The Tenth International *Aspergillus* Meeting Asilomar Conference Grounds Pacific Grove, CA March 12-17, 2013



Aspergillus discodancus created by Erwin Berthier

Aspergillus Genomes Research Policy Group (AGRPG)

An *Aspergillus* Genomics workshop was held at the March 2003 Asilomar Fungal Genetics meeting. From discussions in that workshop it was obvious that our community needed to organize to fully exploit genomics resources. A provisional *Aspergillus* Genomes Research Policy Committee (AGRPC) was conscripted and charged with creating a structure for community-wide coordination and organizing an annual meeting. The First *Aspergillus* Meeting was held in Copenhagen, April 21, 2004, as a satellite meeting of the European Congress on Fungal Genetics-7. In addition to scientific presentations, bylaws were approved, community research directions were discussed and the 2004 AGPRC was elected. The name *Aspergillus* Genomes Research Policy Group was adopted for the community. The objectives of the AGRPG are: (1) Provision of an educational and discussion forum for issues pertaining to *Aspergillus* genomics, in its widest sense, and for the various *Aspergillus* research communities; (2) Influencing grant making bodies and other institutions on behalf of the various *Aspergillus* research communities; (3) Coordinating research activities internationally, as and when required, to further the science base of the *Aspergillus* genus. For more information on the activities of the AGRPG and other *Aspergillus* news see our homepage at FGSC (http://www.fgsc.net/Aspergillus/asperghome.html).

2012 AGRPC

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Asperfest11 will be March 22-23, 2014, in Seville, Spain immediately before ECFG12 (March 23-27) <u>http://congreso.us.es/ecfg12/satellitemeeting.php</u>

The Tenth International Aspergillus Meeting Asperfest 10 Merrill Hall, Asilomar Conference Center March 11-12, 2013

March 11 (Monday)

5:00 - Registration in the Chapel

7:00-10:00 Posters and Welcome Reception in Merrill Hall (sponsored by Novozymes)

7:00-8:00	Students with last name starting A-G be at your poster
8:00-9:00	Students with last name starting H-N be at your poster
9:00-10:00	Students with last name starting O-Z be at your poster

March 12 (Tuesday)

9:00 Welcome, introductions and announcements Michelle Momany

9:15 Session I:

- 9:15 Location, location: compartmentalization and regulation of secondary metabolism Nancy Keller, Univ Wisconsin, Madison
- 9:35 'Dr Jekyll and Mr Hyde': The evolution of the Aspergillus genome Antonis Rokas, Vanderbilt University
- 9:55 Light and time in *Aspergillus nidulans* Reinhard Fischer, Karlsruhe Inst. of Technology

10:15 -10:40 Coffee Break

10:40 Session II: Genomics Projects

- 10:40 AspGD update
 - Martha Arnaud, AspGD, Stanford Univ
- 10:55 Community Resources for *Aspergillus fumigatus*: an NIAID funded genome sequencing project. Liliana Losada, J. Craig Venter Institute
- 11:10 A. nidulans deletion project update
 - Stephen Osmani, Ohio State Univ
- 11:25 Aspergillus JGI Community sequencing projects

Ronald DeVries, CBS-KNAW Fungal Biodiversity Centre

11:40 The Environmental Molecular Sciences Laboratory molecular analysis capabilities for fungal biology

Scott Baker, Pacific Northwest Natl Lab

12:00-1:30PM Lunch

Jennifer Wortman

Gerhard Braus

1:30 Community directions discussion; Elections

1:45 Session III: Talks from Abstracts

1:45 Comprehensive annotation of secondary metabolism biosynthetic genes and gene clusters of *Aspergillus nidulans, A. fumigatus, A. niger* and *A. oryzae*

Diane O. Inglis, Stanford University

2:00 F-box protein 15 (Fbx15) links virulence of *Aspergillus fumigatus* to protein degradation and stress response.

Bastian Jöhnk, Georg August University

2:15 Effect of carbon sources on CreA protein level in *Aspergillus oryzae* Mizuki Tanaka, Tohoku University

2:30 – 2:45 Coffee Break

2:45 Identification of a gene cluster mediating the biosynthesis of the *Aspergillus fumigatus* cell wall and secreted polysaccharide, galactosaminogalactan

Fabrice N. Gravelat, McGill University

3:00 Co-cultivations of fungi: microscopic analysis and influence on protein production Isabelle Benoit, CBS-KNAW

3:15 Discovery of Sexual Reproduction in the Black Aspergilli Heather L. Darbyshir, University of Nottingham

3:30 Pontecorvo lite: A decade of Asperfest

Michelle Momany

Election results; poster prize; other discussion items

Michelle Momany

Ronald deVries & Gerhard Braus

Biochemistry and Metabolism

1. Identification of local and cross-chromosomal biosynthetic gene clusters in filamentous fungi using gene expression data.

Mikael R. Andersen¹, Jakob B. Nielsen¹, Andreas Klitgaard¹, Lene M. Petersen¹, Tilde J. Hansen¹, Lene H. Blicher¹, Charlotte H. Gottfredsen², Thomas O. Larsen¹, Kristian F. Nielsen¹, Uffe H. Mortensen¹. 1) Department of Systems Biology, Technical University of Denmark, Kgs Lyngby, Denmark; 2) Department of Chemistry, Technical University of Denmark, Kgs Lyngby, Denmark; 2) Department of Chemistry, Technical University of Denmark, Kgs Lyngby, Denmark; 2) Department of Chemistry, Technical University of Denmark, Kgs Lyngby, Denmark; 2) Department of Chemistry, Technical University of Denmark, Kgs Lyngby, Denmark; 2) Department of Chemistry, Technical University of Denmark, Kgs Lyngby, Denmark

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-chemistry 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 AN11080 (on chromosome V) as well as identication 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*.

*2. N-glycan profiling of Aspergillus nidulans using solid-phase glycan extraction and mass spectrometry. Diana Anyaogu¹, Shuang Yang², Jakob B. Nielsen¹, Hui Zhang², Michael Betenbaugh³, Uffe Hasbro Mortensen¹. 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* are 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 *Aspergilli* to improve protein production. In the present study the Solid-Phase Glycan Extraction (SPGE) method was used to isolate and purify N-glycans from the secretore 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 .

*3. Heme regulation in Aspergillus fumigatus.

Nicola Beckmann¹, Ernst R. Werner², Hubertus Haas¹. 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 (Δ hemA) 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 Δ hemA was blocked at ALA concentrations below 20 µM, but fully restored by addition of 200 µM on solid as well as in liquid media. Supplementation with protoporphyrin IX (PpIX), the iron free heme precursor, and hemin (chloroprotoporphyrin IX iron(III)) supported growth of Δ hemA, 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 Δ hemA, ALA restriction transcriptionally increased the heme-biosynthetic coproporphyrinogen(III)oxidase and the putative heme receptor CFEM3. Additionally, ALA limitation decreased the resistance of Δ hemA 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.

*4. Key Steps in the Biosynthesis of the Fungal Virulence Factor Gliotoxin. Pranatchareeya Chankhamjon¹, Daniel H. Scharf², Kirstin Scherlach¹, Nicole Remme¹, Andreas Habel¹, Thorsten Heinek², Martin Roth³, Axel A. Brakhage², Christian Hertweck¹.

1) Biomolecular Chemistry, HKI, Jena, Jena, Germany; 2) Molecular and Applied Microbiology,HKI, Jena, Jena, Germany; 3) Bio Pilot Plant,HKI, Jena, 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 in the mediates produced. Here we present the critical role of a specialized glutathione S-transferase (GST), GliG, in the enzymatic sulfurisation and the key step of epidithiol 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 virulence factor, but also outline a new function of microbial GSTs and gain insights into the formation of organosulfur compounds.

5. Induction of sclerotia and Aspergillus section Nigri.

Jens Frisvad, Lene Petersen, Ellen Lyhne, Thomas Larsen, CMB, Dept Systems Biol, Kgs. Lyngby, Denmark

The purpose of this study was to induce sclerotium production in Aspergillus niger and other black Aspergilli. Some species in Aspergillus section Nigri are known for their production of sclerotia, especially A. carbonarius, A. tubingensis (few isolates), A. sclerotioniger, A. sclerotiicarbonarius, A. costaricaensis, A. piperis, 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 hitherto not reported to produce sclerotia. Earlier French beans were suggested as inducers of sclerotium production, but we could not repeat this with any isolate of A. niger. However by using media such as white rice and brown rice or adding different fruits to CYA (Czapek yeast autolysate agar) and incubate at 25 C we were able to induce sclerotium production in certain strains of A. niger. Old strains used for citric acid production, or full genome sequenced strains, were not induced to produce sclerotia, but several fresh strains from different foods did produce abundant sclerotia on the different media, at 25 C, but not 37 C. One older classical citric acid producer from NRRL produced many sclerotia, however. Sclerotium producing isolates also contained aflavinines, confirmed by HPLC-DAD-MS-MS, secondary metabolites only produced in the sclerotia, and detected in A. niger for the first time. Other species, such as A. ibericus, A. neoniger, A. heteromorphus, A. fijiensis, A. luchuensis (formerly A. acidus), A. aculeatinus and A. saccharolyticus could also produce sclerotia on fruit media. The sclerotia contained many sclerotium-specific secondary metabolites.

6. Identification of a gene cluster mediating the biosynthesis of the *Aspergillus fumigatus* cell wall and secreted polysaccharide, galactosaminogalactan.

Fabrice N. Gravelat¹, Mark J. Lee¹, Alexander Geller¹, Dan Chen², Anne Beauvais³, Hong Liu⁴, William C. Nierman², Jean-Paul Latge³, Thierry Fontaine³, Scott G. Filler⁴, Donald C. Sheppard¹. 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 dominant 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, Gtb3 and Ega3) encoded by these genes are predicted to contain conserved domains involved in polysaccharide metabolism. The 2 other proteins (Sph3 and Esr3) 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 spherulin 4-like protein; and esr3, encoding an extracellular serin-rich protein. Phenotypic analysis of both the $\Delta esr3$ and $\Delta sph3$ mutant strains confirmed that deletion of these genes resulted in both impaired GAG production and impaired adherence, similar to the phenotype of the $\Delta uge3$ 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 a 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.

*7. Metabolic adaptations in *Phytophthora infestans* and the role of a phosphagen kinase system in energy metabolism.

Meenakshi Kagda, Howard Judelson. Plant Pathology and Microbiology, University of California, Riverside, CA 92521.

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 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.

8. LaeA sleuthing reveals cryptic gene clusters in pathogenic Aspergilli.

Nancy Keller², Wenbing Yin², Saori Amaike², Katharyn Affeld², JinWoo Bok², Daniel Schwenk³, Dirk Hoffmeister³, Joshua Baccile¹, Ry Forseth¹, Frank Schroeder¹ 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, Beutenbergstraße 11a, 07745 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, hexadehydro-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.

*9. Characterization of fumiquinazoline biosynthesis in Aspergillus fumigatus.

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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 fumiquinazoline 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 metabolomic 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.

10. Engineering Cyclic Peptide Biosynthesis in Poisonous Mushrooms.

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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 phalloidin 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.

*11. Genomic and metabolomic analysis of polyketide production in Ascocoryne sarcoides.

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Polyketides are an important group of secondary metabolites from fungi that exhibit a wide range of structural diversity and function, with many reported to be involved in protection (eg. pigments) and defense (eg. antibiotics). The structural diversity of polyketides is largely attributed to the polyketide synthases that catalyse their synthesis, due to their exceptional assemblage and combinatorial power. An isolate of Ascocoryne sarcoides was found to produce four linear polyketides of similar structure, and these exhibited bioactivity against the Gram positive bacteria Bacillus subtilis. Structural characterisation of one of the polyketides via HREIMS and NMR indicated a new metabolite. The polyketide exhibited varying levels of reduction along the central carbon chain, which is indicative of biosynthesis via a highly reducing iterative polyketide synthase. The genome of the isolate of A.sarcoides was sequenced, assembled and annotated, which enabled genome mining for polyketide synthases. A total of three polyketide synthases were identified, which contained domains associated with highly reducing iterative polyketide synthases. Genetic diversity studies were conducted to ascertain relatedness to functional polyketide synthases of fungi (eg. lovastatin, fumonisin, T-toxin). The gene clusters surrounding the polyketide synthases were also profiled to ascertain genetic mechanisms involved in polyketide production (ie. transporters, regulators, etc). This study has used advances in metabolomics and genomics to conduct an in depth analysis of polyketides from A. sarcoides, and the genes that regulate their production. Future studies will look to functionally characterise these genes, and determine their potential application in the agricultural and biofuel sectors.

*12. Genome mining to identify gene clusters involved in mono- and sesqui- terpene production in the bioactive endophytic fungus, Nodulisporium sp.

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Endophytic fungi are an emerging tool in agriculture, in part, due to their ability to protect their host plant against biotic stresses. The secondary metabolism of endophytic fungi is central to their unique role in defence of their host. Endophytic isolates of a Nodulisporium sp. were found to produce a suite of volatile secondary metabolites, bioactive against a range of agricultural pathogens (fungi and bacteria), pests (insects) and weeds. Profiling the volatile metabolome of isolates of Nodulisporium sp. identified 52 metabolites, with the majority belonging to the mono- (eg. eucalyptol) and sesqui- (eg. β -Elemene) terpene structural class. Terpenes are important defense metabolites of plants and have been researched extensively, along with the terpene synthase genes that regulate their production. However, only a limited amount of research has been conducted on fungal terpenes and their biosynthesis (eg. mycotoxins), while no monoterpene synthases have been identified from fungal species. Terpene synthases are unique in their function, in that they can synthesise multiple terpene structures from the one enzyme, and this potentially offers an endophyte a diverse arsenal against pathogens and pests. The genomes of six isolates of Nodulisporium sp. were sequenced, assembled and annotated, and the genes involved in terpene production identified through genome mining. A total of eight terpene synthases were identified, which is disproportionately high compared to other sequenced fungal species. The gene clusters surrounding the terpene synthases were profiled to ascertain genetic mechanisms involved in terpene production (ie. transporters, regulators, etc). Comparative genomic studies were also employed to evaluate genetic diversity across the terpene synthase gene clusters of the six isolates. This metabolomic and genomic study provides new information about metabolite diversity of terpenes in endophytic fungi and the genes regulating their production. This knowledge is providing the foundation for future functional analysis of this important gene class, and also genetic information for the creation of designer endophytes to improve disease resistance of the host plant.

13. Platforms for secondary metabolite analysis in filamentous fungi.

Uffe H. Mortensen¹, Jakob B. Nielsen¹, Diana C. Anyaogu¹, Dorte K. Holm¹, Lene M. Petersen¹, Morten T. Nielsen¹, Mikael S. Joergensen^{1,2}, Kristian F. Nielsen¹, Pia F. Johannesen², Dominique A. Skovlund², 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 allows 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. Theses 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 knockin/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.

14. Characterization of the 3-methyl orsellinic acid gene cluster in Aspergillus nidulans.

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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 (*pkbR*), with homology to transcription factors. Based on this observation, we decided to overexpress *pkbR*. A qRT-PCR analysis of this strain was used to delineate the borders of the gene cluster as well as to stimulate formation of cichorine, cichorinic 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.

15. Analyzing the impact of compartmentalization on organic acid production in *Aspergillus niger*. **Matthias G. Steiger**^{1,2*}, Marzena L. Blumhoff^{1,2,3}, Diethard Mattanovich^{1,2}, Michael Sauer^{1,2}. 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.

sAspergillus 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. Itaconic 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-aconitic 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.

16. Increased production of fatty acids and triglycerides in Aspergillus oryzae by modifying fatty acid metabolism.

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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. orvzae.

17. Molecular genetic characterization of secondary metabolism pathways in Asperillus species. **Clay Wang**¹, Yiming Chiang¹, Nancy Keller³, Kenneth Bruno⁴, Scott Baker⁴, Chun jun Guo¹, James Sanchez¹, Benjamin Knox⁴, Alexandra Soukup³, Jin Woo Bok³, Manmeet Ahuja², Ruth Entwistle², Liz Oakley², Shu-lin Chang¹, Hsu-Hua Yeh¹,

Mike Praseuth¹, Berl Oakley². 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.

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18. Aspergillus nidulans SNXA^{HRB1} is an SR/RRM family protein that rescues defects in the CDC2/CYCLINB pathway.

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Control of the eukaryotic G2/M transition by CDC2/CYCLINB is tightly regulated. To further characterize this regulation in *Aspergillus nidulans*, we conducted a screen for extragenic suppressors of *nimX2^{cdc2}* that resulted in the identification of the cold-sensitive, G1-arresting *snxA*1 mutation. Our data show that *snxA*1 suppresses defects in regulators of the G2/M transition, including *nimX2^{cdc2}*, *nimE6^{cyclinB}*, and *nimT23^{cdc25}*, but does not suppress the G1/S-arresting *nimE*10^{cyclinB} mutation or any of four S phase mutations. Furthermore, the *snxA*1 mutation or deletion of *snxA* alter localization patterns of NIME^{CYCLINB} at the restrictive temperatures for *snxA*1 and *nimX*2, supporting a role for SNXA in cell cycle control. *snxA* encodes the *A. nidulans* ortholog of *Saccharomyces cerevisiae* Hrb1/Gbp2, nonessential shuttling mRNA binding proteins belonging to the SR (Serine-Arginine Rich) and RRM (RNA Recognition Motif) protein family. *snxA*^{hrb1} is nonessential, its deletion phenocopies the *snxA*1 mutation, and overexpression of gDNAs or of alternatively spliced *snxA* cDNAs rescues *snxA*1 mutant phenotypes. SNXA^{HRB1} is predominantly nuclear, but is not retained in the nucleus during the partially-closed mitosis of *A. nidulans*. We further demonstrate that the *snxA*1 mutation does not suppress *nimX*2 by altering NIMX2^{CDC2}/NIME^{CYCLINB} kinase activity, suggesting that the effects of SNXA1 on NIMX2^{CDC2}/NIME^{CYCLINB} may be due to altered localization of NIME^{CYCLINB}. These data suggest a novel role in G2/M regulation for this SR/RRM family member. This work was supported by the Mississippi INBRE funded by grants from the National Center for Research Resources (5P20RR016476-11) and the National Institute of General Medical Sciences (8 P20 GM103476-11) from the National Institutes of Health.

19. The *Aspergillus nidulans* MAPK module AnSte11-Ste50-Ste7-Fus3 controls development and secondary metabolism.

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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.

*20. Two methyltransferase protein complexes control fungal development and secondary metabolite production. Oezlem Sarikaya Bayram¹, Oezguer Bayram¹, Jong-Hwa Kim², Keon-Sang Chae³, Dong-Min Han⁴, Kap-Hoon Han², 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 novel 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 asexual or sexual 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.70-749, Korea

*21. Functional analysis of sterol transporter in filamentous fungus Aspergillus nidulans.

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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.

*22. Autophagy promotes survival in aging submerged cultures of the filamentous fungus Aspergillus niger. Maria A. Burggraaf^{1,2}, Benjamin M. Nitsche^{1,2}, Gerda Lamers¹, Vera Meyer^{2,3}, Arthur F.J. Ram^{1,2}. 1) Institute of Biology Leiden, Molecular Microbiology and Biotechnology, Leiden, The Netherlands; 2) Kluyver 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 industrialscale 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 batch 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 ata1, ata8 and ata17 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.

*23. Pheromone-induced G2 cell cycle arrest in Ustilago maydis requires inhibitory phosphorylation of Cdk1. Sónia M. Castanheira, José Perez-Martín. Centro Nacional de Biotecnología. CSIC. Darwin 3, Campus de Cantoblanco, 28049 Madrid, Spain.

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, Saccharomyces cerevisiae and Ustilago maydis use a similar MAPK cascade to respond to sexual pheromone and in both cases a morphogenetic response is provided (shmoo and conjugative hypha, respectively). However, while S. cerevisiae arrests its cell cycle in G1 in response to pheromone, U. maydis does this by arresting at G2. The mechanisms and physiological reasons involved in the distinct cell cycle response to pheromone in U. maydis are largely unknown. In this communication we will introduce our attempts to characterize the molecular mechanisms behind pheromone-induced cell cycle arrest in U. maydis .Our results have indicated that inhibitory phosphorylation of Cdk1 is part of the mechanism of the pheromone-induced G2 cell cycle arrest. This inhibitory phosphorylation depends on the essential kinase Wee1. We analyzed the transcriptional pattern of cell cycle related genes in response to overactivation of pheromone pathway (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 cyclinwere downregulated at transcriptional level. Using chimeric promoter fusions we found that transcriptional downregulation was not as important for pheromone-induced cell cycle arrest as expected and we are analyzing other possible regulatory options such as stability or subcellular localization of these regulators.

24. The arrestin-like protein ArtA is essential for ubiquitylation and endocytosis of the UapA transporter in response to both broad-range and specific signals.

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We investigated the role of all arrestin-like proteins of Aspergillus nidulans in respect to growth, morphology, sensitivity to drugs and specifically for the endocytosis and turnover of the uric acid-xanthine transporter UapA. All arrestin null mutants are viable showing wild-type growth and morphology, except one which is affected in conidiospore production, but several have modified profiles in respect to N or C source utilization and drug sensitivity. A single arrestin, ArtA, is essential for HulARsp5-dependent ubiquitination and endocytosis of UapA in response to ammonium or substrates. Genetic analysis further showed that residues 545-561 of the UapA C-tail, which includes a critical di-acidic motif, is required for efficient UapA endocytosis. Mutational analysis of ArtA shows that the N-terminal region (2-123) and both PY elements are essential for its function. ArtA undergoes HulA-dependent ubiquitination at residue Lys343 and this modification is critical for the efficiency of UapA ubiquitination and endocytosis, especially in response to ammonium. Lastly, we show that ArtA is essential for vacuolar turnover of transporters specific for purines (AzgA) or L-proline (PrnB), but not for an aspartate/glutamate transporter (AgtA). Our results are discussed within the frame of recently proposed mechanisms on how arrestins are activated and recruited for ubiquitination of transporters in response to broad range signals, but also put the basis for understanding how arrestins, such as ArtA, regulate the turnover of a specific transporter in the presence of its substrates.

25. Unique protein domains regulate *Aspergillus fumigatus* RasA localization and signaling during invasive growth.

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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 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 every 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.

*26. Colletotrichum orbiculare Bub2-Bfa1 complex, a spindle position checkpoint (SPOC) component in Saccharomyces cerevisiae, is involved in proper progression of cell cycle.

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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 attenuate 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.

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.

*27. The exocyst complex is necessary for secretion of effector proteins during plant infection by *Magnaporthe oryzae*.

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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 immunity 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 Biotrophic 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.

28. A highly conserved sequence motif is required for PkcA localization to septation sites and protein function in *Aspergillus nidulans*.

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Many proteins with diverse functions contribute to cell wall synthesis in polarized 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 periphery of the septation site. The ring constricts in synchrony with the actin/myosin contractile ring and dissipates when septa are fully matured. To determine which domains are important for septum site localization, green fluorescent protein tagged, domain-deleted versions of PkcA were constructed. The domains that are vital to A. nidulans maintenance of cell wall integrity were separately identified by growing the domain deleted stains in the presence of the cell wall stressor calcofluor white. We have determined that the localization signal and the domain responsible for resistance to calcofluor white are distinct. The PkcA septation site localization signal is found within a region having homology with C2 domains of PKC proteins found in other organisms. Observations of both N- and C- terminal truncations support the conclusion that the PkcA septation site localization signal lies within the final 20 amino acids of the C2 domain. Removal of these amino acids causes PkcA mislocalization to the cytoplasm. Furthermore, removal of the localization signal renders the resulting truncated proteins less able to complement calcofluor white hypersensitivity in a strain carrying a mutation in its PkcA gene, highlighting the requirement of proper localization for this aspect of PkcA function.

29. Beyond green mining: analysis of fungal cytochemistry using gold nanoparticles.

Fatemeh Farazkhorasani¹, Martin Prusinkiewicz², Kathleen M Gough¹, **Susan GW Kaminskyj**². 1) Chemistry, University of Manitoba, Winnipeg, Canada; 2) Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada Cells including fungal hyphae and other microorganisms, as well as fungal growth medium including both complex and defined composition, can reduce solutions of HAuCl₄ to elemental gold nanoparticles (AuNPs). As described in 2012 Analyst 137:4934-42, we have shown that AuNPs formed by growing fungal hyphae can be used as analytical substrates for surface-enhanced Raman scattering (SERS) spectroscopic analysis. These SERS spectra are in the same energy range as our Fourier-transform infrared (FTIR) spectroscopic studies that provided information about cell composition. However, SERS is orders of magnitude more sensitive, and analysis is limited to cell components within a few nanometers of the AuNP. Our current interest is the fungal cell wall, which forms a porous interface between the cell and its environment. Cell wall chemistry is intrinsically related to cell-environment interactions, particularly for pathogenesis. The fungal wall is about 25 % of fungal dry weight, and its synthesis and maintenance is estimated to require ~25 % of the fungal genome. Fungal walls are ~ 80 % carbohydrate. Minor structural differences in carbohydrate bonding can cause profound changes in their metabolism, which complicates analysis. Preliminary studies described in the Analyst paper showed that SERS-active AuNPs can be generated by living hyphae. Higher Au concentrations produced larger AuNPs within and on the hypha, but in addition were lethal within 30 min. Lower Au concentrations produced clusters of smaller AuNPs on the cell wall surface, and were not lethal. These were also SERS-active. We are using SERS to probe the wall composition of engineered mutants in the Aspergillus galactofuranose biosynthesis pathway, which plays key roles in fungal growth and drug resistance. We expect the combination of fungal genetic engineering and high sensitivity/high spatial-resolution chemical analysis will provide novel information about fungal growth and infectivity.

30. 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 Kaminskyj**. 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 candidaisis. 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 Calcofluor. 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 Calcofluor 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.

31. Aspergillus nidulans cell walls lacking galactofuranose are more susceptible to glucan degrading enzymes. Biplab Paul¹, Tanya Dahms¹, **Susan Kaminskyj**². 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.

*32. Identification of novel genes involved in induction of appressorium development triggered by plant-derived signals in *Colletotrichum orbiculare*.

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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 triggered 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 appressoria 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 gene Δ single mutants, *cokel2* Δ candidate gene Δ double mutants, and complementation strains. As expected, candidate gene Δ mutants in *cokel2* Δ back ground showed same phenotypes as those of screened *cokel2* double mutants.

33. 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.

34. The GATA-type transcription factor NsdD is a key regulator of conidiation and secondary metabolism in *Aspergillus*.

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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 repressor of conidiation *sfgA*, which is designed to identify a new 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 (AN3152), 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 *flbA~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 (β and α). Finally, the subsequent transient promoter analysis using the *brlA* promoter::luciferase fusion constructs have revealed that NsdD negatively regulates the *brlA* β 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 *Aspergilli*.

*35. Stability of a G protein alpha subunit in genetic backgrounds lacking the G beta subunit or a cytosolic guanine nucleotide exchange factor.

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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^{WT}) or constitutively active, GTPase-deficient gna-1 allele (gna-1^{Q204L}). Overexpressing gna-1^{WT} (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*^{Q204L} (GTP bound) in the $\Delta gnb-1$ or $\Delta ric8$ 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.

36. Calcium imaging and measurement during growth and response to stresses in Aspergillus fumigatus.
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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 ($[Ca^{2+}]_c$) concentration; (2) appropriately integrate homeostatic and stress-responsive adaptations; and (3) undergo normal calcium signaling. There is evidence for calcium signaling regulating numerous processes including spore germination and hyphal tip growth. The low resting level of [Ca²⁺], (50-100 nM) is maintained by Ca²⁺-pumps and -antiporters, and cytoplasmic Ca²⁺-buffering. However, [Ca²⁺], 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 $[Ca^{2+}]_c$ 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 $[Ca^{2+}]_c$ 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 germlings to high external calcium induces dramatic and very dynamic changes in $[Ca^{2+}]_c$ with the generation of localized $[Ca^{2+}]_c$ transients and waves. Furthermore, there is considerable heterogeneity in the $[Ca^{2+}]_c$ responses of different germlings 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 signaling in filamentous fungi.

*37. THE velvet regulators in Aspergilli.

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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 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.

*38. Identification of two Golgi-localized putative UDP-galactofuranose transporters with overlapping function in Aspergillus niger.

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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 glycoproteins (galactomanno-proteins) [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; 2600 GA Delft, The Netherlands

39. Exploiting transcriptomic signatures of Aspergillus niger to uncover key genes important for high protein traffic through its secretory pathway.

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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 situation. 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 endoplasmaticum reticulum including well-known target genes of the HacA transcription factor, e.g. bipA, clxA, prpA, tigA and pdiA. 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 HacA 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.

40. Peering into the secret-ory life of Aspergillus nidulans with a little help from classical genetics

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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 sequencing. 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 secretion, and therefore, for hyphal growth. Thus, we begin with a sequenced, well-characterized heat-sensitive X^{ts} mutation in an A. nidulans Golgi gene. An X^{ts} strain is mutagenised to isolate suX suppressor mutations, reversing the absence of growth resulting from X^{ts} at the restrictive temperature. Less interesting intragenic reversion/pseudo-reversion events are identified by the inability of any given suX X^{ts} strain to produce single mutant X^{s} progeny when crossed to a wild-type. These mutations are next sequenced and archived. The remaining extragenic suppressors are allocated to one of the eight A. nidulans chromosomes by parasexual analysis, exploiting the rarity of mitotic recombination. Next, meiotic crosses between the suX X^{ts} strain and a panel of parental strains carrying markers in the suX chromosome are analysed to detect genetic linkage. Once linkage is detected, suX is further mapped to the smallest feasible chromosomal interval. Candidate genes in the annotated genome interval, hopefully conspicuous at this stage to the educated eye, or, as a last resort, the whole interval between the genetic boundaries, are sequenced to identify the suppressor. The combination of gene mapping with sequencing eliminates the cumbersome identification of a single causative mutation (aka 'a needle in a haystack') hidden amongst the genetic variability of the mutant and parental strains, inherent to whole genome sequencing approaches.

41. Aspergillus nidulans septin interactions and post-translational modifications.

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Septins are cytoskeletal elements found in fungi, animals, and some algae, but absent in higher plants. These evolutionarily conserved GTP binding proteins form heteroligomeric 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 welldefined 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 antiacetylated 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 coiledcoil 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.

*42. Generation of pathogenic diploids from heterogeneous conidial populations of Aspergillus flavus. Farhana Runa¹, Ignazio Carbone¹, Deepak Bhatnagar², Gary Payne¹.

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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, protoplast 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 diploids. Pathogenicity assays using Galleria mellonella showed that diploids are more virulent than the parental haploids. Our results suggest that conidial populations of A. flavus are predominantly homokaryotic but a small percentage of conidia are heterokaryotic. Within a heterokaryon, a diploid nucleus could be formed by fusion of two haploid nuclei, which may allow the generation of a pathogenic strain.

*43. Investigating Cell Cycle-Regulated Control of Appressorium Morphogenesis in the Rice Blast Fungus *Magnaporthe oryzae.*

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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.

44. Control of Multicellular Development by the Physically Interacting Deneddylases DEN1/DenA and COP9 Signalosome.

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Deneddylases remove the ubiquitin-like protein Nedd8 from modified proteins. An increased deneddylase activity has been associated to various human cancers. In contrast, we show here that a, several UPR stress transcriptomes and a carbon-source induced secretion transcriptome. Overall, 40 genes were commonly up-/down-regulated under these three 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 deneddylase activity. A deneddylase disequilibrium impairs multicellular development and suggests that control of deneddylase activity is important for multicellular development.

45. Visualization of apical membrane domains in *Aspergillus nidulans* by <u>Photoactivated Localization Microscopy</u> (PALM).

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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 µm, 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.

46. Identification and characterization of new alleles required for microtubule-based transport of nuclei, endosomes, and peroxisomes.

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Eukaryotic cells use the microtubule-based molecular 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 fluorescently-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 two 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.

*47. Cell biology of conidial germination during colony initiation in the human pathogen, *Aspergillus fumigatus*. A. Contreras-Valenzuela^{1*}, D. MacDonald², M. Bromley², N. D. Read¹. 1) Fungal Cell Biology Group, Institute of Cell Biology, University of Edinburgh, Edinburgh EH9 3JH, UK; 2) Institute of Inflammation and Repair, University of Manchester, Manchester M23 9LT, UK.

We are analysing the cell biology of colony initiation in the human pathogen *Aspergillus fumigatus*. Conidial germination, conidial swelling and germ tube elongation have been quantified. Live-cell imaging techniques have been used to image, track and quantify different organelles (e.g. nuclei, mitochondria, vacuoles and peroxisomes) during germination after labelling them with different fluorescent proteins and vital dyes. No evidence has been obtained for the conidia or germ tubes of *A. fumigatus* forming interconnected networks by means of conidial anastomosis tube fusion. To analyse this in more detail, I am using a more sensitive assay with strains expressing cytosolic GFP, as well as auxotrophs to force heterokaryon formation. I have also being using cell cycle inhibitors to establish the relation between nuclear division and germination in this filamentous fungus.

48. A Hook protein is critical for dynein-mediated early endosome movement in Aspergillus nidulans.

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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 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.

*49. Cellular morphogenesis of Aspergillus nidulans conidiophores: a systematic survey of protein kinase and phosphatase function.

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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 HsI1/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.

Comparative and Functional Genomics

50. Exploring the biomass modifying enzymes of new filamentous fungal isolates from Vietnam, using secretome and transcriptome analyses.

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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 thermotolerance, 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.

51. The Aspergillus and Candida Genome Databases: Recent Developments and Future Plans.

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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.

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

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.identify their role in the degradation process.Tackling biofuel bottlenecks through genome wide association studies in *Saccharomyces cerevisiae*.

53. Alternative structural annotation of Aspergillus oryzae and Aspergillus nidulans based on RNA-Seq evidence. **Gustavo C Cerqueira**¹, Brian Haas¹, Marcus Chibucos², Martha Arnaud³, Christopher Sibthorp⁴, Mark X Caddick⁴, Kazuhiro Iwashita⁵, Gavin Sherlock³, 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 trancript 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:// http://www.aspergillusgenome.org).

***54. Functional analysis of roles of expanded genes in fruiting body development in Coprinopsis cinerea. Jinhui Chang**, Hoi Shan Kwan. School of life sciences, The Chinese University of Hong Kong, Hong Kong

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 comparing to simple fungi. Among these clusters, we chose to further analyze the group of Posttranslational modification related genes. We predicted and compared the PTM 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 siRNA 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 imported roles in light signal transduction pathway and possess different functions in different developmental stages.

55. Integrated transcriptional profiling and analysis for identification of *Cryptococcus neoformans* genes regulated during human cryptococcal meningitis.

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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 *in vivo* and *in vitro* conditions, we selected two strains of *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 *in vivo* and *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 *in vivo* compared to YPD and 39 genes overlapped between the CSF and *in vivo* condition. Some of the overlapping genes in CSF and *in vivo* have been reported to be related to the virulence composite of *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 *C. neoformans* taken directly from the CSF of two human patients. The comparisons between in vivo and in vitro samples helped us to identify a group of genes that may be important for surviving, adapting and proliferating of *C. neoformans* in the CSF of the human host.

56. Spliceosome twintrons ("stwintrons") revealed by fungal nuclear genomes.

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The spliceosome is an RNA/protein complex, responsible for 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 splicesomal introns. We have predicted four putative instances of "introns within introns" in nuclear genes of fungi. We call these "swtintrons" for "spliceosomal twin introns". Putative stwitrons 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 Magnaportacea. 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

57. Mechanisms of adaptation to host rice cells by the blast fungus.

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To infect rice, the devastating blast fungus *Magnaporthe oryzea* has distinct morphogenetic stages that allow it to breach the surface of the host leaf and invade the plant tissue. How 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* trehalose-6phosphate 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 $\Delta tps1$ strains that can form functional appressoria and penetrate the rice surface but fail to grow beyond the first infected cell. Impaired invasive growth of $\Delta tps1$ strains is due to loss of glucose sensing, inactivation of the NADPH-dependent genetic switch, and altered carbon assimilation. Moreover, NADPH-requiring antioxidation systems are shut down in $\Delta tps1$ 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.

58. Tackling biofuel bottlenecks through genome wide association studies in Saccharomyces cerevisiae.

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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 genegene interactions in phenotypic variation. The results provide a platform for synthetic engineering of hydrolysate tolerance in diverse yeast strains.

*59. Functional analysis of catalase-peroxidase encoding genes in the fungal wheat pathogen *Zymoseptoria tritici*.

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Zymoseptoria tritici is the new name of the foliar wheat pathogen cereal *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 *MgCat*_1, encoding a secreted catalase-peroxidase, plays an important role in the pathogenicity of *Z. tritici*. *Mg*_Cat-1 mutants hardly induced any disease symptoms and expression analysis of *Mg*_Cat-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 *Mg*_Cat-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*.

60. Genomics of fungal interactions for bioenergy crops.

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Bioenergy crops depend on plant interactions with fungi including pathogens and symbionts. Saprobic fungi determine efficiency of plant material bioconvertion. Genomics offer 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.

61. Genome-wide analysis of eleven white- and brown-rot Polyporales provides insight into mechanisms of wood decay.

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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, 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.

62. Comprehensive annotation of secondary metabolism biosynthetic genes and gene clusters of *Aspergillus nidulans*, *A. fumigatus*, *A. niger* and *A. oryzae*

Diane O. Inglis, Martha B. Arnaud, Jonathan Binkley, Gustavo Cerqueira, Prachi Shah, Marek Skrzypek, Farrell Wymore, Jennifer R. Wortman and Gavin Sherlock

Secondary metabolite production by *Aspergillus* species is an expanding area of research and the genome sequence for multiple *Aspergillus* species provide a wealth of predictive information about secondary metabolite production. Genomic mining techniques and overexpression strategies have enabled researchers to discover novel secondary metabolites produced by *Aspergillus* and other filamentous fungal species and the genes involved in their biosynthesis. The *Aspergillus* Genome Database (AspGD) provides information and make functional annotations about the gene products of *A. nidulans, A. fumigatus, A. niger* and *A. oryzae*. The annotation of these genes in one centralized database is critical for making use of the published literature for secondary metabolism.

AspGD uses the Gene Ontology (GO) vocabulary to make these functional annotations, including many that involve secondary metabolism in these fungal species. The GO is a standardized vocabulary that AspGD and other groups use to describe the function of gene products and enables comparisons among orthologs of different species. During the course of routine curation, we found that GO terms to precisely describe the function of these secondary metabolism genes in AspGD had not yet been created. We, therefore, performed a major overhaul of the annotations in the Biological Process (BP) aspect of the GO for all genes that have recorded functions in secondary metabolite production. These new GO BP annotations include specific terms for the each of the secondary metabolites involved and AspGD has leveraged these new annotations made to each species to make predictions about new genes with roles in secondary metabolism in the less characterized *Aspergillus* species. Secondary metabolite biosynthetic genes frequently occur as clusters in the genome. We used the anti-SMASH and SMURF algorithms as a starting point for the identifying cluster in *A. nidulans*, *A. fumigatus*, *A. niger* and *A. oryzae* for manual curation. The resulting clusters were further analyzed by genomic context using tools available at AspGD to manually annotate and refine the secondary metabolite biosynthetic gene clusters of *Aspergillus*. This work will further enable researchers to identify potentially important novel secondary metabolites in *Aspergillus* species.

63. Systematic analysis of the uncharacterized genes, which widely conserved among filamentous fungi, in *Aspergillus oryzae*.

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The genome sequences of Aspergillus oryzae revealed huge number of uncharacterized genes, which were occupied about 50% of A. oryzae genes. 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 gene 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 at least 7 species among 14 filamentous fungi as the cff 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 hydroxyurea, camptothecin, micafungin 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.

64. Aegerolysin proteins from Aspergillus species.

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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. eryngei), 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. Aegerlysins 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. Joint Genome Institute (JGI) has recently sequenced eight Aspergillus species (A. tubingensis, A. brasiliensis, A. acidus, A. glaucus, A. versicolor, A. sydowii, A. wentii and A. zonatus) as a result of community sequencing proposal (CSP2011). The genome sequences are available at MycoCosm (JGI) (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.

*65. RNA silencing in poplar anthracnose fungus Colletotrichum gloeosporioides.

Simeng Li, Yonglin Wang, Chengming Tian.

anthracnose is one of the most destructive diseases on *Poplus sp*, whose causal agent is *Colletotrichum gloeosporioides*. Although the fungus is a broad-host range plant pathogen, only dozens of genes involved in pathogenesis have been identified and characterized. In order to establish a high-throughput platform, this study delivered the double-stranded RNA(dsRNA) expression cassette into protoplasts to trigger silencing for functional genomics research in *C. gloeosporioides*. A new silencing vector pSD-SUR1 based on RNA-silencing vector (pSD1) with a convergent dual promoter was introduced. In this silencing system, the target gene was proposed to be transcribed as a chimeric RNA which activates the system. As an indicator of gene silencing, GFP fluorescence is used to evaluate efficiency of this silencing system. The fluorescence observation showed GFP fluorescence significantly decreased in some of the silenced strains, comparing with the recipient strain. The GFP mRNA transcript levels in the strains were analyzed using quantitative RT-PCR. The results showed that the reduction range of controls in *gfp* expression was from 30% to 80%, suggesting an effective gene silencing signal transduction pathways and transcriptional factor were inserted respectively into the vector pSD-SUR1 and to be silenced. In conclusion, RNA silencing system opens up new opportunity for exploring gene function in the fungus *C. gloeosporioides*.

66. 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.

67. Evolutionary genomic analysis of cytochrome P450 proteins in the subphyla Pezizomycotina. **Venkatesh Moktali**, Seogchan Kang. The Pennsylvania State University, University Park, PA 16802

a central repository for gene and protein information about several *Aspergillus* species. Scientific curators at AspGD collect and record gene and protein

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 phylogenomic 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.

*68. Comparative Genomics of L and S Morphotypes of Aspergillus flavus.

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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 hydrolase 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 vegetative compatibility 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 transitively 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.

69. A novel approach for functional analysis of genes in the rice blast fungus.

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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 genes in Magnaporthe oryzae by constitutive expression under a strong promoter. Employing a binary vector (pGOF), carrying EF1ß promoter, we generated a total of 4,432 transformants by Agrobacterium tumafaciens-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 promotor regions of putative lethal genes, MoRPT4 and MoDBP5, 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.

70. Novel transcriptomics approaches for metabolic pathway engineering target identification in *Aspergillus*. **Peter J. Punt**, Martien Caspers, Marvin Steijaert, Eric Schoen, Machtelt Braaksma. Microbiology, TNO, Zeist, Netherlands

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 much more efficient transcriptomics approaches such as RNAseq. Combination of these approaches with novel experimental design and statistical methods for targetgene 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 clonebased transcriptomics approach for the identification of genes relevant for itaconic acid production in Aspergillus. Fungal Genetics and Biology 48 (6), pp. 602-611.

*71. A RNA-Seq directed functional genomics screen to identify novel cell wall genes in the hyphal tip of Neurospora crassa.

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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-Seg of the filamentous fungus Neurospora crassa. The growing 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 mRNA 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 set includes Zn-Cvs transcription factors (NCU04866 & NCU04663), Glycoside Hydrolase 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.

72. Comparative reannotation of 21 Aspergillus genomes.

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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 exonintron 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 genomes 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.

*73. Genome-wide analysis of small RNA machineries in fungal kingdom.

Jiayao Wu¹, Jaeyoung Choi², Fred O. Asiegbu¹, Jari P.T. Valkonen¹, Yong-Hwan Lee^{1,2}. 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: genomeic 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 Argonauts with plant and animal, but longer in the length and less 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. Phylogentic analysis indicates that most fungal Argonuates belong to AGO-like subfamily. However, fungal Dicers could to 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.riceblast.snu.ac.kr/).

Gene Regulation

*74. Molecular cloning and differential expression of two novel Family 1 β-glucosidases genes from the rare fungus Stachybotrys microspora.

Salma Abdeljalil, Houcine Lazzez, Ali Gargouri. Centre of Biotechnology of Sfax, Sfax-Tunisia

The cellulolytic system of the fungus Stacchybotrys microspora is characterized by the existence of several β glucosidases. From a compilation of fungal β -glucosidases belonging to family GH1, we designed primers to isolate β 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 Smbgl1A while the third one is a part of a second gene named Smbgl1B. RT-PCR analysis showed the first gene is induced by cellulose and repressed by glucose while Smbgl1B 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 SmBgl1A deduced aminoacid sequence. The predicted secondary structure of Smbgl1 confirmed its appurtenance to GHI family: the presence of a classical (β/α)8 barrel and all the characteristic of subsite -1 (glycone site).

75. Aspergillus nidulans galactofuranose biosynthesis affects antifungal drug sensitivity. Md. Kausar Alam, Susan Kaminskyj.

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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 *niiA*(p) promoter. For repression and expression, *alcA*(p)-regulated strains were grown on complete medium with glucose or threonine, whereas *niiA*(p)-regulated strains were grown on minimal medium with ammonium or nitrate. Expression was assessed by qPCR and colony phenotype. The *alcA*(p) and *niiA*(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 niiA(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. Previously, we showed that wild type Af*ugmA* can restore an *A. nidulans ugmA* deletion strain to wild type phenotype. Our current results show that certain amino acid residues in *A. fumigatus* UgmA are critical for Gal-f generation: constructs wCharacterization of tannic acid-inducible and hypoviral-regulated *CpsHsp*1 expression level of the chestnut blight fungus *Cryphonectria parasitica*.

*76. The transcriptional factors XYR1 and CRE1 regulate the expression of Cellulolytic and Xylanolytic genes at carbon source dependent-manner in *Hypocrea jecorina* (*Trichoderma reesei*).

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The ascomycete *Hypocrea jecorina* (anamorph of *Trichderma reesei*) is a 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 activity of cellulases and xylanases in the $\Delta xyr1$ and $\Delta cre1$ mutants and compare with the parental *T. reesei* (QM9414), in three different carbon sources. The strains were grown in Mandels-Andreotti medium, supplemented with cellulose, sophorose 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, cel3c, cel3e, xyn2* and *swo* genes showed a significantly high expression in the mutant $\Delta cre1$ when compared with the parental QM9414 and low expression of the *cel1a, cel3d* and *cel61b* genes was observed when compared the mutant $\Delta xyr1$ with the QM9414 on cellulose, sophorose and glucose. Overall, all of cellulase and xylanase genes showed higher expression in mutant $\Delta cre1$ and low expression in mutant $\Delta xyr1$ in all studied conditions, when compared to QM9414. Concerning to enzymatic profiles, the activity of CMCase, β -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.

*77. Characterization of tannic acid-inducible and hypoviral-regulated *CpsHsp*1 expression level of the chestnut blight fungus *Cryphonectria parasitica*.

J.-H. Baek¹, J.-A. Park¹, J.-M. Kim², S.-M. Park¹, D.-H. Kim¹. 1) Institute for Molecular Biology and Genetics, Center for Fungal Pathogenesis, Chonbuk National University, Jeonju, Chonbuk, South Korea.; 2) Department of Bio-Environmental Chemistry, Wonkwang University, Iksan, Chonbuk, South Koreaith mutated *A. fumigatus* sequences failed to rescue the An*ugm* Δ phenotype.

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-Hsps_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 viruscontaining CpsHsp1-null progeny displayed characteristics of invasive feeding hyphae, near 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 hypovirulent strain UEP1 was compared with that in the wild-type strain EP155/2. Northern blot analysis of RNA from UEP1 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 UEP1 strains by Northern blot analysis and Western blot analysis.

*78. Removal of C4-methyl Sterol Accumulation in a SREBP-null Mutant of *Aspergillus fumigatus* Restores Hypoxia Growth.

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The human pathogen *Aspergillus fumigatus* adapts to stress encountered in the mammalian host based on evolutionary mechanisms derived from its ecological niche as a composter or saprobe. SrbA, a member of the helix-loop-helix family of transcription factors, plays a significant role in *A. fumigatus* hypoxia adaptation, antifungal drug responses, and virulence. SrbA is a direct transcriptional regulator of several key enzymes in the ergosterol biosynthesis pathway, which has been

verified by *in vivo* ChIP-SEQ analyses. The sterol intermediate profile of $\Delta srbA$ revealed a significant accumulation of C4methyl sterols, which correlates with the loss of *erg25* (C4-sterol methyl oxidase) mRNA abundance in the SrbA-null mutant. We hypothesized that this C4-methyl sterol accumulation may contribute to the significant phenotypes observed in $\Delta srbA$. We have characterized the two genes predicted to encode C4-methyl sterol oxidases (Erg25) in *A. fumigatus*. Genetic deletion of both *erg25* genes, A and B, is lethal in *A. fumigatus*, while single genetic deletions of the respective genes are viable. Although loss of both *erg25A* and *erg25B* resulted in accumulation of C4-methyl sterols, $\Delta erg25A$ accumulated far more C4-methyl sterol intermediates than $\Delta erg25B$, suggesting that Erg25A is the predominant C4-sterol methyl oxidase in *A. fumigatus*. No dramatic *in vitro* or *in vivo* phenotypes under various stress conditions were observed in $\Delta erg25A$ or $\Delta erg25B$ mutants; however, a moderate reduction in hypoxia growth was observed in $\Delta erg25A$. Generation of a strain that constitutively expresses *erg25A* in the $\Delta srbA$ background biochemically relieved a majority of the C4methyl sterol buildup in $\Delta srbA$. Significantly, restoration of *erg25A* mRNA levels in $\Delta srbA$ with a promoter replacement fully restored the *in vitro* hypoxia growth defect of $\Delta srbA$. These results indicate that *erg25* transcriptional regulation by SrbA and management of C4-methyl sterol intermediate accumulation is highly important for hypoxia stress adaptation in *A. fumigatus*. Future studies will explore the impact of reducing C4-methyl sterol levels in $\Delta srbA$ on *A. fumigatus* virulence.

79. Screening of a non-essential protein kinase collection reveals the importance of carbon starvation in CreA derepression and cellulase production in *Aspergillus nidulans*.

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The purpose of this study was to understand the signaling pathways involved in the regulation of cellulase production in the model filamentous fungus *Aspergillus nidulans*. In particular, focusing on the role of non-essential protein kinases (NPK) in the modulation of CreA-mediated hydrolase repression.

Screening a collection of 103 NPKs mutants for reduced growth on avicel identified nine NPKs linked to nutrient sensing, cellular energy status and cell growth. The reduced ability of these NPK mutants to grow on avicel was correlated with a reduction in endocellulase activity and *egIA/B* transcription. Xylanase activity was also demonstrated to be reduced in several NPK mutants.

The construction of a wild-type CreA::GFP strain, under the control of the native promoter, enabled the localisation of the cellulase repressor protein. CreA derepression and the loss of nuclear localisation on avicel correlated with carbon starvation conditions.

Several of the identified NPKs were related to the cAMP signaling pathway including PkaC, SchA and YakA. The wild-type PkaC was demonstrated to be hyperactivated under avicel and starvation conditions. Genome-wide transcriptional profiling of the wild-type, $\Delta pkaC$ and $\Delta schA$ strains post growth on avicel revealed the influence of these NPKs on cellular metabolism, CAZy enzymes, transcription factors and transporters.

Sexual crosses between the NPKs mutants and the *ΔcreA* strain revealed the recovery of cellulase activity, while crosses between the NPKs and the CreA::GFP strains showed impaired CreA derepression under avicel or starvation conditions. These results demonstrate the role of starvation in the induction NPKs involved in CreA derepression and hydrolytic enzyme production, while additional external signals exist that induce the cellulase transcriptional activators. Financial support: FAPESP and CNPq, Brazil.

*80. 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 Davis², Richard Todd¹ 1) Department of Plant Pathology, Kansas State University, Manhattan, USA; 2) Department of Genetics, University of Melbourne, Melbourne, AUS

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 CrmA-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.

*81. Expression Analysis of Genes Associated with *Trichoderma harzianum* Mycoparasitism Process Against *Sclerotinia sclerotiorum*.

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Fungal species Trichoderma harzianum, are studied as plant pathogens biocontrol agents in soil, and its mechanism of biocontrol is a complex process, that can occur through different mechanisms or a combination thereof, including mycoparasitism. The expression of two T. harzianum genes, Epl-1 and Exoglucanase (exo83) were analyzed using RTqPCR. Were performed direct confrontation assays in plate between T. harzianum (ALL42) x T. harzianum and T. harzianum x Sclerotinia sclerotiorum. RNA was extracted from both, before and after hyphae contact. T. harzianum (ALL42) was capable to inhibit the growth of S. sclerotiorum in plate confrontation assay. Scanning electron microscopy showed that T. harzianum (ALL42) promoted morphological alterations of S. sclerotiorum cell wall. RNAs (1 µg) from each sample, were first treated with DNase I (Fermentas) to remove genomic DNA. After this step, cDNAs strands were synthesized using the First Strand cDNA kit MaximaTM Synthesis, according to the manufacturer's instruction. After dilution of 1/50 times the cDNA were used for Real-time PCR analysis using equipment CFX96TM using Bio-Rad ® SsoFastTM EvaGreen Supermix (Bio-Rad) for signal detection, in accordance with manufacturer's instruction. A gene encoding actin was amplified as a reference to normalize the total amount of cDNA present in each reaction. The gene expression level was calculated according to the method 2^{-ΔΔCT} (Livak & Schmittgen, 2001), where the transcription rate of T. harzianum x T. harzianum before contact was used as a control sample. These calculated gene expression results were used for construction of graphs showing the differences in expression of these genes during the comparison. The results showed an increase of up to 6 fold for Epl-1 gene and approximately 22 fold for the Exoglucanase gene during contact with the host, indicating an extremely important role of both genes in *T. harzianum* mycoparasitism process. Mutants for both genes are under construction in order to access the role of these genes in biological control.

*82. HapXcess and C-terminal truncation impairs Aspergillus fumigatus' iron homeostasis.

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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 sidG and siderophore transporter-encoding mirB) within one hour of induction. In agreement, elevated hapX expression decreased the cellular accumulation of protoporphyrin IX, the ironfree precursor of heme, and increased production of the extracellular siderophore TAFC. 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 sufficiency. These data demonstrate tight iron-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 xy/P 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.

83. The CCAAT-Binding-Complex mediates Iron Regulation in Aspergillus fumigatus.

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Iron is essential for a wide range of cellular processes but its excess is toxic. Therefore, microorganisms evolved finetuned mechanisms for uptake and storage of iron, to sustain iron homeostasis. In the opportunistic fungal pathogen *Aspergillus fumigatus*, the bZIP-type transcription factor HapX mediates adaption 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 sufficiency, demonstrating iron-independent 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 I282-B09 to HH.

*84. Redundant Nuclear Localization Signals Mediate Nuclear Import of the Aspergillus nidulans Transcription Activator of Nitrogen Metabolic Genes AreA

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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 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.

*85. Diverse classes of small RNAs originating from genomic hotspots, tRNA and the mitochondrial genome in Phytophthora infestans.

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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 sRNA '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 lle 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 PiAvr2, which is located just 231 bp from a class II transposon. The presence of sRNAs mapping to both PiAvr2 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: Crinkler effector genes, arrays of duplicated genes, potentially antisense overlapping transcripts, and genes containing transposon insertions. Our present task is to reveal the role that sRNAs might play in their regulation.

*86. Fugal-specific sirtuin *HstD* coordinates the secondary metabolism and development via the *LaeA*. **M. Kawauchi**^{1,2}, K. Iwashita^{1,2}. 1) Dept. Mol. Biotech., Grad. Sch. Adv. Sci. Mat., Hiroshima Univ., Hiroshima, Japan; 2) Natl. Res. Inst. Brewing, Hiroshima, Japan

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 *LaeA*, which is the most studied coordinator for the regulation of secondary metabolism and development, was induced in the $\Delta HstD$ strain, and we found a significant genetic interaction between *HstD* and *LaeA* 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 *LaeA* 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.

87. 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.

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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 conidia, 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 microscope revealed both invasive growth-type and sectored-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 sectored isolate. 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 sectored 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.

*88. Similar is not the same: Differences in the function of the (hemi-) cellulolytic regulator XInR (XIr1/Xyr1) in filamentous fungi.

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The (hemi-) cellulolytic transcriptional activator XInR (XIr1/Xyr1) is a major regulator in fungal xylan and cellulose degradation as well as in the utilization of D-xylose via the pentose catabolic pathway. XInR 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 XInR. 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 β-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 α -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* $\Delta xyr1$. 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.

89. Cooperative regulation of *Aspergillus nidulans* cellulase genes by transcription factors McmA and ManR/CIrB.

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Expression of the endoglucanase A gene (eglA) in A. nidulans is inducible by cellobiose. 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 Zn₂Cys₆ 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 CIrB 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/CIrB. In this presentation, we provide evidences that McmA and ManR/CIrB cooperatively regulate induction of cellulase genes. Effects of mcmA mutation and manR/clrB deletion on expression of cellulase genes were examined by qRT-PCR. Expression of egIA, egIB, and cbhA was highly induced by cellobiose in the wild type strain. The induction was significantly impaired by the mcmA mutation and abolished by the manR/cIrB deletion, indicating that the cellulase genes under control of McmA and ManR/CIrB are overlapped. Binding of His-tagged McmA and Flag-tagged ManR/CIrB-DBD (DNA Binding Domain), which were produced in E. coli and purified, to the CeRE containing region of the ealA 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. ManR/CIrB-DBD alone showed very weak binding to the region. When both McmA and ManR/CIrB-DBD were added, a slower-migrating and more abundant shifted band was appeared, which suggested cooperative binding of McmA and ManR/ClrB-DBD. Presence of McmA and ManR/ClrB-DBD 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/CIrB 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 Biooriented Industry.

*90. Protein kinase A signaling in *Aspergillus fumigatus*: Identification of downstream targets.

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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.

91. Conditional expression of the phospho-transmitter gene *ypdA* and the signaling interaction of YpdA with response regulators; SskA and SrrA in *Aspergillus nidulans*

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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 (SskA 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 alcA* promoter (*CypdA* strain) and analyzed their phenotype. We constructed mutant strain from *CypdA* by deleting the response regulator gene *srrA* (*CypdA/ΔsrrA*) and *sskA* (*CypdA/ΔsrkA*). When *ypdA* was downregulated, *CypdA* showed remarkable growth retardation and formed abnormal hyphae, and *CypdA/ΔsrrA* unexpectedly showed more severe growth retardation than the parent *CypdA*, in contrast, the growth retardation of *CypdA/ΔsskA* partly recovered. It is suggested that the growth retardation of *CypdA/ΔsrrA*Δ*sskA*) from *CypdA* by deleting the HogA pathway. We further constructed a mutant (*CypdA/ΔsrrA*Δ*sskA*) from *CypdA* by deleting the two RR genes. Here, we discuss two-component signaling under the inhibitory conditions of signaling between YpdA and response regulators.

*92. The transcription factor, AtrR, regulates the expression of ABC transporter genes and ergosterol biosynthesis genes in aspergilli.

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We previously demonstrated that a novel Zn(II)2Cys6 transcriptional factor, 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 ApatrR ortholog (AfatrR) in Aspergillus fumigatus was similarly hypersensitive to azole drugs. However, little is known about target genes regulated by AfAtrR. In this study, we comprehensively examined the target genes regulated by AfAtrR using next-generation DNA sequencing technology (RNA-seq). RNA-seq analysis indicated that AfAtrR similarly regulated at least one ABC transporter. In addition, surprisingly, AfAtrR 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 AfSrbA were nearly identical with those regulated by AfAtrR. Therefore, we investigated difference in function between AtrR 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.

93. Down Regulation of sidB Gene by Use of RNA interference Technology in the Filamentous Fungi Aspergillus nidulans.

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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 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 μ M). 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.

94. RNA silencing of *pacC* increases *afIR* 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 afIR/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 pacCRNAi construct under the control of a thiamine inducible promoter. Expression of pacC and af/R transcripts was then measured via RT-gPCR in cultures grown under alkaline or acid conditions. At pH 4, between pacCRNAi inducing and repressing conditions, an afIR/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 pacCRNAi was suppressed, the afIR/pacC ratio was 0.2 indicating that pacC production was higher than that of afIR. afIR 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 pacCRNAi was induced, the afIR/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.

*95. Suppressor mutagenesis of a Δ *laeA* mutant reveals novel regulators of secondary metabolism in *Aspergillus nidulans*.

Alexandra Soukup, Jerry Luo, Jin Woo Bok, Nancy P. Keller. UW-Madison, Madison, WI.

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 LaeA. Deletion of *laeA* 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 *ΔlaeA* mutant resulted in identification of 17 plasmids containing inserts ranging from one to four genes. Further analysis of the suppressor plasmids confirmed of a subset to increase SM production both in the original *laeA* deletion strain and in wild type backgrounds.

96. Effect of carbon sources on CreA protein level in Aspergillus oryzae

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Aspergillus oryzae has an ability to produce copious amounts of amylolytic enzymes, although transcriptional induction of these enzymes is repressed in the presence of glucose. The carbon catabolite repression in filamentous fungi is regulated by the transcription factor CreA. In *Aspergillus nidulans*, CreB, CreC and CreD were also identified as the carbon catabolite repression regulating factors, and these factors are presumed to be involved in ubiquitination or deubiquitination of CreA. However, there is little information on the expression profile of CreA at protein level. In this study, we generated the 4HA-fused CreA expression strain of *A. oryzae*, and expression level of CreA protein was monitored by Western blot analysis.

The steady-state CreA protein level in maltose medium was lower compared with those in glucose and fructose media. The reduction of CreA protein level in maltose medium was also observed in 4HA-CreA constitutive expression strain driven by enolase gene promoter. To investigate the effect of carbon sources on CreA protein level in detail, the mycelia grown in fructose medium was transferred to various carbon sources media. The CreA protein level was rapidly decreased after transfer to maltose and xylose media, suggesting that CreA protein was degraded under condition for induction of glycoside hydrolase-encoding genes. In contrast, reduction of CreA protein level was not observed under maltose plus glucose condition, suggesting that CreA protein was stabilized in the presence of glucose. These results suggest that the level of CreA protein is regulated by carbon sources in *A. oryzae* at the post-translational process. This study was supported by the Program for Promotion of Basic Research for Innovative Bioscience (PROBRAIN).

*97. Further Characterization of Surface Recognition Mechanisms in Magnaporthe oryzae.

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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 helics are essential for Msb2 functions. Site-directly 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.

*98. 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.

99. ChIP-seq: an inexpensive and powerful method for studying genome-wide chromatin remodeling and transcription regulation in fungi.

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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 Cyc8-Tup1 co-repressor complex in *Saccharomyces cerevisiae*. Examples on how ChIP-seq applications may be broadly applied to address common questions regarding transcription regulation will also be presented.

100. FigA, a putative member of low-affinity calcium system, is involved in both asexual and sexual differentiation in *Aspergillus nidulans*.

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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 influx system (LACS). In the filamentous fungus Aspergillus nidulans, we identified the homologs of HACS—the voltage gated channel CchA and the stretch activated channel MidA, which formed a complex that played important roles in conidial development, hyphal polarity establishment, and cell wall components in low-calcium environmental condition. In comparison, loss of FigA, a putative member of LACS, showed very severe defects in conidiation and in self-fertility during sexual development under either low or high calcium environmental condition. Interestingly, extracellular Ca2+ was unable to improve these figA defects substantially. Most importantly, the quantitative PCR results revealed the expression of the major asexual development regulator brIA and sexual development regulator nsd, steA had been remarkable regulated in figA mutant. In addition, the

localization of Fig1::GFP revealed that FigA was highly accumulated at the center of septum on the mature hypha, and the sites between vesicle-metulae, between metulae-phialide. These data implied that figA likely played important roles in cellular trafficking and communication during in both asexual and sexual differentiation in Aspergillus nidulans. *Correspondence to be addressed: linglu@njnu.edu.cn This work was financially supported by the National Natural Science Foundation of China (NSFC) 31200057 to Z.S, 31070031 to L.L.

101. Engineering and characterizing protein secretion in Aspergillus niger.

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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. Competition with native proteins for rate limiting steps in the secretory pathway, transcription, mRNA 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 reduce 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.

Pathogenic and Mutalistic Interactions

*102. Two G protein-coupled receptors, GprC and GprD, regulate density-dependent development in *Aspergillus flavus*.

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Aspergillus flavus is an opportunistic pathogen of several plant hosts, including maize. This interaction is mediated in part by oxygenated polyunsaturated 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 (GPCR) are responsible for receiving and transducing oxylipin signals in *A. flavus*. We have deleted and overexpressed two GPCRs, *gprC* and *gprD*, and found that they are important in regulating density-dependent development, which is thought to involve oxylipin signaling. Specifically, depletion of both *gprC* and *gprD* locks 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 GprC and GprD are important for pathogenicity, and heterologous expression of GprC and GprD in *Saccharomyces cerevisiae* is being used to address questions concerning direct ligand-receptor activation.

*103. Detoxification of nitric oxide by flavohemoglobin and the denitrification pathway in the maize pathogen *Fusarium verticillioides*.

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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 nitrate reductase (dNaR, 16-fold increase), dissimilatory nitrite reductase (dNiR, 226-fold), and P450 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. 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.

*104. 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-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 rvegrass seedlings. However, deletion of vapA 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 H_2O_2 but conidia of the yapA mutant are very sensitive to H_2O_2 . 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.

105. Deep RNAseq of wheat leaf infection by M. graminicola identifies phase-specific in planta expressed genes and varying transcriptional contributions of fungal chromosomes.

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Mycosphaerella graminicola is the causal agent of Septoria tritici blotch disease of wheat. Infection of leaves by M. graminicola involves a characteristic long period of symptomless intercellular growth of at least 8-10 days prior to the formation of necrotic leaf lesions. The genome sequence of the model isolate of M. graminicola, IPO323, was recently published by the research community in conjunction with the JGI and has been shown to contain 21 chromosomes. We have performed a deep RNAseq analysis to investigate fungal gene expression in vitro (in Czapek-Dox (CDB) and Potato Dextrose broth) and throughout phases of plant infection: day 1 (d1) germination on the leaf surface, day 4 (d4) slow growth in the absence of symptoms within the leaf, day 9 (d9) symptoms of disease become visible, day 14 fungal growth rate increases and finally day 21 when the fungus is sporulating asexually in fully necrotic plant tissue. Sequencing was performed on the Illumina Hiseq platform. The RNA-seq data was analysed using the Tuxedo tools (Trapnell et al., 2012). Tophat2 was used to map the reads against the M. graminicola genome. Transcript abundance (in FPKM) was determined using Cufflinks. Significant changes in transcript expression across all 21 pairwise comparisons were determined using cuffdiff (FDR<0.05, p-value <0.01). Remarkably even by d1 of plant infection > 600 differentially expressed genes were detected relative to growth in CDB culture. Many of the most strongly expressed genes from d1 to d9 of infection encode predicted secreted proteins, mostly of unknown function. No genes whatsoever displayed differential expression between d4 and d9 of infection and only 1 between d1 and d4. This implies that the fungus responds to the plant early and maintains a consistent level of gene expression throughout early symptomless infection. Amongst the genes up-regulated specifically in planta, were those that appeared to be present in gene clusters surrounding polyketide synthases, suggesting the up-regulation of specific secondary metabolites during infection. Finally overall read mapping to chromosomes highlighted the fact that the eight smallest chromosomes (Chr14-21) were significantly less transcriptionally active than the 13 larger "core" chromosomes, which may be consistent with their being dispensable for asexual plant infection.

106. Aspergillus fumigatus trehalose-6-phosphate regulates innate immune responses and virulence through modulation of fungal cell wall composition.

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Mechanism(s) behind the attenuated fungal virulence of trehalose biosynthesis pathway mutants are not fully understood. We observed previously that TPS2/OrIA, 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 Δ orIA 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, Δ orIA, in a murine model of X-linked chronic granulomatous disease (X-CGD) is mediated by an increased susceptibility of Δ orIA to polymorphonuclear leukocyte (PMN) killing. In the absence of PMNs in the xCGD murine model, Δ orIA exhibited restored fungal burden and virulence similar to wild-type inoculated animals. Null mutations in putative trehalose biosynthesis proteins TsIA and TsIB in the Δ orIA 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 Δ orIA 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.

*107. Penetration-specific effectors from *Phytophthora parasitica* favour plant infection.

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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 occuring 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.

*108. Fungal lipoxygenases: a novel instigator of asthma?

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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 lipoxygenase 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 lung epithelial cells, participates in the production of leukotriene and other eicosanoids, and exacerbates 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* lipoxygenase, 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 levels.

109. Molecular characterization of fungi associated with superficial blemishes of potato tubers in Al-Qasim region, Saudi Arabia.

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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 guality. 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 recorded in this study. Fusarium, Penicillium, Ilyonectria, Alternaria and Rhizoctonia were the most common genera collected from different blemish types. Using ITS region sequencing all collected fungi identified the species level. All Fusarium strains colled during this study were use to detect its pathogenicity against 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.

110. F-box protein 15 (Fbx15) links virulence of Aspergillus fumigatus to protein degradation and stress response.

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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 (<u>Skp1 cullin-1 F-box protein</u>) 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 which 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 antiphagocytic gliotoxin. 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 sigalosome are putative Fbx15 interaction partners. We propose an interdependent stabilization of Fbx15 and the COP9 sigalosome, which is required to link protein degradation and stress response to virulence.

*111. 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; $\Delta aarA$, $\Delta pksP$ and $\Delta sidA$. 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 $\Delta aarA$ and $\Delta pksP$ 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 PPTAs role in siderophore biosynthesis and not the activation of other secondary metabolism pathways that is critical for the virulence of *A. fumigatus*.

*112. The Ustilago maydis MAP Kinase signaling pathway: Identification of direct MAP kinase targets by phospho-peptide enrichment.

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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 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 kinases 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 kpp2 and kpp6. We then used a two-step chromatographic procedure combining phosphoprotein enrichment using AI(OH)3-based metal oxide affinity chromatography (MOAC), followed by tryptic digest of enriched phosphoproteins, and TiO2-based MOAC for phosphopeptide enrichment. This enabled detection of low abundant phosphorylated peptides using LC-MS/MS and allowed direct identification and sitespecific 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 presented.

*113. Characterization of effectors of the barley pathogen Rhynchosporium commune.

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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 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 *Rrs1*.

After obtaining the genome sequence of *R. commune* it turned out that NIP1 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.

*114. The Velvet gene is required for mutualism between *Epichloë festucae* and perennial ryegrass. **Mostafa Rahnama**^{1,2}, Richard Gardner¹, Damien Fleetwood². 1) School of Biological Sciences, University of Auckland, Auckland, New Zealand; 2) Forage Improvement Group, AgResearch, Auckland, New Zealand

The velvet gene (*veA* or *velA*) is a key factor in the regulation of fungal development, 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 remaining 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 calcolfluor 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)-triggered defense response may be occurring in Δ *velA* mutant associations. We are currently analysing the transcriptomes of wild type and mutant *E. festucae/Lolium perenne* symbiota to determine the *velA* regulon and elucidate the mechanism of host death.

*115. Genetic exchange in an arbuscular mycorrhizal fungus; *Rhisophagus irregularis*.

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Rhizophagus irregularis is a model species of an arbuscular mycorrhizal fungi (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 coenocytic organism, 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 mix of nuclei is random. In addition the nuclei are exchange with the surrounding cytoplasm. This lead to a guestion whether mitochondria from both parental isolates are transmitted to the offspring. In order to answer those questions I performed an in vitro experiment, where 6 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.

*116. Transcriptional regulatory circuits necessary for appressorium-mediated plant infection by *Magnaporthe oryzae*.

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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 transcription factor, MST12 (ScSte12 homogue) is part of the PMK1 MAP kinase signalling pathway, which is required for appressorium formation and invasion. The Mst12 null mutant is able to form completely normal melanised appressoria but it is non pathogenic. The Mst12 null mutant is unable to form a penetration peg and therefore to cause disease in the rice plant. To understand the mechanism of the penetration peg formation, we have recently carried out genome-wide comparative transcriptional profiling analysis for mst12 null mutant using RNA-seq and HiSeq 2000 sequencing. In this way, we will show the transcriptional signature associated with penetration peg differentiation in the rice blast fungus. Moreover we will show the set of genes that are likely to be MST12 regulated and therefore help define the regulatory circuits necessary for appressorium-mediated plant infection by plant pathogenic fungi.

*117. Diversity and Phylogeny of genus *Suillus* (Suillaceae, Boletales) from Pakistan (Asia). Samina Sarwar, Abdul Nasir Khalid. Botany, University of the Punjab, Lahore, Punjab, Pakistan.

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 & ITS6R and ITS2 & ITS8F were used to amplify the ITS1 and ITS2 along with partially 5.8S gene. Out of these, twelve (13) different Suillus species were found. Among them two (2) species seem undescribed and three (3) as new records for Pakistan. Their Phylogenetic relationships have also been discussed.

*118. The NADPH Oxidase Complexes in Botrytis cinerea.

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Reactive oxygen species (ROS) are generated in all aerobic environments and therefore play a major role for 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 germling 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.

119. Molecular diagnosis to discriminate pathogen and apathogen species of the hybrid *Verticillium longisporum* on the oilseed crop *Brassica napus*.

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The cruciferous fungal pathogen *Verticillium longisporum* represents an allodiploid 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 isogene 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 allodiploid 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.

120. From antagonism to synergism: roles of natural phenazines in bacterial-fungal interactions between Pseudomonas aeruginosa and Aspergillus fumigatus

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Secreted small molecules are increasingly recognized to mediate many types of bacterial-fungal interactions in nature and the clinical environment, 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.

Population and Evolutionary Genetics

121. Clonality and sex impact aflatoxigenicity in Aspergillus populations.

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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 aflatoxinproducing 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; or (4) female fertility/sterility that impacts the frequency of sexual reproduction. Population shifts that result in changes in ploidy or nuclear DNA composition (homokaryon versus heterokaryon) 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 aflatoxigenesis 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 aflatoxigenicity in these agriculturally important fungi.

*122. Discovery of Sexual Reproduction in the Black Aspergilli.

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The black aspergilli are members of the genus *Aspergillus* that are typically characterized by the production of dark or black asexual conidia (classified as section *Nigr*). The group includes *Aspergillus niger*, which is of particular industrial importance because of its safe use status and ability to produce a wide range of enzymes and organic acids. All members of the black aspergilli have previously only been known to reproduce by asexual means. However, as a result of combined molecular and cultural experimental studies it can now be revealed that at least one member of the black aspergilli, *Aspergillus sclerotiicarbonarius*, is able to complete a sexual cycle. Wild type isolates of *A. sclerotiicarbonarius* were found to retain the ability to form sclerotia, structures associated with both dormancy and sexual reproduction, and strains of complementary MAT1-1 and MAT1-2 could be identified based on the presence of mating-type genes. Crossing strains of opposite mating type, and an extended period of incubation, resulted in the production of sclerotia containing multiple ascocarps, with asci and viable ascospores, within the matrix of a sclerotium. This is consistent with past studies of phylogenetically related species in the *Aspergillus* section *Flavi* (teleomorph genus *Petromyces*). Progeny analysis is being undertaken based on data arising from comparative genome sequencing of parental isolates, mating-type distribution and phylogenetic analysis. The discovery of a heterothallic sexual cycle in *A. sclerotiicarbonarius* provides insights into the evolution of asexuality in the black aspergilli. It is hoped that ongoing molecular genetic studies into the early sexual morphogenesis may provide an insight into the regulation of sexual reproduction in the black aspergilli.

*123. The evolution of Sfp1 mediated, cell size control in Ascomycete fungi.

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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 underlied 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 ribosome 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 sfp1 Δ 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 stp1 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.

124. Profiling conditionally dispensable chromosomes of the plant-pathogenic fungus *Zymoseptoria tritici* (syn. *Mycosphaerella graminicola*).

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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 genome annotation we mapped all RNAseq reads to the genome of *Z. tritici* and predicted gene transcripts. By combining our results with the latest genome annotation we set up a new transcript list, which was used in 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*.

*125. Detection of Mitochondrial DNA Heteroplasmy in the progeny of crossed genetically divergent isolates of Arbuscular Mycorrhizal Fungi.

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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-rnl) 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 the 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 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.

126. Fertility in Aspergillus fumigatus and the identification of an additional 'supermater' pair.

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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 AfIR928, 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.

*127. Population shifts and mating-type heterokaryosis in Aspergillus flavus.

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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, aflatoxigenic 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.

Education and Professional Development

None.

Other topics

128. Co-cultivations of fungi: microscopic analysis and influence on protein production.

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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 XInR knock out strains. XInR 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.

129. Improving heterologous protein production in Aspergillus vadensis.

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Aspergillus vadensis 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. vadensis* two strategies were tested: (i) identification of new promoters for high gene expression, and (ii) overexpression of the xylanolytic regulator, XInR. 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. vadensis* was transformed with *xlnR*, *xlnD* (encoding beta-xylosidase) 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. XInD 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. vadensis*. Highlights from this study will be presented.

130. 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 (several 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 α -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.

131. A new method for fungal genetics: flow cytometry of microencapsulated filamentous microcolonies.

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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.

*132. Antifungal Pisum sativum defensin 1 Induces a non-Apoptotic Death in Aspergillus nidulans.

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Psd1 is a basic, cysteine-rich plant defensin isolated from Pisum 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 cerebroside 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 µM 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 ΔΔ8-desaturase (ANID_04592) and sphingolipid C9methylase (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 the mutant strains. Unraveling the mechanisms of cell death induced by antifungal peptides may lead to the identification of new targets that drive antimycotic selectivity.

133. Multiple gene integration system using a self-excisable marker cassette in Aspergillus oryzae.

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We have previously reported that Cre/loxP-mediated marker recycling system in which Cre enzyme is directly introduced into fungal cells to excise the marker gene flanked by loxP sites [1]. In addition, we have developed the Cre-mediated marker recycling with mutant loxP sequences to introduce a number of biosynthetic genes into A. oryzae, and achieved a high yield production of kojic acid by sequential introduction of genes for oxidoreductase and its transporter [2]. In this study, we further attempted to improve the marker recycling system in more convenient manner by applying a selfexcisable marker cassette that allows the removal of selectable marker together with Cre expression construct. We adopted the adeA gene as a selectable marker in this study, because the adeA deficient mutant shows orange colony, which allows us to easily discriminate the marker-rescued strains. The A. nidulans adeA gene was used to replace the Neurospora crassa pyr4 flanked by mutant lox sequences in the expression plasmid previously constructed [2]. Cre expression construct was designed to place the cre gene downstream of A. oryzae xylanase G2-encoding xynG2 promoter sequence. The xynG2 promoter is repressed in the presence of glucose resulting in the absence of Cre expression, and is induced in the presence of xylose to drive Cre expression. The Cre expression construct was inserted between the adeA gene and a mutant lox site in the expression plasmid. A. oryzae adeA deletion mutant was transformed by using the resulting plasmid in the presence of glucose, and then the transformants were transferred to the medium containing xylose as a sole carbon source. After growth on the xylose medium, colonies with orange color were observed and were proved to show adenine auxotrophy, indicating that a self-excisable marker cassette was efficiently function to

remove the selectable marker. Further PCR analysis supported the excision of both the marker and Cre expression construct in the resulting recombinants.

[1] Mizutani et al., Appl. Environ. Microbiol., 78, 4126–4133 (2012).

[2] Ebara et al., 9th Aspergillus meeting, Marburg, Germany (2012).

*134. Prezygotic and postzygotic control of uniparental mitochondrial DNA inheritance in *Cryptococcus neoformans*.

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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 α , most progeny derived from such mating events inherit the mitochondrial DNA from the **a** parent. The homeodomain protein complex Sxi1 α /Sxi2**a**, formed in the zygote after **a**- α 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 Sxi1 α /Sxi2**a**, and the cooperation between these prezygotic and postzygotic factors help achieve stricter uniparental mitochondrial inheritance in this eukaryotic microbe.

*135. A new method for gene mining and enzyme discovery.

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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. circinellolides* 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 β -glucosidase. We expressed this gene in *Pichia pastoris* and found that the recombinant protein has high β -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.

136. Overproduction of phleichrome by synthetic inducers and cloning of polyketide synthase genes in phytopathogenic fungus *Cladosporium phlei*.

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Phleichrome pigment produced by a *Cladosporium phlei* 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 elsinochrome and several other 4,9-dihydroxyperylene-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. Among 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.

137. Occurrence of dsRNA mycovirus (LeV-FMRI 2427) in edible mushroom *Lentinula edodes* and its meiotic stability.

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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 (LeV) and sequence comparison of the clone amplicon showed the identical sequence to the known RdRp genes of LeV 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.

138. Community Resources for Aspergillus fumigatus: an NIAID funded genome sequencing project.

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Previous genome sequencing projects have offered the first glimpse into the A. fumigatus

genome structure and organization. They have also highlighted the challenges associated with identification of functional features in fungal genomes. Eight years after the first *A. fumigatus* genome was sequenced, we still do not have the complete set of protein coding and non-coding genes in the genome. The insufficient quality of the genome annotation of *A. fumigatus* is a major limitation to gene expression analysis, reverse genetics, and other functional studies. Furthermore, the genome sequences of only two strains, AF293 and A1163, are currently available, which limits our view of the intraspecific genetic variation. Understanding the variation within this pathogenic species is critical for better detection and management of invasive aspergillosis and can greatly facilitate the development of new drugs and diagnostic devices. More polymorphic markers are needed for epidemiological and population studies as well as for antifungal resistance genotyping. To address these limitations, we employed Next generation Sequencing (NGS) technologies to improve genome annotation by RNAseq under a wide range of conditions and to characterize diversity in *A. fumigatus* at the genomic level by sequencing almost 50 different isolates. In this talk, we will present the status of the NIAID funded project and report our findings as they pertain to gene content and gene expression variations, SNP analysis, and gene model improvements.

*139. Chemically Induced Haploinsufficiency Screens to Identify Drug Mechanism of Action in *Aspergillus Fumigatus*.

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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. 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⁻/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.

*140. Identification of fungi associated with maize (Zea mays L.) growing in different planting dates at northern Tamaulipas, Mexico.

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Summary Maize is one of the most important crops for Mexico based on cultural, economic and gastronomic implications. Grain production shows significant losses during harvest at field and after, during storage, transport, processing and human or cattle consumption due several factors where outstand fungi which produces a broad range of mycotoxins as aflatoxins and fumonisins. Mycotoxyns are secondary metabolites with toxic and lethal effects on living organisms exposed to contaminated food and they are mainly produced by the genus Aspergillus (A. flavus and A. parasiticus). Here we identified fungal pathogens associated to grain produced by eight maize hybrids planted in four planting dates during 2010 in Rio Bravo, Tamaulipas, Mexico and also we detected and guantified aflatoxins produced by Aspergilli. The fungi isolates were identified based on macro and microscopic traits in acidified potato-dextrose-agar as well as following the method of Hoffman and Winston (1987) where the primers ITS (internal transcribed spacers) and PFPRIMF3 and PFPRIMR4 primers and PCR were used. The amplified sequences were analyzed at NCBI database (National Center for Biotechnology Information). We identified based on morphology and molecular methods the genera Aspergillus, Fusarium and Penicillium infecting maize grains and the former genus showed the highest incidences. The 98% of the maize samples were contaminated by aflatoxins which ranged from 7.19 to 75.72% of total aflatoxins. Aflatoxin concentrations found in maize were higher than the maximum permissible values according the Mexican Government regulations (NOM-247-SSA1-2008) (< 20 ppb). The maize hybrid G-8285 showed the highest fungal incidences while the lowest damaged hybrids were Bisonte, H-437, and H-439. Our results demonstrated the efficiency of molecular methods for identification of toxigenic fungi associated to maize growing at different sowing dates at northern Mexico and current and high incidences of aflatoxins in the crop.

*141. 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, Ca²⁺/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 CaCl₂, Calcofluor White and caspofungin. Pre-treatment of wild-type cells with CaCl₂ and CFW, activates the Ca²⁺/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.

142. Temperature- and pH characteristics of endo-cellulases in Rhizophlyctis rosea.

Bo Pilgaard¹, Frank Gleason², Peter Busk¹, 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 true fungi from soil. We have previously confirmed the presence of cellulases in Rhizophlyctis 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 Rhizophlyctis 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 Rhizophlyctis 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.

*143. Production and characterization of esterases from *Chaetomium thermophilum* and their applicability in biomass conversion.

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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 thermophilic 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 (*CtAxeA*) 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 CtAxeA 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 potential application in biomass bioconversion to e.g. higher value chemicals or biofuels.

144. Comparative activity-based protein profiling of pathogenic Aspergillus fumigatus and non-pathogenic Neosartorya fischeri and Aspergillus clavatus exposed to human serum.

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Pulmonary Invasive Aspergillosis is a devastating lung infection caused by the pathogenic fungus Aspergillus fumigatus. Unlike other microbial pathogens, A. fumigatus does not have true virulence factors because it did not evolve in the human host environment. Furthermore, virulence associated genes are shared between closely related and more distantly related fungi. The presence of these genes in non-pathogenic species suggests protein regulation and/or activation in response to environmental stimuli may contribute to pathogenicity. We used Activity-Based Protein Profiling (ABPP) in order to compare a subset of proteins of three closely related aspergilli, A. fumigatus, Neosartorya fischeri, and A. clavatus in human serum culture over time. ABPP is a chemical biology tool for the interrogation of enzyme activity in complex biological systems or proteomes. ABPP targets the active proteome of a biological system by covalently modifying enzyme active sites with small molecules (Activity-Based Probes (ABP)); therefore, tagged proteins pertain to active biological function that can be profiled under a variety of conditions. Proteins were covalently captured by general cysteine reactive and serine hydrolase specific ABPs followed by affinity purification and LC-Mass Spectrometry based identification; 800, 500 and 400 probe-reactive proteins were identified in A. fumigatus, N. fischeri, and A. clavatus respectively. Additionally, the relative abundance of probe-labeled proteins was drastically different between the three species in the presence and absence of human serum and over time. Over 350 unique probe-labeled proteins, including virulence associated and stress response proteins, were observed in A. fumigatus, while only 50% of expected orthologous proteins were measured in N. fischeri and A. clavatus. Processes associated with probe-labeled proteins unique to and over-represented in A. fumigatus include cell wall biosynthesis, signaling cascades, and stress response. We show that ABPP can be used to compare probe-reactive proteins between multiple organisms over multiple conditions and hypothesize that probe-reactive proteins uniquely measured in A. fumigatus may reveal active mechanisms necessary for invasive infection.

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