of normal oxidative and cationic stress adaptation and repair mechanisms. In particular, hydrogen peroxide detoxification mechanisms are inhibited by elevated salt concentrations. This leads to the accumulation of intracellular reactive oxygen species, and ultimately to accelerated necrotic death. Ectopic expression of key detoxification mechanisms in C. albicans cells decreases the efficacy of killing by human neutrophils.

723. Phosphoproteomic analysis of the aquatic fungus Blastocladiella emersonii during germination. J. Crestani, S. Lopes Gomes, Biochemistry, IQ, USP, Sao Paulo, Brazil.

The aquatic fungus Blastocladiella emersonii presents an interesting life cycle with two cell differentiation stages, the germination and the sporulation, during which drastic morphological and biochemical changes are observed. During germination, protein synthesis is inhibited, a transient increase of AMP levels is observed, activation of PKA is detected, as well as mobilization of cellular glycogen and an efflux of calcium. These results suggest a possible phosphorylation/dephosphorylation control, probably through cell signaling networks, which were not characterized up to now. Therefore, the present work aims to elucidate these signaling phenomena by using a phosphoproteomic analysis of B. emersonii during the germination. Firstly, we compared the phosphoproteomic profile from two distinct cell types of B. emersonii, the zoospores and the germling cell (at 45 min of germination) by using two-dimensional gels stained with Pro-Q Diamond phosphoprotein dye. The comparison revealed about 82 phosphoproteins from germling cells and 44 phosphoproteins from zoospores. These preliminary results suggest that phosphorylation events may be involved during early germination. To detect the signaling processes and the proteins involved in these events we utilized IMAC-IMAC phosphoproteomic methodology to obtain an enriched phosphopeptide sample from each stage of germination and early vegetative growth (0, 25, 45, 60 and 90 min). Enriched phosphopeptide samples will be detected by using a LTQ Velos Orbitrap mass spectrometer; filtered using DTASelect and analyzed using Ascore and Debunker. The results of this work will contribute to improve the knowledge of the cellular regulatory processes in this early diverging fungus. Financial support: FAPESP and CNPq.

724. Towards an accurate genome: high-throughput proteogenomic validation of Stagonospora nodorum genes via sub-cellular proteomics. Kejal Dodhia1, Robert Syme2, Thomas Stoll2, Marcus Haste2, James Hane1, Angela Williams5, Eiko Furuki4, Jeffrey Gorman1, Richard Oliver1, Kar-Chun Tan1. 1) The Australian Centre for Necrotrophic Fungal Pathogens, Environment & Agriculture, Curtin University, Perth, Bentley 6102, Australia; 2) Protein Discovery Centre, Queensland Institute of Medical Research, Herston, Qld 4029, Australia; 3) Plant Industry, Commonwealth Scientific and Industrial Research Organisation, Private Bag No, 5, Wembley WA 6913, Australia.

Stagonospora nodorum is the causal agent of stagonospora nodorum blotch on wheat. S. nodorum was the first of the Pleosporales fungi to have its genome sequence published and genes annotated. However, in silico gene annotation can be erroneous. Therefore, experimental evidence is often needed to refine gene annotations. Proteogenomics is an emerging high-throughput technique, which is a “direct-to-genome mapping” technique whereby the mass spectra from protein analyses are mapped onto the predicted gene set and/or the 6-frame whole genome translation. In this study, we performed a comprehensive proteogenomic analysis of the secreted, intracellular and cell-wall/membrane sub-proteomes of S. nodorum using a two-dimensional liquid chromatography (2D-LC) LTQ Orbitrap MS approach. This study has verified a total of 3580 genes from all sub-proteomes. Of these, 113 had not been experimentally verified previously. When combined with previous proteomic data, 4377 (35% of the total predicted gene set) genes were verified. In addition, all mass spectra were matched to a 6-frame genome translation database to identify evidence of gene model conflicts. The study has found that 2629 genes showed evidence of frame conflicts and extensions of coding exons into annotated introns or untranslated regions. At least 43 potential new genes were identified.


Fungal polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) hybrid enzymes produce a broad array of ecologically and medicinally relevant compounds. The enzymes are evolutionarily conserved, and their structures and functions are determined by their domain compositions, which are the result of evolutionary events, such as recombination and horizontal gene transfer. To identify conserved domain compositions in PKS-NRPS hybrid enzymes, a database of fungal protein sequences was screened for sets of domain pairs that co-occur, and their occurrence was assessed by using a sliding window scan over the domain pairs. Similarly, domain pairs that do not co-occur were identified by using a second scan with the reverse window direction. The results were verified by using a conservative approach that included the sequence alignment and functional analysis of representative enzymes. The results of this work will contribute to a deeper understanding of the evolutionary processes that have shaped these enzymes.

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natural products. To date, only a dozen gene clusters could be matched to the requisite PKS-NRPS pathways and the programming of the multifunctional enzymes is still enigmatic. The (heterologous) expression of chimeras of PKS (lovastatin synthase, LovB) and NRPS (cytochalasin synthetase, CheA) in *Aspergillus terreus* did not result in the production of polyketide-amino acid hybrid molecules and suggests a potential incompatibility of a fungal highly reducing PKS (hrPKS) with the NRPS component of fungal PKS-NRPS hybrids. Furthermore, the heterologous expression of a shortened CheA (truncated after the C domain) in A. oryzae did not lead to cyclized products. To rationalize the unexpected outcome of the gene fusion (and shortening) experiments, we accomplished extensive bioinformatic analyses of fungal PKS-NRPS hybrids and LovB-type PKS. Hence, a noncanonical function of C-terminal condensation (C) domains in truncated PKS-NRPS homologues and an evolutionary imprint of the PKS-NRPS domains, which reflect the evolutionary history of the entire megasynthase, was inferred. Moreover, the participation of not only the adenylation (A) domain but also the C domain to amino acid selection was shown to be likely. These findings shed new light on the complex code of this emerging class of multifunctional enzymes and will greatly facilitate future combinatorial biosynthesis and pathway engineering approaches towards natural product analogues.

726. Secondary metabolism and development is mediated by LlmF control of VeA subcellular localization in *Aspergillus nidulans*. Jonathan M. Palmer1, Jeffrey Theisen2, Rocio Duran2, Scott Grayburn2, Ana Calvo2, Nancy Keller1. 1) Medical Microbiology and Immunology, Univ Wisconsin, Madison, WI; 2) Biological Sciences, Northern Illinois University, DeKalb, IL.

Secondary metabolism and development are linked in *Aspergillus* through the conserved regulatory velvet complex composed of VeA, VeB and LaeA. The founding member of the velvet complex, VeA, shuttles between the cytoplasm and nucleus in response to alterations in light. Here we describe a new interaction partner of VeA identified through a reverse genetics screen looking for LaeA-like methyltransferases in *Aspergillus nidulans*. One of the putative LaeA-like methyltransferases identified, LlmF, is a negative regulator of sterigmatocystin production and sexual development. LlmF interacts directly with VeA and the repressive function of LlmF is mediated by influencing the localization of VeA, as over-expression of LlmF decreases the nuclear to cytoplasmic ratio of VeA while deletion of *llmF* results in an increased nuclear accumulation of VeA. We show that the methyltransferase domain of LlmF is required for function, however LlmF does not directly methylate VeA in vitro. This study identifies a new interaction partner for VeA and highlights the importance of cellular compartmentalization of VeA for regulation of development and secondary metabolism.

727. Overproduction of phleichrome by synthetic inducers and cloning of polyketide synthase genes in phytopathogenic fungus *Cladosporium pheili*. K.-K. So1, N.-L. Nguyen1, J.-M. Kim1, Y.-S. Jeong1, D.-H. Kim1. 1) Institute for Molecular Biology and Genetics, Center for Fungal pathogenesis, Chonbuk National University, Jeonju, Jeonbuk, South Korea; 2) Department of Bio-Environmental Chemistry, Wonkwang University, Iksan, Jeonbuk, South Korea.

Phleichrome pigment produced by a *Cladosporium pheili* is a pathogenic toxin of timothy plant (*Phleum pretense*). Phleichrome reacts with oxygen molecules following light activation to produce highly toxic reactive oxygen species. Phleichrome is structurally similar to elsiochrome and several other 4,9-dihydroxypyrrole-3,10-quinone fungal toxins. Phleichrome has a huge potential to be used as photodynamic agent for treatment of cancer and viral infection. Using the UV mutagenesis method we were able to obtain two mutant strains that overproduced phleichrome in different culture conditions compared with the wild type strain. In addition, we synthesized two different diketopiperazines as inducers and confirmed that diketopiperazines significantly enhanced phleichrome biosynthesis in a dose dependent manner. To gain insight into the metabolic pathway of phleichrome production, we performed to clone and sequence several polyketide synthase (PKS) genes. While the three representative types of PKS, two, one, and one gene for reducing-, partially reducing-, and non-reducing type PKS, respectively, were cloned, sequenced, and characterized. Biological characterization of these genes is underway to determine its role in the production of phleichrome and open the possibility of metabolically engineering this pathway for overproduction of the desired substance.

728. Symbiotic fungal endophytes that confer tolerance for plant growth in saline soil. Zakia Boubakir1, Elizabeth Cronin2, Susan GW Kaminskiyj3. Biology, Univ Saskatchewan, Saskatoon, Saskatchewan, Canada.

Fungal endophytes are plant symbionts, and appear to be ubiquitous in plants growing in natural soils. Pioneering work by RS Redman and RJ Rodriguez showed that class II fungal endophytes confer tolerance to harsh growth environments. These endophyte strains are expected to enhance plant growth and improve nutrient uptake under normal and saline conditions, although currently the mechanism(s) is unknown. Soil salinity is one of the most serious agricultural problems that restrict plant growth and crop yield in many areas of the world. Saskatchewan has large areas of saltinized soils as well as many saline lakes. In this study, we are characterizing endophyte fungi isolated from saltinized soils in southern Saskatchewan. These include potash mine tailings, which are ~95% NaCl. In the spring of 2012 we collected 90 plant samples from 9 sites, from which we isolated ~450 endophyte fungi. Here, we will present a preliminary characterization of isolate Skj422.08. This strain has been shown to confer NaCl tolerance for tomato and wheat that were grown in soil mix watered with fresh water, then stressed with 200 mM or 12 g/L NaCl (tomato), or 300 mM or 18 g/L NaCl (wheat). Skj422.08 also improved growth when tomato and wheat were grown from seed in 150 mM or 9 g/L NaCl. This project is funded in part by Mosaic Co, a major producer of potash in Saskatchewan, as well as phosphate and micronutrients in other parts of North America, and worldwide.


Radioisotope contamination poses a threat to both ecosystem functioning and public health. Compared with plants, fungi can accumulate much higher amounts of heavy metals and radionuclides in their fruiting bodies. This was seen after the reactor accident of Chernobyl in 1986, when it became clear that fungal uptake of radionuclides such as 60Co, 90Sr, and most importantly 137Cs may reach harmful levels if consumed. It is thus of crucial importance to study radionuclide uptake into fungi to evaluate subsequent migration in the environment, thus allowing for meaningful ecological risk assessment. Usually, this is being performed by analysing stable isotopes of the same elements, whenever these are available. Due to their lower criticality, this reduces costs and risk of associated experiments. It is generally assumed that the effects of both are identical. However, comparative analyses have seldom been performed, and never with fungi. Ionizing radiation is known to cause effects ranging from DNA and Protein damage to increased oxidative stress and possibly apoptosis in a number of organisms. Especially in gene expression studies, certain reactions such as regulation of protein and nucleic acid repair mechanisms might thus deviate between stable and radioisotope exposure. Thus, differential gene expression of the fully sequenced model organism S. commune was analysed using MACE [Massive Analysis of CDNA Ends] under treatment with stable 133Cs and the b-decaying 137Cs and compared with untreated samples. Differential gene expression analysis points out factors responding to either radiation or Cs ions, thus differentiating between metal ion stress and radiation effects. While several carbohydrate metabolism genes and especially hydrophobin genes are specifically regulated following radiation, most expected responding factors, such as genes involved in stress response or ion and water transport, are
730. The completion of meiosis in Ustilago maydis requires an Ndt80 ortholog. C. E. Doyle[1], H.Y. K. Cheung[1], B. J. Saville[1,2]. 1) Environmental & Life Sciences Graduate Program, DNA Building, Trent University, 2140 East Bank Dr., Peterborough, ON, Canada; 2) Forensic Science Program, DNA building, Trent University, 2140 East Bank Dr. Peterborough, ON, Canada.

Meiosis in the model fungal plant pathogen Ustilago maydis requires growth in the plant host; as such, control of meiosis responds to cues received during pathogenic development. To begin investigating this process, an ortholog of the Saccharomyces cerevisiae meiotic control protein, Ndt80 (non-dityrosine) was identified in Ustilago maydis. It was hypothesized to control progression through meiosis and has been designated mcg1 (meiosis control gene 1). To assess its role in meiosis, mcg1 deletion mutants were constructed in compatible U. maydis haploid strains by replacing the gene with different selectable markers. This allowed the impact of mcg1 deletion on pathogenesis, teliospore development and the completion of meiosis to be determined. Infections with compatible Dmcg1 strains were fully pathogenic, but teliospores produced from these crosses displayed a distinctive, abnormal morphology and meiotic segregation assays indicated that they germinated without undergoing meiosis. This suggests that Mcg1 is involved in the regulation of meiosis and teliospore formation. To investigate this possibility that Mcg1 accomplishes this function by acting as a transcription factor, the upstream region of U. maydis genes was searched for variants of sites known as middle sporulation elements (MSE, the binding site of S. cerevisiae Ndt80). 89 genes with upstream MSEs were screened by RT-PCR, using RNA from dormant teliospores of wild-type and Dmcg1 strains. The results suggested that transcript levels for 43 of these genes differed in wild-type, relative to Dmcg1 teliospores, which indicated that the expression of these genes was affected, either directly or indirectly, by Mcg1. Further screens using RT-qPCR allowed the confirmation of genes with increased transcript levels, as well as those with decreased transcript levels, in the Dmcg1 teliospores relative to the wild-type teliospores. The upstream regions of these genes were screened for the presence of conserved sequence elements. In parallel, Mcg1 was aligned with putative orthologs to identify conserved regions. Based on these alignments, mcg1 genes containing targeted mutations were synthesized. Together, these analyses begin the determination of how in planta transitions in U. maydis development are controlled.

731. Impact of changes in the target P450 CYP51 enzyme associated with altered triazole-sensitivity in the Wheat pathogen Mycosphaerella graminicola. Eileen Scott[1], Regula Frey[2], Helge Sierotzkii[1], Michael Csuka[1]. 1) Syngenta, Biological Sciences, Jealotts’ Hill International Research Centre, Bracknell, United Kingdom; 2) Syngenta Crop Protection Munchwilen AG, Research Biology Centre, Schaffhauuserstrasse, Stein, Switzerland.

The triazoles are a widely used class of fungicides, targeting the cytochrome P450 sterol 14α-demethylase Cyp51. They are hence also known as the 14α-demethylase inhibitors, the 14-DMIs. Despite heavy use of this chemical class in the field over a considerable period of time, catastrophic resistance has not been observed in the economically important plant pathogen M. graminicola. Rather, there has been a slow shift toward reduced sensitivity. A large number of mutations in the Cyp51 gene have been previously associated with this shift in sensitivity to DMIs, although other resistance mechanisms such as alteration in sterol biosynthesis and fungicide uptake and efflux may also play a role. It has been hypothesized to control changes in resistance levels with specific Cyp51 mutations in field isolates. However, due to the genetic diversity of Mycosphaerella, the possible effect of non-target site mutations and issues with expression in exogenous fungi making solid conclusions has been problematic.

In order to accurately assess the contribution of each of the target site substitution mutations found in the field associated with 14-DMI resistance we have introduced mutations individually and in combination into the endogenous Cyp51 gene in a uniform genetic background (M. graminicola genome sequenced strain, IPO323). Here, we present the findings of the comparative efficacy of varying triazole structures against this comprehensive collection of mutants.

732. Molecular Evolutionary Analysis and Synteny of Fungal GAL Genes. Julien S Gradigno[1], C. L Anderson[2], R. A Wilson[1], E. N Moriyama[1,4]. 1) School of Biological Sciences, University of Nebraska - Lincoln, Lincoln, NE; 2) Department of Computer Science and Engineering, University of Nebraska - Lincoln, Lincoln, NE; 3) Department of Plant Pathology, University of Nebraska - Lincoln, Lincoln, NE; 4) Center for Plant Science Innovation, University of Nebraska - Lincoln, Lincoln, NE.

In many fungal species (including Saccharomyces, Candida, Schizosaccharomyces and related genii), genes involved in successive steps of a metabolic pathway are often physically clustered in the genomes. Within genes involved in the Leloir pathway for galactose catabolism, such clustering is considered to facilitate niche adaptation via rapid gene inactivation. This pathway involves three structural genes - GAL1, a galactokinase, GAL7 a uridylyl transferase and GAL10, a bifunctional protein with two epimerase domains. The products of the GAL80, GAL4 and GAL3 genes functionally protein with two epimerase domains. The products of the GAL80, GAL4 and GAL3 genes cooperatively regulate expression of the structural genes. GAL1 and GAL3 are highly similar (>90% identity) and likely arose from an ancient duplication event. GAL1, 7 and 10 are known to cluster in many divergent fungal lineages, including Saccharomyces, Candida, Schizosaccharomyces, and Cryptococcus. To further investigate potential syntenic patterns in a wider range of fungal lineages including filamentous species, we identified orthologous GAL proteins from over 60 fungi. An initial set of orthologue candidates was generated using a combination of BLAST, reciprocal BLAST and profile hidden Markov model searches. Sequences meeting the percent identity and coverage thresholds established for each protein were subsequently aligned using MAFFT. We then reconstructed maximum likelihood phylogenies and, where necessary, compared predicted secondary structures to produce the orthologue database. Location information was obtained from the respective source database (NCBI, JGI or the Broad Institute). We confirmed that GAL1, GAL7 and GAL10 are not clustered in all 53 species of filamentous fungi we studied. While in 7 species closely related to S. cerevisiae as well as C. albicans, as previously reported, the two GAl10 domains exist as a single fused protein, they exist as separate structures against this comprehensive collection of mutants.


Meiotic drives (MD) are nuclear genetic loci ubiquitous in eukaryotic genomes that cheat the Mendel laws by disturbing segregation in their favor. All known MDs are composed of at least two linked genes, the distorter that acts as a toxin by disrupting the formation of gametes, and the responder that acts as an antitoxin and protects from the deleterious distorter effects. In fungi, MDs are known as Spore Killers (SK). In the model ascomycete Podospora anserina, MD has been associated with deleterious effect during ascospore formation of the Het-s strain and in Neurospora crassa a resistance gene (respondent) to the Sk-2 and Sk-3 distorters has been identified. MDs are easily studied in P. anserina thanks to the ascus structure as SKs are identified by the presence of 2-spored ascus in crosses between strains. Here, we identify and characterize by targeted deletion in P. anserina Skp1 and Skp2, two MD
34. Alkaliphilic fungi from soda lakes and soda soils. Alexey A. Grum-Grzhimaylo, Alfons J.M. Debets1, Marina L. Georgieva2, Elena N. Bilanenko3. 1) Wageningen University, Wageningen, The Netherlands; 2) Lomonosov Moscow State University, Moscow, Russia.

Alkaliphilic fungi growing optimally at pH exceeding neutral values have received little scientific attention and generally it is believed that only prokaryotic organisms are able to survive harshly elevated ambient pH values. To date, only a handful of alkalophilic fungi (i.e. fungi growing optimally at pH > 9) have been reported. The few studies devoted to fungi growing at high pH lack a systematic molecular phylogenetic analysis. Our study aims to reveal the taxonomic distribution of alkalophilic filamentous fungi we isolated from soils at different sites near soda lakes. We intend to test if the alkalophilic trait has occurred independently throughout the fungal kingdom or rather once in a single lineage. Soda lakes and soils with pH ranging from 8 to as high as 11 are believed to be the natural habitats for alkaliphiles. The high pH is maintained mainly due to strong buffering capacity of carbonate salts presented there. We used alkaline agar medium (the pH is buffered at around 10) with antibiotic as a selective medium for screening for potential alkaliphilic fungi in the collected soil samples. By these means we isolated 99 ascomycetous strains which were capable of growing, to different extents, at pH 10. Two thirds of the total number turned out to be anamorphic fungi displaying only asexual sporulation (mostly Acremonium-like) while only 19 strains were holomorph homothallic being able to develop the full life cycle. Seventeen isolates produced only sterile mycelium without reproductive structures under laboratory conditions. We sequenced five genes (SSU rDNA, LSU rDNA, RPB2, TEFlalpha, ITS region of rDNA) to pinpoint the taxonomic positions of all isolated. After phylogenetic reconstructions all our strains had tendency to group in two different lineages within the Ascomycota. The first lineage is the Plectosphaerellaaceae family (insertae sedis within subphylum Hypocreomycetidae) which harbors 39 alkalophilic isolates while the second lineage is Emeriellopas-clade (order Hypocreales, subphylum Hypocreomycetidae) within the Acremonium cluster containing 30 strains. The remaining 30 isolates are presumably alkalitolerant members of the Pleosporinae and Sordariinae lineages known to be ubiquitous soil fungi.

35. A new method for gene mining and enzyme discovery. Y. Huang1,2, P. Busk3, M. Grell1, H. Zhao2, L. Lange2. 1) Section for Sustainable Biotechnology, Department of Biotechnology, Department of Biotechnology and Environmental Engineering, Aalborg University Copenhagen, Denmark; 2) Environmental Microbiology Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, Sichuan 610041, PR China; 3) University of the Chinese Academy of Sciences, Beijing 100049, PR China.

Peptide pattern recognition (PPR) is a non-alignment based sequence analysis principle and methodological approach, which can simultaneously compare multiple sequences and find characteristic features. This method has improved the understanding of structure/function relationship for enzymes within the CAZy families, which would make it easier to predict the potential function of novel enzymes, creating bigger promises for industrial purposes. Mucor circinelloides, member of the former subdivision Zygomycota, can utilize complex polysaccharides such as wheat bran, corncob, xylan, CMC and avicel as substrate to produce plant cell wall degrading enzymes. Although the genome of M. circinelloides has been sequenced, only few plant cell wall degrading enzymes are annotated in this species. In the present project, PPR was applied to analyze glycoside hydrolase families (GH family) and mining for new GH genes in M. circinelloides genome. We found 19 different genes encoding GH3, GH5, GH6, GH7, GH9, GH16, GH38, GH43, GH47 and GH125 in the genome. Of the three GH3 encoding genes found, one was predicted by PPR to encode a b-glucosidase. We expressed this gene in Pichia pastoris and found that the recombinant protein has high b-glucosidase activity (4884 U/mL). In this work, PPR provided targeted short cut to discovery of enzymes with a specific activity. Not only could PPR pinpoint genes belonging to different GH families but it did also predict the enzymatic function of the genes.

36. Occurrence of dsRNA mycovirus (LeF-FMRI 2427) in edible mushroom Lentinula edodes and its meiotic stability. J-M. Kim1, 2,3, H-Y. Yun1, M.-S Yang1, D.-H. Kim1. 1) Department of Bio-Environmental Chemistry, Wonkwang University, Iksan, Jeonbuk, South Korea; 2) Institute for Molecular Biology and Genetics, Center for Fungal Pathogenesis, Chonbuk National University, Jeonju, Jeonbuk, South Korea.

The dsRNA was first found in the malformed cultures of Lentinula edodes strain FMRI 2427, one of three most popular sawdust cultivating commercial strains of shiitake. This dsRNA was also found in the healthy-looking fruiting bodies and actively growing mycelia. Cloning of partial genome of dsRNA revealed the presence of RdRp sequence of a novel L. edodes mycovirus (LeF) and sequence comparison of the clone amplicon showed the identical sequence to the known RdRp genes of LeF found in strain HKA. Meiotic stability of dsRNA was examined by the measuring the ratio of the presence of dsRNA among the sexual monokaryotic progenies. More than 40% of monokaryotic progenies still contained the dsRNA indicating the persistence of dsRNA during sexual reproduction. Comparing mycelial growth of monokaryotic progenies suggested that, although variations in growth rate existed among progenies, there appears no direct relationship of mycovirus infection to the growth rate.

37. Analysis of fungal communities associated with grapevine wood diseases, based on fungal ITS pyrosequencing. Nicolas Lapalu1,2,3, Angélique Gautier1,2, Laetitia Brigitte1,2, Jessica Vallance1, Emilie Bruez1, Joelle Amselem1,2, Hadi Quesneville1, Valérie Lavial1,2, Marc-Henri Lebrun1,2, Patrice Rey1,2,3. 1) INRA-URGI, Versailles, France; 2) INRA, BIOGER, Thiverval Grignon, France; 3) INRA, Santé Végétal, Bordeaux, France.

The Grapevine Trunk Diseases (GTDs) are the most common diseases of grapevine wood inducing a slow decay leading to plant death. Due to the environmental impact, chemical treatments are no longer authorized, and prevention or trunk removal are the last available control methods. Fighting against these slow evolving diseases requires a better knowledge of fungal and bacterial communities associated with GTDs. Our approach is based on fungal species identification using ITS (Internal Transcribed Spacer) sequences obtained by pyrosequencing (Roche 454) of grapevine wood samples. DNAs were extracted from different parts of grapevine trunks and amplified using fungal specific ITS primers. A workflow was set up to analyze pyrosequencing data, allowing taxonomic assignment with a database extracted from Genbank and curated with the FungalTExtractor (Nilsson H et al. 2010). The pipeline links tools, including cleaning and extracting ITS sequences to limit the impacts of sequencing errors on clustering and assignation steps. Then, Operational Taxonomic Units (OTUs) detection and taxonomic assignments were performed with the QIIME package (Caporaso JG et al. 2010). Samples from different vineyards (infected or not) were selected from several samples, were analyzed. Different ITS PCR primers and technical replicates were tested using controls corresponding to the mixture of fungal DNAs from diverse known species. These controls highlighted interests and limits of PCR amplions pyrosequencing and the relevance of the bioinformatics methods to extract accurate data to fit to the context of taxonomy.


Therapeutic treatment for systemic mycoses is severely hampered by the extremely limited number of antifungals. Treatment for fungal infections in the...
central nervous system is further compounded by the poor CNS penetration of most antifungals due to the blood-brain barrier. Only a few fungistatic azole drugs, such as fluconazole, show reasonable CNS penetration. Here we demonstrate that sertraline (brand name Zoloft), the most prescribed antidepressant, displays potent antifungal activity against Cryptococcus neoformans, the major causative agent of fungal meningitis. In the in vitro assays, this neurotrophic drug is fungicidal to all natural Cryptococcus isolates tested at clinically relevant concentrations. Furthermore, sertraline interacts synergistically or additively with fluconazole against Cryptococcus. Importantly, consistent with our in vitro observations, sertraline alone reduces the brain fungal burden at a comparable efficacy as fluconazole in a murine model of systemic cryptococcosis. It works synergistically with fluconazole in reducing the fungal burden in brain, kidney, and spleen. In contrast to its potency against Cryptococcus, sertraline is less effective against strains of Candida species and its interactions with fluconazole against Candida strains are often antagonistic. Therefore, our data suggest the unique application of sertraline against cryptococcosis. To understand the antifungal mechanisms of sertraline, we screened the whole genome deletion collection of Saccharomyces cerevisiae for altered sertraline susceptibility. Gene ontology analyses of selected mutations suggest that sertraline perturbs translation. In vitro translation assays using fungal cell extracts show that sertraline inhibits protein synthesis. Taken together, our findings indicate the potential of adopting this antidepressant in treating cryptococcal meningitis.

379. Analysis of gene expression of proteases of Trichoderma spp during confrontation with plant pathogens in vivo. Valdirene Monteiro1, Eva Couto1, Fenix Oliveira1, Marcela Suriani2, Raphaela Georg3, Cirano Ulhoa1. 1) Universidade Estadual de Goiás, Anápolis, Brazil; 2) Universidade Federal de Goiás, Goiânia, Brazil.

The genus Trichoderma with teleomorismo in Hypocrea comprises a group of saprophytic fungi and micoparasitas widely used as biological control agents of soil borne plant pathogens. Among the mechanisms proposed for the biocontrol of Trichoderma species are mycoparasitism by production of cell wall degrading enzymes of plant pathogens, antibiotic production volatile and non-volatile competition for nutrients, rhizosphere competent and induction of defense responses in plants. In recent years, several efforts have been made to better understanding of molecules involved in mycoparasitism. In this study we assessed the expression of protease genes of Trichoderma species by qRT-PCR and the gene for alpha-tubulin as a housekeeping gene. Differential expression was assessed from Trichoderma viride and Trichoderma harzianum during contact and after contact with the host hyphae of fungal pathogens Rhizoctonia solani and Sclerotinia sclerotiorum. The "primers" were designed based on the sequences of the genes encoding these enzymes deposited in the database DOE Joint Genome Institute (http://www.jgi.doe.gov/). The protease genes of both species were upregulated in protease expression, Carboxypeptidase, cysteine protease, Subtilisin peptidase, trypsin, protease Serino metallopeptidase. Initial results showed that during the contact of Trichoderma viride and Rhizoctonia solani on metallicprotease gene is 80% higher than that expressed in the same condition for Trichoderma harzianum. When observing the condition after contact between T. viride and Sclerotinia sclerotiorum the metalloprotease gene does not have its expression as required presenting less 60% expressed in relation to genes aspartate, carboxy, and subtilisin serine proteases. The expression was very different between the clashes of Trichoderma and plant pathogens as well as for contact times. Leading us to believe that specific classes of proteases are required at different stages of contact of Trichoderma and pathogen. Person's test was performed to analyze the correlation between the expression of these genes and other genes of proteases involved in mycoparasitism as glucanases and chitinases.

740. Analysis of differential protein profile during antagonism of Trichoderma harzianum and Sclerotinia sclerotiorum. Valdirene Monteiro1, Roberto Silva2, Cirano Ulhoa2. 1) Universidade Estadual de Goiás, Anápolis, Anápolis, Brazil; 2) Universidade Federal de Goiás, Goiânia, Brazil; 3) University of São Paulo.

Trichoderma sp. are effective biocontrol agents for several soil-borne fungal plant pathogens including Fusarium sp., Rhizoctonia solani, and Sclerotinia sclerotiorum some species are also known for their abilities to enhance systemic resistance to plant diseases as well as overall plant growth. The biocontrol exercised by Trichoderma can occur by means of several antagonistic mechanisms such as nutrient competition, antibiotic production and mycoparasitism. Mycoparasitism has been proposed as the major antagonistic mechanism displayed by Trichoderma sp. in the present study were examined the differential production of proteins in three different conditions. These conditions were Trichoderma harzianum vs mycelium of fungal pathogens Sclerotinia sclerotiorum; Trichoderma harzianum vs esclerotia of fungal pathogens Sclerotinia sclerotiorum and Trichoderma harzianum vs leaves beans infected with Sclerotinia sclerotiorum. The differential expression of proteins in different conditions of antagonism was analyzed by two-dimensional difference gel electrophoresis (2D-DIGE). Initial analyzes showed profiles varied between interactions. The proteins which were expressed were so different and trypsin-like SM1 between conditions T. harzianum and bean leaves and T. harzianum and mycelium. There is a higher expression of glucanases and chitinases in the condition of the interaction between T. harzianum and sclerotia. It was observed the presence of three proteins predicted molecular weight of 15kDa that are present in all tested conditions at a comparable efficacy as fluconazole in a murine model of systemic cryptococcosis. It works synergistically with fluconazole in reducing the fungal burden in brain, kidney, and spleen. Preliminary transcriptome data shows that the sets of upregulated genes differ between the two nematodes tested, suggesting a nematode-specific defense response. Whereas B. willibaldi induced the expression of genes hypothetically involved in secondary metabolite production, such as a non-ribosomal peptide synthase and a terpene synthase; A. avenae induced previously characterized nematototoxic fungal lectins, such as CGL2, CCL1 and a chimeric RicinB-fold protein. To characterize this inducible defense response in more detail, we developed a reporter system in C. cinerea consisting of the cgII promoter coupled to the gene encoding the red fluorescent protein dTomato. The reporter C. cinerea strain was grown into a microfluidics device with compartmentalized channels and confronted with A. avenae. This system allowed us to microscopically monitor the induction of the effector molecule within the mycelium over time. Preliminary results indicate that the induction can spread within hyphae but is locally confined. The expression of the reporter protein dTomato, driven by the cgII promoter, was first detected after 10 h of nematode predation. In conclusion, our findings show, first, that the defense of C. cinerea against fungivorous nematodes is regulated at the level of transcription. Second, we could demonstrate that the transcriptional induction of defense effector proteins in response to predation is not only locally confined but also predator-specific.
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