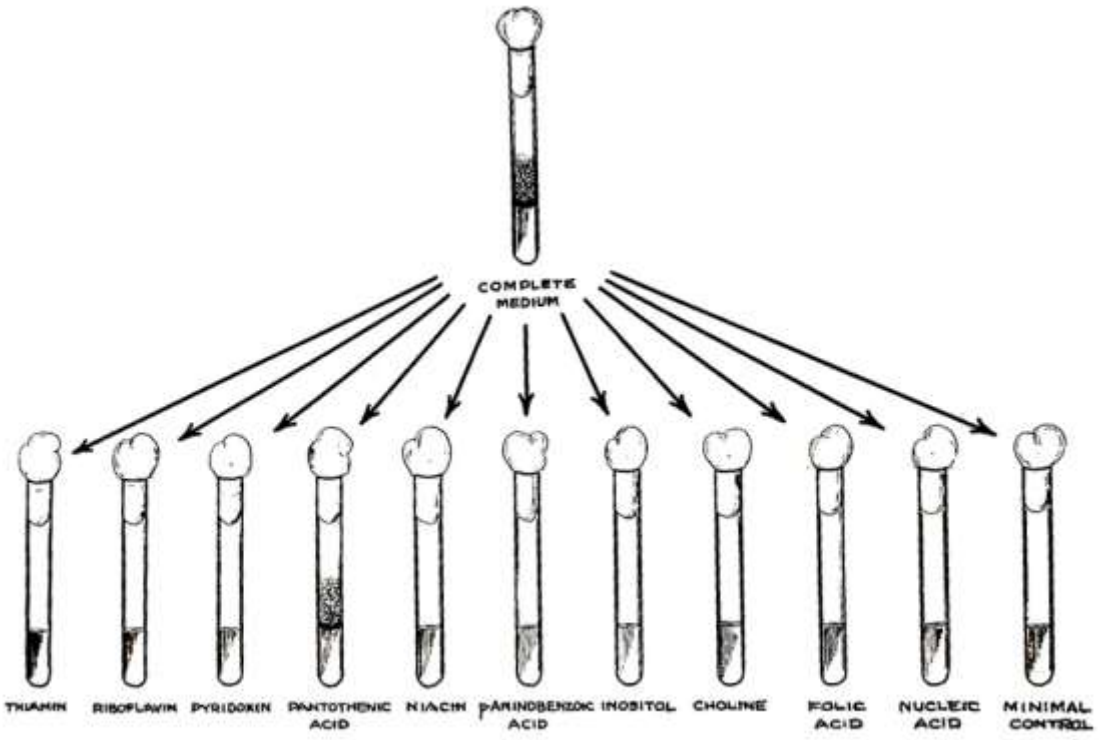
Neurospora 2014

March 6 - 9
Asilomar Conference Center
Pacific Grove
California



PROGRAM
and
ABSTRACTS

About the cover image: This image was one of four designed and commissioned by George Beadle and was published in at least two places: American Scientist 34:31-53 (1946) 'Genes and the chemistry of the organism' and Fortschritte der Chemie organischer Naturstoffe 5:300-330 (1948) 'Some recent developments in chemical genetics'. The drawing demonstrates the characterization of a strain auxotrophic for pantothenic acid. The original drawings are kept at the FGSC.

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Scientific Organizers

N. Louise Glass
Plant and Microbial Biology
University of California - Berkeley

Luis F. Larrondo
Dept Genética Molecular y Micro
Pontificia Universidad Católica de Chile

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Brief Schedule

DAY	MORNING	AFTERNOON	EVENING
Thursday		Arrival	Dinner
March 6		Registration	Mixer (Kiln)
Friday	Breakfast	Lunch	Dinner
March 7	Plenary Session I	Plenary Session II	Poster Session I
Saturday	Breakfast	Lunch	Banquet
March 8	Plenary Session III	Plenary Session IV	Poster Session II
Sunday	Breakfast	Lunch	
March 9	Plenary Session V	Departure	

All Plenary Sessions will be held in the Fred Farr Forum. Posters will be displayed in Kiln throughout the meeting. They may be set up Friday and be displayed until the end of the poster session/reception on Saturday evening.

Neurospora 2014

Scientific Program

Friday March 7th

7:30 – 8:30 Breakfast, Crocker

Morning (Fred Farr Forum) Cell Biology and Morphogenesis Chairs: *Nick Read and Greg Jedd*

8:30-8:40	Welcome and Announcements	
8:40-9:00	**Rosa Mouriño**	Contractile force and intracellular traffic. MYO-2 and MYO-5 in *Neurospora crassa*
9:00-9:20	**Stephan Seiler**	Regulation of NDR kinases pathways during septum formation
9:20-9:40	**Frank Kempken**	BEM46 and auxin biosynthesis in *Neurospora crassa*
9:40-10:00	**Eddy Sanchez**	Pre-exocytic vesicular organization: *Neurospora crassa* YPT-1 regulates vesicular traffic at the Golgi and at the Spitzenkörper
10:00 – 10:30	Coffee Break	
10:30-10:50	**Barry Bowman**	What is the structure and function of "the vacuole" in *Neurospora crassa*?
10:50-11:10	**Steve Free**	Regulation of female development in *Neurospora crassa*
11:10-11:30	**Jeff Townsend**	Comparative transcriptomics, functional analysis and systems biology of perithecial development
11:30-11:50	**Tom Hammond**	Efficient detection of unpaired DNA during meiosis requires a component of the homologous recombination machinery
12:00-14:00	Lunch, Crocker	
	Business Meeting and Presentation of Awards	

Afternoon Metabolism, Signaling and Development Chairs: *Wilhelm Hansberg* and *Maria Celia Bertolini*

14:30-14:50	**Dan Ebbole**	Asexual sporulation in Neurospora and conservation and divergence of developmental pathways in fungi
14:50-15:10	**Nick Read**	The dynamic mode-of-action of antifungal peptides
15:10-15:30	**Arnaldo Videira**	Mechanisms of programmed cell death in Neurospora
15:30-15:50	**Clifford Slayman**	K+ regulation in *Neurospora*: Three proteins do it all
15:50-16:20	Coffee Break	
16:20-16:40	**Kathy Borkovich**	G-proteins and post-transcriptional regulation
16:40-17:00	**Masayuki Kamei**	MSN-1 is a transcription factor regulated by MAK-1-MAP kinase in *Neurospora crassa*
17:00-17:20	**Matt Sachs**	eRF1 and eIF4A3 mRNA stability in *Neurospora* is controlled by NMD, EJC, and CBP factors through 3'UTR introns
17:20-17:40	**John Galazka**	(**Perkins Award**) Chromosome conformation capture followed by high-throughput sequencing reveals association between chromatin domains and loss of heterochromatin associations in *dim-5* and *hpo*

Evening

18:00 Dinner, Crocker
19:00-22:00 Poster Session (**Kiln**)

Saturday March 8th

7:30-8:30 Breakfast, Crocker

Morning **Gene Expression and Epigenetics** **Chairs:** ***Dan Ebbole and Jeff Townsend***

8:30-8:50 **Zach Lewis** CRF5 is required for DNA repair in Neurospora

8:50-9:10 **Shin Hatakeyama** The relationship between mutagen sensitivity and life span

9:10-9:30 **Quiying Yang** Quelling and DNA damage-induced small RNA production

9:30-9:50 **Logan Decker** Molecular dissection of meiotic silencing of unpaired DNA

9:50-10:20 Coffee Break

10:20-10:40 **Durgadas Kasbekar** Does sequence heterozygosity underlie suppression of meiotic silencing by wild Neurospora strains? Does Neurospora contain nucleus-limited genes?

10:40-11:00 **Andy Klocko** **(Perkins Award)** Neurospora importin-α (Nucleoporin-6) directs chromatin modifying complexes to sub-nuclear chromatin targets

11:00-11:40 **Michael Freitag** **(Beadle and Tatum Award)** Centromeric chromatin structure in filamentous fungi: DNA sequence, protein complement and phylogenomics

12:00-13:00 Lunch, Crocker

Special Session

13:15-14:30 **Navigating FungiDB** **Jason Stajich**

Afternoon **Circadian Clock and Environmental Sensing** **Chairs:** ***Kwangwon Lee and Christian Heintzen***

14:40-15:00 **Jennifer Hurley** Conserved RNA helicase FRH acts non-enzymatically to support the intrinsically disordered Neurospora clock protein FRQ

15:00-15:20 **Rigzin Dekhang** The *Neurospora crassa* transcription factor ADV-1 is a component of the circadian output pathway

15:20-15:40 **Bill Belden** Chromatin in circadian and light regulated gene expression

15:40-16:00 **Qun He** Suppression of WC-independent frequency transcription by RCO-1 is essential for Neurospora circadian clock

16:00-16:30 Coffee Break

16:30-16:50 **Stela Virgilio** Connection between glycogen metabolism regulation, light and the circadian clock in *Neurospora crassa*

16:50-17:10 **Christian Heintzen** An RNA-Seq study of circadian and non-circadian responses to light and temperature in Neurospora

17:10-17:30 **Kwangwon Lee** Local adaptation of the Neurospora circadian clock

17:30-17:50 **Christian Hong** Mathematical modeling of circadian rhythms in *Neurospora crassa*

Evening

18:00-19:30 Banquet, Crocker

19:45-20:45 **Banquet Speaker (Fred Farr Forum)**

Eric Selker One step at a time: My reflections on some of the 'good old days' of Neurospora molecular genetics

20:45-22:00 Post-Banquet Social/ Poster Session (Kiln)

Sunday, March 9th

7:30-8:30 Breakfast, Crocker

Morning **Genomics, Evolution and Methodology in the Neurospora Post-Genomic Era**
Chairs: *Jay Dunlap and Hanna Johannesson*

8:30-8:50 **James Galagan** Modeling the Neurospora Clock Regulatory Network

8:50-9:10 **Scott Baker** A re-sequencing journey through Neurospora classical mutant strains

9:10-9:30 **Hanna Johannesson** Adaptive introgression slows down molecular degeneration of the mating-type chromosome in *Neurospora tetrasperma*

9:30-9:50 **Pierre Gladieux** Speciation genomics of *Neurospora discreta*

9:50-10:20 Coffee Break

10:20-10:40 **Eric Bastiaans** Fusion and laboratory evolution of selfish nuclei

10:40-11:00 **Marcus Roper** Nuclear dynamics in Neurospora

11:00-11:20 **J. Philipp Benz** A comparative systems analysis of polysaccharide utilization in *Neurospora crassa* reveals molecular mechanisms of carbon adaptation as well as new factors with biotechnological applications

11:20-11:40 **Kevin McCluskey** Whole genome sequence underlies a new paradigm for genetics and discovery at culture collections

Plenary session Abstracts

Contractile force and intracellular traffic. MYO-2 and MYO-5 in *Neurospora crassa*.

Rosa R. Mouriño-Pérez; Arianne Ramírez-del Villar, and Ramon O. Echauri-Espinosa. Departamento de Microbiología. Centro de Investigación y Educación Superior de Ensenada (CICESE). Ensenada, B. C., México.

Myosins are motor proteins associated with actin filaments. Depending on the class, myosins can have transport or scaffolding functions. There are several kinds of myosins in eukaryotic cells but in *N. crassa* there are just three, MYO-1 (Class I), MYO-2 (Class II) and MYO-5 (Class V). We studied the localization and dynamics of the class II and class V myosin and their regulatory protein CDC-4 in *Neurospora crassa* by tagging them with GFP/mChFP and observing them by confocal and TIRF microscopy. Additionally, we deleted the *myo-2* and *myo-5* genes to observe its phenotypic consequences. MYO-2-GFP localizes at developing septa approximately six minutes before any sign of plasma membrane invagination, decorating actin filaments, it is part of the septal actomyosin tangle and also of the contractile actomyosin ring. MYO-2-GFP is not present in the apex, and its mutation seems to be essential. MYO-5-GFP is present in the apex co-localizing with the Spitzenkörper (Spk) and forming a cloud of fluorescence around it. It is also possible to observe it very close to the plasmalema in the apical dome. In basal parts of the hypha, there is just a faint fluorescence. MYO-5-GFP is present during septum formation; it is recruited when the actomyosin ring starts constriction. The absence of MYO-5 produces alterations in growth and hyphal morphogenesis, although polarized growth is maintained. Δ*myo-5* mutant forms small and compact colonies compared with the wild type stain (WT). Δ*myo-5* mutant produces 22 % of the WT biomass. Conidiation is strongly affected (0.09% of WT). Δ*myo-5 mutant* branches mainly apically and has a smaller hyphal diameter (less than 33.33 %) than the WT. The lack of MYO-5 has a strong effect in cell growth; it seems to participate in the secretion pathway and in the Spk organization. Although MYO-2 is present in the Spk, it appears to be a crucial protein in the organization of the actin cytoskeleton during septation.

Regulation of NDR kinase pathways during septum formation.

Yvonne Heilig[†], Anne Dettmann[†], Rosa R. Mourino-Pérez [#] and Stephan Seiler[†]. [†]Albert-Ludwigs University Freiburg, Germany. [#]Center for Scientific Research and Higher Education of Ensenada (CICESE), Mexico

Regulation of cell polarity and cytokinesis is highly complex and involves a large number of components that form elaborate interactive networks. Fungal nuclear Dbf2p-related (NDR) kinases function as effector kinases of the morphogenesis (MOR) and septation initiation (SIN) networks and are activated by the pathway-specific germinal centre (GC) kinases POD6 and SID1, respectively. We characterized a third GC kinase, MST-1, that connects both kinase cascades. Genetic and biochemical interactions with SIN components and life imaging identify MST-1 as SIN-associated kinase that functions in parallel with the GC kinase SID-1 to activate the SIN-effector kinase DBF-2. Aberrant cortical actomyosin rings are formed in Δ*mst-1*, which result in miss-positioned septa and irregular spirals, indicating that MST-1-dependent fine-tuning of the SIN is required for proper formation and constriction of the septal actomyosin ring. However, MST-1 also interacts with several components of the MOR network and modulates MOR activity at multiple levels. These data specify an antagonistic relationship between the SIN and MOR during septum formation that is, at least in part, coordinated through the GC kinase MST-1.

BEM46 and auxin biosynthesis in *Neurospora crassa*

K. Kollath-Leiß, C. Bönniger, P. Sardar & F. Kempken. Abteilung Botanische Genetik und Molekularbiologie, Botanisches Institut und Botanischer Garten, Christian-Albrechts-Universität zu Kiel, Olshausenstr. 40, 24098 Kiel, Germany; fkempken@bot.uni-kiel.de

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Pre-exocytic vesicular organization: *Neurospora crassa* YPT-1 regulates vesicular traffic at the Golgi and at the Spitzenkörper.

Sanchez-Leon, Eddy[1*], Bowman, Barry[2] and Riquelme, Meritxell[1]. [1]Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada (CICESE). Ensenada, Baja California-Mexico. [2]Department of Molecular, Cell & Developmental Biology, University of California, Santa Cruz, California, USA.

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How are vacuoles formed in *Neurospora crassa*? The role of Rab GTPases.

Barry Bowman, Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz CA, 95064

The lysosomal compartments in eucaryotic cells are acidified by a proton pump, the vacuolar ATPase. In *N. crassa*, the vacuolar ATPase is very abundant in a previously undescribed organelle found near the hyphal tip. This organelle is typically spherical with a diameter of 2-3 mm. Surprisingly, it does not appear to be acidified (fails to accumulate carboxy-CFFDA). It also can rapidly change shape, forming an elongated tube that appears to bleb off small vesicles. We are exploring the hypothesis that this organelle in involved in the biogenesis of the vacuole. Delivery of proteins to organelles is partly mediated by Rab GTPases. We have constructed strains of *N. crassa* which have Rab proteins fused to GFP or RFP (Rab 1, 2, 4, 5, 7, 8, and 11). The Rab proteins that are predicted to traffic to vacuoles, such as Rab 7, are also found in the organelles with high concentration of vacuolar ATPase. However, the Rab proteins show a complex pattern of distribution – different vesicles contain different relative amounts of Rab proteins. Vesicle trafficking and formation of vacuoles may be a highly dynamic process. Vesicles may be very heterogeneous in protein composition and somewhat promiscuous in fusion with other vesicles.

Regulation of female development in *Neurospora crassa*.

Stephen J. Free and Jennifer Chinnici. SUNY University at Buffalo, Department of Biological Sciences, Buffalo, NY 14260.

By screening the single gene deletion library, and using co-segregation, complementation and RIP experiments, we have identified a number of genes that are required for female development in the filamentous fungus *Neurospora crassa*. The deletion mutants were characterized phenotypically and with perithecia grafting experiments. The genes required for female development could be assigned into four general groups: 1) Signal transduction pathway genes. We find that several different signal transduction pathways are required to regulate female development. All three MAP kinase gene pathways are required as well as a pheromone activated pathway and the PACC signal transduction pathway. 2) Transcription factor genes. Several transcription factors were found to be required to regulate female development. 3) Anastomosis and autophagy genes. Autophagy and cell-to-cell fusion were found to be required for female development, and the grafting experiments demonstrated that these genes are required to mobilize and move resources from the vegetative hyphal network into the developing perithecia to support female development. 4) Other miscellaneous genes. The focus of the presentation is on the PACC pathway. Deletion mutants for the *pal-A*, *pal-B*, *pal-C*, *pal-F*, and *pal-H* were found to be defective in female development. Characterization of a *pac-C*^{RIP} mutant demonstrated that *pac-C* is also required for female development. The PAC-C pathway mutants are defective in two aspects of female development. First, they are unable to generate protoperithecia in response to environmental cues, but they can form protoperithecia in response to the nearby presence of a strain of the opposite mating type. Second, the fertilized protoperithecia from a PAC-C pathway mutant is unable to complete female development and is unable to produce asci. Protein localization experiments with GFP-tagged PAL-A constructs show that the *N. crassa* PAC-C pathway includes a signaling complex associated with the ESCRT complex. Thus, the *N. crassa* PAC-C signal transduction pathway is similar to the PacC/Rim101 pathway previously characterized as regulating pH-dependent activities in *Saccharomyces cerevisiae* and *Aspergillus nidulans*., but in *N. crassa*, the pathway plays a key role in inducing female development in response to environmental cues

Comparative transcriptomics, functional analysis and systems biology of perithecial development.
Zheng Wang, Francesc Lopez-Giraldez, Nina Lehr, Marta Farré, Ralph Common, Frances Trail and Jeffrey P. Townsend

Neurospora can serve as a tractable model for understanding genetic basis of sexual development in multicellular organisms. Applying a reverse-genetic approach to advance such a model, we used random and multitargeted primers to assay gene expression across perithecial development in *Neurospora crassa*. We found that functionally unclassified proteins accounted for most upregulated genes, whereas downregulated genes were enriched for diverse functions. Moreover, genes associated with developmental traits exhibited stage-specific peaks of expression. For instance, expression of a gene encoding a protein similar to zinc finger, *stc1*, was highly upregulated early in perithecial development, and a strain with a knockout of this gene exhibited arrest at the same developmental stage. A similar expression pattern was observed for genes in RNA silencing and signaling pathways, and we found that strains with knockouts of these genes were also arrested at stages of perithecial development that paralleled their peak in expression. The observed stage specificity allowed us to correlate expression upregulation and developmental progression and to identify regulators of sexual development. Bayesian networks inferred from our expression data revealed previously known and new putative interactions between RNA silencing genes and pathways, and provide a powerful approach for continued systems biological analysis of development. Overall, our analysis provides a fine-scale transcriptomic landscape and novel inferences regarding the control of the multistage development process of sexual crossing and fruiting body development in *N. crassa*.

Efficient detection of unpaired DNA during meiosis requires a component of the homologous recombination machinery.
Pegan Sauls[1], Dilini Samarajeewa[1], Jay Pyle[1], Kevin Sharp[1], Zach Smith[1], Morgan McCall[1], Hua Xiao[2], Patrick Shiu[2], Kevin Edwards[1], Erik Larson[1], and Tom Hammond[1]. [1]School of Biological Sciences, Illinois State University, Normal, Illinois, 61790. [2]Division of Biological Sciences, University of Missouri, Columbia, Missouri, 65211.

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Asexual sporulation in Neurospora and conservation and divergence of development pathways in fungi.
Daniel Ebbole, Shengli Ding, Brian Shaw, Heather Wilkinson, Dawoon Chung, and Charles Greenwald. Texas A&M University, College Station, Texas.

The genetic pathways leading to macroconidia formation in Neurospora and conidia production of Aspergillus have served as model systems for asexual sporulation in fungi. The morphological differences between these two models suggest that they are not homologous pathways. However, the flbC orthologous transcription factor activates conidiation in both fungi and the medA orthologs play critical roles in conidophore development. This talk will review our latest work to define the pathway leading from activation of macroconidiation to the activation of cell wall degrading enzymes essential for cleavage of interconidial cell walls to allow for conidial separation. This pathway will be compared to asexual development pathways across the Ascomycota and a discussion of the origins of macro- and microconidiation in Neurospora and other fungi.

The dynamic mode-of-action of antifungal peptides
Muñoz, A.[1], Alexander, A.[1], Zhao, C.[1], Vendrell[2], M., Marcos, J.F.[2], Read, N.D.[1]. [1] Manchester Fungal Infection Group, Institute of Inflammation and Repair, University of Manchester, Fungal Cell Biology Group, University of Edinburgh, Edinburgh, UK. [2]Centre for Inflammation Research, Queens Medical Research Institute, Little France, Edinburgh EH16 4T, UK. [3]Food Science Department, Instituto de Agroquímica y Tecnología de Alimentos (IATA) - CSIC, Valencia, Spain

Few drugs are available to treat life-threatening fungal infections, and resistance against these drugs is rising. Synthetic antifungal peptides (AFPs) are being actively explored as novel pharmaceuticals. We are investigating the mode-of-action of various small rationally designed peptides. For this purpose have used the fungal model *Neurospora crassa* combined with live-cell imaging of fluorescently labelled AFPs and other live-cell probes, inhibitor treatments, mutant analyses and the synthesis of peptides with different fluorescent labels. PAF26 is a *de novo*-designed hexapeptide possessing two well-defined motifs: N-terminal cationic and C-terminal hydrophobic regions. We have characterized how each motif is responsible for PAF26's dynamic antifungal mechanism of action involving the electrostatic interaction with cells, cellular internalization, and cell killing. PAF26 increases cytosolic free Ca^{2+} ($[Ca^{2+}]_c$) and several Ca^{2+} signalling/homeostatic mutants are resistant to the AFP. Our results provide new mechanistic insights into the mode-of-action of AFPs that should help us design new synthetic AFP-based drugs with improved activity and stability.

Mechanisms of programmed cell death in Neurospora.

Arnaldo Videira, Instituto de Biologia Molecular e Celular, Porto, Portugal

Neurospora crassa undergoes programmed cell death (PCD) as a result of heterokaryon incompatibility or following treatment with different substances. We have been exposing the fungus to the sphingolipid phytosphingosine or the kinase inhibitor staurosporine, which reduce viability and induce ROS production, glutathione efflux and DNA condensation and fragmentation, hallmarks of PCD. Respiratory chain (complex I) mutants are more resistant to phytosphingosine than the wild type strain. The analysis of the transcription profile of phytosphingosine-treated cells indicates that a down regulation of genes encoding mitochondrial proteins may be a major effect of the drug and further confirms the mitochondrial involvement in phytosphingosine-induced cell death.
A specific staurosporine transporter of the ABC type was identified following a transcriptomic analysis of drug-treated cells. The protein is highly induced upon *N. crassa* exposure to staurosporine and its absence renders cells hypersensitive to the drug. Staurosporine also provokes defined alterations in intracellular calcium in a process mediated by the phospholipase C signaling pathway that includes calcium influx and its mobilization from and to internal stores. In agreement, staurosporine sensitivity is altered in deletion mutants lacking proteins implicated in calcium handling.
Modulation (enhancement) of PCD can be achieved by treating cells with a combination of death inducers with drugs targeting specific proteins involved in the death process that can be identified with genomic approaches. Those combinations also affect other fungi and human cells, indicating their anti-fungal and anti-tumor potential.
Supported by Fundação para a Ciência e Tecnologia (FCT) and Fundação Luso-Americana para o Desenvolvimento (FLAD).

K^+ regulation in *Neurospora*: Three proteins do it all

Alberto Rivetta[1], Kenneth Allen[2], and Clifford Slayman[1]. [1]Department of Cellular and Molecular Physiology, and [2]Department of Genetics, Yale School of Medicine, New Haven, CT 06520 U.S.A.

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G-proteins and post-transcriptional regulation.

Alexander Michkov, Shouqiang Ouyang, Arit Ghosh and Katherine Borkovich. Department of Plant Pathology and Microbiology. University of California, Riverside

Heterotrimeric G proteins consist of α, β and γ subunits. We have previously characterized three Gα subunits (GNA-1, GNA-2 and GNA-3), one Gβ (GNB-1) and one Gγ (GNG-1) subunit in *Neurospora crassa*. Interestingly, Δgnb-1 and Δgna-1 mutants share some common defects, which may be explained by the reduced GNA-1 protein levels observed in the Δgnb-1 mutant. Previous studies in our laboratory showed that levels of gna-1 mRNA are similar in wild type and the gnb-1 mutant, consistent with a post-transcriptional mechanism. Results from ribosomal profiling experiments demonstrate that loss of gnb-1 influences both global and gna-1 mRNA association with polysomes, most likely through inhibition of translational initiation. Experiments are in progress to determine the mechanism underlying this control.

MSN-1 is a transcription factor regulated by MAK-1 MAP kinase in *Neurospora crassa*.

Masayuki Kamei, Masakazu Takahashi, Akihiko Ichiishi, and Makoto Fujimura. Faculty of Life Sciences, Toyo University, Gunma, Japan

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eRF1 and eIF4A3 mRNA stability in *Neurospora* is controlled by NMD, EJC, and CBP factors through 3'UTR introns.

Ying Zhang and Matthew S. Sachs, Department of Biology, Texas A&M University, College Station, TX 77843

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Perkins Award Lecture

Chromosome conformation capture followed by high-throughput sequencing reveals associations between chromatin domains and loss of heterochromatin associations in *dim-5* and *hpo*

Jonathan M. Galazka and Michael Freitag. Department of Biochemistry and Biophysics, Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331

Eukaryotic genomes are organized into distinct chromatin domains that generate specific three-dimensional arrangements within the nucleus. These arrangements result from the interplay between basic physical forces and the action of protein factors, but the molecular details of these forces and interactions are still poorly understood. Some regions of the Neurospora genome are organized into dense heterochromatic foci containing trimethylated H3 lysine 9 (H3K9me3). H3K9me3 is generated by the DIM-5 methyltransferase and recognized by Heterochromatin Protein 1 (HP1), which aids in condensation of chromosome regions into heterochromatic domains. We hypothesized that intra- and interchromosomal heterochromatic domains physically associate into foci and that these associations help order the remainder of the genome. Furthermore, we hypothesized that the elimination of heterochromatin by the deletion of *dim-5* or *hpo* would result in loss of heterochromatic foci and thus physical re-structuring of the genome. Here we compare the three-dimensional structure of the WT Neurospora genome to that of *dim-5* and *hpo* deletion strains by application of chromosome conformation capture (3C) followed by high-throughput Illumina sequencing, also called "HiC". HiC relies on the ligation of physically proximal regions of genomic DNA and allows the construction of a high-resolution and systematic three-dimensional model of the genome. Though still limited by sequencing depth, our preliminary data show the association of heterochromatin domains and the loss of some associations in the *dim-5* and *hpo* deletion strains.

CRF5 is required for DNA repair in Neurospora.

Evelina Y. Basenko and Zachary A. Lewis. Department of Microbiology, University of Georgia, Athens, GA 30602

Many DNA repair mechanisms involve regulators of chromatin structure. To identify new genes involved in DNA repair or other aspects of the DNA damage response, we screened the Neurospora knockout collection for MMS (methyl methanesulfonate)-sensitive deletion strains. This approach uncovered over 200 MMS-sensitive strains, including many that lack putative regulators of chromatin structure. We characterized strains lacking *crf5 (chromatin remodeling factor 5),* a member of the SNF2 family of ATP-dependent chromatin remodeling enzymes. *crf5* mutants exhibit hypersensitivity to MMS and to the topoisomerase I inhibitor camptothecin, but are not hypersensitive to UV light or bleomycin. *crf5; mei-3* double mutants exhibit synthetic growth defects, even in the absence of DNA damage. These data suggest that CRF5 and MEI-3 have complementary functions. Exposure to MMS triggers post-translational modification of CRF5, which is correlated with an increase in nuclear abundance of CRF5 and changes in CRF5's nuclear localization pattern. We used a proteomics approach to identify CRF5-interacting proteins. Our data suggest that CRF5 interacts with a conserved WD40 domain protein to facilitate proper DNA repair in Neurospora. Our ongoing genetic and molecular analyses of CRF5 and its interacting proteins will provide additional insights into the role of this putative ATP-dependent chromatin remodeler in the Neurospora DNA damage response.

The relationship between mutagen sensitivity and life span

Shin Hatakeyama, Laboratory of Genetics, Faculty of Science, Saitama University, JAPAN

To uncover the mechanism of the maintenance of the genome integrity, we've been isolated mutants showing mutagen sensitivity based on they might be deficient in repair function of damaged DNA. Many *Neurospora* mutants, *i.e. uvs*- (ultraviolet sensitive-), *mus*- (mutagen sensitive-), *mei*- (meiosis), and *upr*- (ultraviolet photoreactivation), were isolated. Indeed, as we've predicted, most mutants had deficiencies in their responsible genes which included in each of DNA repair systems, *i.e.* nucleotide excision repair, alternative nucleotide excision repair, homologous recombination repair, translesion synthesis, checkpoint control, and so on. Still, there are some mutagen sensitive mutants where the responsible genes do not belong to any known DNA repair system. Among them, some mutants show not only mutagen sensitivities, but also short life span. For example, the *mus-10* and *uvs-5* strains show sensitivities to MMS (methyl methanesulfonate) and UV, also stop hyphal growth within approximately three weeks. Interestingly, in contrast that mitochondrial feature of wild-type strain looks like tubular shape, these mutants harbor fragmented mitochondria. Since their mutations identified as defects in genes which function in mitochondrial fusion, we discussed that short life span might be caused by imbalance of mitochondrial fusion and fission. However, the relationship between mutagen sensitivity and senescent phenotype is left to be resolved. Furthermore, there are another strains show senescent phenotype such as *nd*, *sen* and *uvs-4*, which also show mutagen sensitivity. Through resolving the correlation among the mutagen sensitivity, the short life span and the mitochondrial morphology of these mutants, part of the mechanism of the cellular aging may be clear.

Mechanism of quelling and siRNA production in Neurospora.

Qiuying Yang, Yi Liu. Department of Physiology, University of Texas Southwestern Medical Center, Texas, USA

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Molecular dissection of meiotic silencing by unpaired DNA

Logan M. Decker[1], Thomas M. Hammond[2], Hua Xiao[1], Erin C. Boone[1], Lindsay A. Reustle[1], David G. Rehard[1], Seung A. Lee[1], Tony D. Perdue[3], Patricia J. Pukkila[3], and Patrick K. T. Shiu[1]. [1]Division of Biological Sciences, University of Missouri, Columbia, MO; [2]School of Biological Sciences, Illinois State University, Normal, IL; [3]Department of Biology, University of North Carolina, Chapel Hill, NC.

In *Neurospora crassa*, genes unpaired during meiosis are transiently silenced by a process called meiotic silencing by unpaired DNA (MSUD). It is one of several surveillance mechanisms in place to protect the genome integrity of the fungus. MSUD requires common RNAi factors, including SAD-1, an RNA-directed RNA polymerase that converts aberrant RNAs into double strands; SAD-3, a helicase thought to increase SAD-1's processivity on RNA templates; DCL-1, a Dicer protein that cleaves a dsRNA into siRNAs; QIP, an exonuclease that turns siRNA duplexes into single strands; and SMS-2, an Argonaute protein that destroys complementary mRNAs. All these proteins, possibly with the help of SAD-2, form a silencing complex in the perinuclear region and it inspects/processes RNA molecules as they depart the nucleus. We will discuss our current understanding of these and other silencing proteins and how they interact to form an intricate defense mechanism.

Does sequence heterozygosity underlie suppression of meiotic silencing by wild Neurospora strains? Does Neurospora contain nucleus-limited genes?

Durgadas P. Kasbekar, CDFD, Hyderabad 500001, India (kas@cdfd.org.in)

Bichpuri-1 *a* (B) and Spurger *A* (S) are among the four wild-isolated *N. crassa* strains identified whose crosses with the OR-derived testers *::Bml*^{r} and *::mei-3* showed suppression of meiotic silencing of the *bml* and *mei-3* genes, and typified the "Sad type" wild strains. Crosses of 68 other wild strains with the testers showed suppression of silencing in *mei-3*, but not *bml*, and typified the intermediate "Esm type". Only eight wild strains behaved like OR and showed silencing in crosses with both the testers. They were the "OR" type. A B x S cross followed by 10 generations of sibling crosses was used to create isogenic *mat A* and *mat a* strains in the B/S background, and to make new *mei-3* testers. If a tester- heterozygous cross in this background shows meiotic silencing, it would support our hypothesis that the Sad or Esm phenotype arises from heterozygosity for sequence polymorphism in the cross with the OR-derived testers. The polymorphisms might reduce pairing and silence meiotic silencing genes.
Additionally, we are introgressing *N. crassa* translocations into *N. tetrasperma* to produce self-fertile [(*T*) + (*N*)] strains that should generate [(*T*) + (*N*)] and [(*Dp*) + (*Df*)] progeny. If any [(*Dp*) + (*Df*)] is less self-fertile than the corresponding [(*T*) + (*N*)], it would provide evidence for "nucleus-limited" genes, i.e., where the wild-type allele (*WT*) fails to complement a null allele (Δ) in a [(*WT*) + (Δ)] heterokaryon. Such genes have not yet been found, but they are predicted by the putative nucleus-limited phenotype of the *N. crassa scon*^{c} mutant (Butler and Metzenberg 1972 J Bacteriol. 109:140-51), and the MatIS process in *Aspergillus nidulans* (Czaja *et al.* 2013 Genetics 193:1149-1162).

Perkins Award Lecture

Neurospora importin-α (Nucleoporin-6) directs chromatin modifying complexes to sub-nuclear chromatin targets

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The silenced genomic material of heterochromatin plays essential roles in development, chromosome function, and genome defense. In many species, including *Neurospora crassa*, heterochromatin is decorated by methylation of Lysine 9 of histone H3 (H3K9me3) and by cytosine methylation. In Neurospora, a five protein complex named DCDC is responsible for H3K9me3, which in turn directs DNA methylation. Here, we identify and characterize a gene that is important for DCDC function, namely ***d***efective ***i***n ***m***ethylation-3 (***dim-3***). *Dim-3* encodes the nuclear import chaperone *nup-6*. Our *dim-3* mutant, whose critical defect is in an ARM repeat of NUP-6, has a substantial, but incomplete, loss of DNA methylation and H3K9me3. Surprisingly, nuclear transport of all known proteins involved in histone and DNA methylation appears normal in *dim-3* strains. DCDC complex formation also seems normal, but the *nup-6*$^{dim-3}$ allele causes the DCDC members DIM-5 and DIM-7 to be mislocalized from heterochromatin. Interestingly, NUP-6^{dim-3} is displaced from the nuclear membrane, potentially causing DCDC mislocalization. Surprisingly, the histone acetyltransferase GCN-5 also has an altered localization in a *dim-3* strain, raising the possibility that NUP-6^{dim-3} targets multiple chromatin complexes. We suggest that NUP-6 directs chromatin complexes to their genomic targets at the nuclear membrane upon nucleocytoplasmic transport.

Beadle and Tatum Award Lecture

Centromere structure in the filamentous fungi: DNA sequence, protein complement and phylogenomics

Kristina M. Smith, Pallavi Phatale, Jonathan M. Galazka, Lanelle R. Connolly, Steven Friedman, and Michael Freitag. Department of Biochemistry and Biophysics, Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331

Centromere assembly and inheritance are dynamic and organism-specific. Protein complexes that are part of the kinetochore contain highly conserved signature proteins, yet other proteins are highly divergent even between strains within one taxon. These findings suggest the existence of both conserved and divergent protein interactions during centromere and kinetochore assembly and maintenance. We study the centromere-specific histone H3 (CENP-A/CenH3), which forms an alternative nucleosome and serves as a platform for centromere assembly. CenH3 proteins contain hypervariable N-terminal tails and conserved histone fold domains (HFD). C-terminally tagged *Podospora anserina* CenH3 (PaCenH3-GFP) can complement the lack of Neurospora CenH3 (NcCenH3) in mitosis and meiosis. No drastic growth defects were observed after performing race tube assay on progeny from homozygous crosses with PaCenH3-GFP. Progeny at expected ratios were obtained from PaCenH3-GFP crosses but ChIP-seq revealed reduced maintenance of CenH3 protein at centromeric regions after even a single meiosis in most progeny. To analyze the molecular defect, we carried out domain swapping experiments and generated proteins with selected CenH3 point mutations. Only a few amino acids within the HFD appear crucial during meiosis. We also addressed the possibility that centromeric DNA sequences mutate more rapidly than has been previously assumed by characterizing the centromeric DNA of *Neurospora*, *Fusarium* and *Zymoseptoria* species.

We hypothesize that DNA-binding regions of centromere proteins evolve rapidly and adaptively to balance mutation of the underlying DNA sequence.

Conserved RNA helicase FRH acts Nonenzymatically to Support the Intrinsically Disordered Neurospora clock protein FRQ.

Jennifer M. Hurley[1], Luis F. Larrondo[2], Jennifer J. Loros[1,3], Jay C. Dunlap[1*1] Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH 03755, USA [2]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile [3] Department of Biochemistry, Geisel School of Medicine at Dartmouth, Hanover, NH 03755, USA

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The *Neurospora crassa* Transcription Factor ADV-1 is a Component of the Circadian Output Pathway.

Rigzin Dekhang[1], Cheng Wu[1], Erin L. Bredeweg[2], Kristina Smith[2], Matthew Peterson[3], Jillian Emerson[4], Jay Dunlap[4], Michael Freitag[2], James Galagan[3], Matthew Sachs[1], and Deborah Bell-Pedersen[1]. [1]Department of Biology, Texas A&M University, College Station, TX. [2]Department of Biochemistry and Biophysics, Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR. [3]Department of Biomedical Engineering and Microbiology, Boston University, Boston, MA. [4] Departments of Genetics and Biochemistry, Dartmouth Medical School, Hanover, NH.

Circadian clocks confer advantages to organisms by helping them anticipate daily changes in their environment. The core oscillator, in Neurospora, called the FRQ/WCC oscillator (FWO), functions as a negative feedback loop, analogous to circadian oscillators in higher eukaryotes. However, little is understood about how the circadian oscillator signals through output pathways to control overt rhythms in any system. The White Collar complex (WCC), a complex of two GATA-type transcription factors, is a core component of the FWO. Using ChIP-seq, we demonstrated that light-activated WCC directly binds to the promoters of about 200 target genes, including 24 transcription factors (TFs) (Smith, et al. 2010). These TFs potentially regulate the expression of second tier clock outputs. To test this hypothesis, we examined the circadian phenotypes of strains deleted for each of the 24 TFs, and found that deletion of ADV-1 abolishes circadian rhythms in spore development. In addition, *adv-1* mRNA and ADV-1 protein accumulate with a circadian rhythm that is dependent on the FWO. Furthermore, deletion of ADV-1 has no effect on the function of the FWO. Together, these data suggested that ADV-1 functions within an output pathway from the clock, and regulates rhythms in downstream target genes. To elucidate the pathways regulated by ADV-1, we used ChIP-seq to determine the direct ADV-1 targets, and RNA-seq in WT and ΔADV-1 strains to identify genes controlled by ADV-1. We found about 30% of the ADV-1 targets are predicted clock-controlled genes. In addition, the ADV-1 targets were enriched for genes involved in both sexual and vegetative development. Consistent with these data, we discovered that several of these genes are clock-controlled, and require ADV-1 for their rhythmicity.

Smith, K. M., et al. (2010). "Transcription factors in light and circadian clock signaling networks revealed by genomewide mapping of direct targets for neurospora white collar complex." Eukaryot Cell **9**(10): 1549-1556.

Chromatin in circadian and light regulated gene expression

William J. Belden, Department of Animal Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901

The circadian oscillator controls time-of-day gene expression by a network of interconnected feedback loops and is reset by light. The requisite for chromatin regulation in eukaryotic transcription necessitates temporal regulation of histone-modifying and chromatin-remodeling enzymes for proper clock and light-activated gene expression. In *Neurospora* strains lacking the ATP-dependent chromatin-remodeling enzyme CHD1, there is an increase in the amount of DNA methylation at the circadian-regulated and light-activated *frequency* locus. Removal of Lysine Methyltransferase 1 (KMT1), DIM-5, that adds methyl groups to histone H3 lysine 9 (H3K9me1/2/3) or KMT2, SET1 that catalyzes H3K4me1/2/3 both are devoid of DNA methylation at *frq* and suppress the hypermethylation phenotype in *chd1* deletion strains. Loss of either SET1 and/or DIM-5 affects circadian and light-regulated expression. H3K9me3 and H3K4me3 occur at the *frq* and peak approximately 30 minutes after light-activated expression. The data support a model of facultative heterochromatin at *frq* where SET1 is needed for DIM-5 recruitment or it functions as an anti-silencing factor.

Suppression of WC-independent *frequency* transcription by RCO-1 is essential for *Neurospora* circadian clock.

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Rhythmic activation and repression of clock gene transcription is essential for the functions of eukaryotic circadian clocks. In the circadian oscillator of *Neurospora*, *frequency* (*frq*) transcription requires the WHITE COLLAR (WC) complex, and *frq* mRNA level is extremely low in *wc* mutants. Here we show that the transcriptional repressor RCO-1 is an essential circadian clock component by regulating *frq* transcription. In *rco-1* mutants, both overt and molecular rhythms are abolished, and *frq* mRNA levels are constantly high. Surprisingly, *frq* mRNA levels were also constantly high in the *rco-1 wc* double mutant. In addition, WC complex binding to the *frq* promoter is dramatically reduced in the *rco-1* single mutant despite high WC levels. These results indicate that RCO-1 regulates *frq* transcription by suppressing WC-independent transcription and by promoting the binding of WC complex to the *frq* promoter to permit rhythmic activation of *frq*. Furthermore, we found that RCO-1 is required for maintenance of the normal chromatin structure at the *frq* locus and that RCO-1 acts together with the chromatin remodeling factor CHD-1 and histone H3K36 methyltransferase SET-2 in suppressing WC-independent *frq* transcription. Together, our results uncover a previously unexpected regulatory mechanism for clock gene transcription.

Connection between glycogen metabolism regulation, light and the circadian clock in *Neurospora crassa*

Stela Virgilio[1], Oneida Ibarra[2], Ana Carolina Boni[1], Deborah Bell-Pedersen[2], and Maria Célia Bertolini[1]. [1]Instituto de Química, UNESP, Departamento de Bioquímica e Tecnologia Química, UNESP, Araraquara, SP, Brazil. [2]Department of Biology, Texas A&M University, College Station, TX, USA. stela.virgilio@gmail.com

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An RNA-Seq study of circadian and non-circadian responses to light and temperature in Neurospora.

Yamini Arthanari[1], Gareth Muirhead[1], Suzanne Hunt[1], Seona Thompson[1], Gustavo Antonio Urruzia[2], Sue Crosthwaite[1], Christian Heintzen[1]. [1]Faculty of Life Sciences, The University of Manchester, Oxford Road, Manchester, M13 9PT, United Kingdom, [2]Max Planck Institute of Immunology and Epigenetics, Stübeweg 51, D-79108 Freiburg, Germany

Circadian clocks are molecular oscillators that enable organisms to adapt to and predict daily rhythms in the environment. In *Neurospora crassa* the circadian clock is centred on the *frequency* and *white-collar* genes whose products establish a circuitry of positive and negative feedback interactions known as the circadian FRQ-White Collar Oscillator (FWO). The FWO generates molecular rhythms, whose approximately 22-hour periodicity can be entrained to the 24-hour rhythms generated by the daily light and temperature fluctuations. To begin to identify genes that participate in clock, light and temperature signalling pathways, we used high-throughput sequencing of RNA (RNA-Seq) to compare transcriptome profiles of wild-type and FWO knockout strains in different light and temperature environments. We will report on the identification of novel clock, light and temperature controlled transcripts and present data that suggest a central role for one of the identified genes.

Local adaptation of the Neurospora circadian clock.

Kwangwon Lee, Rutgers University, Department of Biology, Center for Computational and Integrative Biology, Camden, NJ 08102

Cellular 24-hr clocks, collectively called circadian clocks, provide crucial time information to an organism for the survival in nature. The molecular mechanisms of the eukaryotic cellular clock have been successfully characterized in the model species *Neurospora crassa*. Furthermore, the large collection of natural isolates of Neurospora from all over the world makes Neurospora an ideal system to study molecular mechanisms of natural variation of circadian rhythms. In the previous study, we have demonstrated how molecular variations of known clock genes products play roles in the circadian 'period' variation of the natural strains. In the current study, we tested on the roles of 'entrainment' as a fitness trait using the natural isolates of *N. discreta* that are collected from different latitudes and from different continents. Our data shows that *N. discreta* has an authentic circadian oscillator that is similar to that of *N. crassa*. We also found that there are continent-specific photic-entrainment phenotypes among *N. discreta* PS4B (phylogenetic species 4, group B), whose genotype is identified from the strains collected all around the world. By whole genome association studies, we identified list candidate genes that might contribute to the entrainment phenotype. To identify novel molecular component for the variation of the entrainment phenotype, we created a mapping population by crossing strains between North American and African. As expected the identified photic-entrainment phenotype was polygenic trait. By association study, we identified few candidate genes. I will discuss the current understanding of the roles of local adaption of photic-entrainment of circadian rhythms.

Mathematical modeling of circadian rhythms in *Neurospora crassa.*

Christian Hong, Department of Molecular & Cellular Physiology, University of Cincinnati

Autonomous circadian oscillations arise from transcriptional-translational feedback loops of core clock components. The period of circadian oscillator is relatively insensitive to changes in physiological temperature and nutrients (e.g. glucose), which are referred to as temperature and nutrient compensation, respectively. Recently, a transcription repressor, CSP-1, was identified as one of the circadian components in Neurospora crassa. The transcription of csp-1 is under the circadian regulation. Intriguingly, CSP-1 represses a circadian transcription factor, WC-1, forming another negative feedback loop. This feedback mechanism is suggested to maintain the circadian period in a wide range of glucose concentrations. We constructed a mathematical model of the Neurospora circadian clock incorporating the above WC-1/CSP-1 feedback loop, and investigated molecular mechanisms of glucose and temperature compensation.

Sunday, March 9th

Modeling the Neurospora Clock Regulatory Network.
James Galagan, Boston University.

The Neurospora core circadian clock component WCC directly regulates over 200 genes including many transcription factors. These clock-controlled regulators are expected, in turn, to control additional downstream genes and therefore transmit and perhaps modulate the sinusoidal signal of the core clock. As part of the Neurospora PO1 consortium, we have analyzed ChIP-Seq and corresponding transcriptomic data for over a dozen clock-controlled regulators. The data reveal that these regulators potentially form a highly interconnected network of regulatory interactions whose functions are largely unknown. In this presentation we will present an overview of our analysis of the ChIP-Seq data, the topography of the regulatory network implied by these data, and results from our ongoing efforts to develop a predictive computational model of the clock controlled regulatory network based on these data.

A resequencing journey through *Neurospora* classical mutant strains. Scott E. Baker[1,2] and Kevin McCluskey[3]. [1]DOE Joint BioEnergy Insitute and [2]DOE Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA and [3]Fungal Genetics Stock Center, University of Missouri, Kansas City, Kansas, City MO

Despite the explosion of genomic and reverse genetic resources for *Neurospora crassa*, a significant number of mutant phenotypes originally defined by "classical" genetic and biochemical methods remain anonymous, i.e. they are not associated with physical loci. The DOE Joint BioEnergy Institute Fungal Biotechnology Team is interested in understanding the biological pathways involved in the secretion of heterologous expressed biomass deconstruction enzymes. We expect that genes whose products are involved in biological processes that include protein translation, cell morphology, central metabolism and protein secretion may also impact heterologous gene expression and secretion. There are several hundred "anonymous" mutant strains of *N. crassa* whose phenotypes impact biological processes of interest. We are working with the Joint Genome Institute to resequence 600 mutant *N. crassa* strains from the Fungal Genetics Stock Center. Currently, we have sequenced over 400 mutant strains of *N. crassa*. In most cases, we are able to identify strong candidate mutations based on genetic mapping data. We will highlight a subset of our results, relating them to the existing genetic and biochemical data.

Adaptive introgression slows down molecular degeneration of the mating-type chromosome in *Neurospora tetrasperma*
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In this study, we used a population genomics approach to understand the roles that selection, recombination and introgression have played in shaping the evolutionary history of the filamentous ascomycete *Neurospora tetrasperma***.** We sequenced the genomes of 92 strains of *N. tetrasperma* and the genomes of two heterothallic species of Neurospora (*N. hispaniola* and *N. sitophila*). With this data, together with publicly available datasets, we resolved the relationship of *N. tetrasperma* to other Neurospora species and the relationship of lineages within the *N. tetrasperma* clade. Comparisons of *N. tetrasperma* genomes revealed large regions of suppressed recombination on the mating-type chromosomes of all lineages of *N. tetrasperma*, that cover over 80% of the chromosome in some lineages. We used population genomic analyses to compare the evolutionary history of the recombining and the recently evolved non-recombining regions and found that suppressed recombination of the mating-type chromosome in *N. tetrasperma* has resulted in a decrease in genetic diversity and a higher rate of molecular degeneration relative to recombining regions of the genome. We also found that introgression has been a common occurrence in the region of suppressed recombination in multiple lineages of *N. tetrasperma*. These introgressed regions on the mating-type chromosome have become fixed in *N. tetrasperma* lineages and have likely been driven to fixation by selective sweeps. We hypothesize that the fixation of these introgressed regions from heterothallic species has resulted in a temporary regeneration of degenerated regions of the mating-type chromosomes in *N. tetrasperma*.

Speciation genomics of Neurospora discreta

Pierre Gladieux, Christopher Hann-Soden, David Kowbel, John Taylor. Department of Plant and Microbial Biology, University of California, Berkeley

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Fusion and the laboratory evolution of selfish nuclei.

Eric Bastiaans, Wageningen University, The Netherlands

Multicellular organization is a highly cooperative state prone to invasion of selfish genotypes (cheaters) that use the resources provided by the multicellular organism without contributing their fair share to non-reproductive functions of the organism (e.g. cancers). Kin selection, often realised through regular single -celled bottlenecks (and in some organisms by an early germline separation), is a solution to prevent selection for cheating. In fungi, the lack of an early germline separation and the potential to fuse with other individuals make cheating a realistic threat. However the heterokaryon incompatibility recognition mechanism limits fusion to almost only clonally related individuals. Perhaps because of this selfish genotypes are not easily picked up in nature.
In order to test the hypothesis that cheating is a realistic threat to multicellular growth in fungi, we used an experimental evolution approach with *Neurospora crassa* that maximised the potential for cheating genotypes by selecting under low relatedness and completely local competition, i.e. under a high inoculation density of spores, in the absence of heterokaryon incompatibility. Within less than 300 generations all eight replicate lines contained genotypes that matched our criteria for cheating: they had increased relative fitness (measured as proportion of spores produced) when in competition with a cooperative ancestral type, but spore production in monoculture was significantly decreased. So there is a clear trade-off between competitive fitness and production of asexual spores when grown without the ancestral cooperative type.
Contrary to predictions about the evolution of social behaviour that cheating genotypes will completely eradicate the social behaviour (the tragedy of the commons), we found a stable polymorphism in all evolved lines: a relatively cooperative type producing many spores when grown in monoculture, and the cheating type described above. We are currently studying the conditions leading to this apparently balanced polymorphism in our evolving lines.

Nuclear dynamics in Neurospora

Marcus Roper[1], Patrick Hickey[2], Anna Simonin[3], and N. Louise Glass[4]. [1]University of California, Los Angeles, [2]NIPHT Ltd., [3]U. Western Sydney, [4]UC Berkeley

The hyphae of *Neurospora crassa* function as conduits for transporting organelles, nutrients and cytoplasm at speeds of tens and even hundreds of microns per second. I will discuss both the adaptive benefits of this transport for the fungal mycelium, and the how hyphae resolve the tradeoffs between acting as transport channels and performing their other functions, like growth and remodeling. Specifically, I will discuss how live cell imaging and mathematical modeling are revealing (i) the role of rapid nuclear transport in allowing N. crassa to tolerate internal genetic diversity, such as that created by mutations that accumulate when nuclei divide, and (ii) how rapidly growing hyphae alternate between phases of nuclear transport and stasis.

A comparative systems analysis of polysaccharide utilization in *Neurospora crassa* reveals molecular mechanisms of carbon adaptation as well as new factors with impact in biotechnological applications

J. Philipp Benz, N. Louise Glass, John E. Dueber, Chris R. Somerville, Energy Biosciences Institute, University of California Berkeley, Berkeley, CA 94704, USA

The need for a stable and sustainable source of energy is one of the grand challenges of our generation. Using lignocellulosic plant biomass as a renewable feedstock e.g. for biofuel production can contribute substantially on our way to meet this goal. However, for highest efficiency the utilization of all major constituent sugars is required. To this end, enzyme cocktails adapted to the individual composition of given feedstocks would be desirable as well as the engineering of downstream organisms able to ferment all major monosaccharides. In particular fungi are among the most promising tools to deconstruct plant cell wall polysaccharides due to their importance in global carbon recycling, but much remains to be learned about their enzyme production and sugar utilization mechanisms. A more profound knowledge of the fungal interaction with different plant substrates will therefore be highly informative for efforts to achieve a cost-effective biofuel production process.

In the presented work, we performed a systematic analysis of how *Neurospora crassa* perceives the presence of the three major plant-derived polysaccharides cellulose, xylan and pectin. The comparison of the individual responses allowed us to deduce fundamental knowledge on fungal adaptation strategies to the carbon composition in its environment. These included specific adaptations, such as a re-organization of the secretory pathway upon sensing of cellulose, but also commonalities, such as a set of enzymes that is cross-induced on all carbon-sources and could represent part of a polysaccharide scouting system.

Our study moreover revealed new factors involved in specific sugar utilization pathways. Here, we report on the identification and biochemical characterization of the first eukaryotic high-affinity MFS-type transporter for the uptake of galacturonic acid (GAT-1), which cannot be consumed by currently used yeast strains. To test its applicability in biotechnological sugar fermentation processes, GAT-1 was recombinantly expressed in yeast in combination with fungal galacturonic acid metabolism enzymes, to generate a transporter-dependent uptake and catalysis system into downstream products with high potential for utilization as platform chemicals: L-galactonate and *meso*-galactaric acid.

Whole genome sequence underlies a new paradigm for genetics and discovery at culture collections.

Kevin McCluskey[1], Aric Wiest[1], and Scott Baker[2]. [1]Fungal Genetics Stock Center, University of Missouri- Kansas City. [2]DOE Joint BioEnergy Insitute and DOE Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA

Whole genome sequence analysis is being applied to classical mutant strains of *Neurospora crassa* to associate a mutant open reading frame with classically defined and genetically characterized mutations. Already nearly 450 strains representing over 300 different mutant loci are sequenced or in the sequencing pipeline. Among these are 219 loci where a single mutant strain is being sequenced and over 70 loci for which multiple strains or alleles are being studied.

In addition to the target information (the identification of the unique classical mutation associated with each anonymous but genetically defined locus), whole genome sequencing provides a wealth of information about gene and genome diversity. This information can be used in a number of ways. For the study of an individual ORF, the diversity among multiple strains represents a de facto mutant screen, with different alleles demonstrating for each polymorphic position the relative importance of that residue in the associated protein. Similarly, many strains contain secondary mutations, both characterized and unknown, and the nature of these secondary mutations is valuable both with regard to protein sequence diversity, and also in evaluating the role of different loci in in vitro growth versus growth in planta.

Finally, whole genome sequence provides living microbe collections, like the FGSC, an alternate to traditional taxonomic organization. When specific traits are desired for study or exploitation, discovery based on whole genome sequence can leverage gene ontology annotation to leapfrog directly to isolates having desirable characteristics without having to screen many strains lacking the trait of interest.

Poster Abstracts

1) CZT-1 is a novel transcription factor controlling cell death and natural drug resistance in *Neurospora crassa*

A. Pedro Gonçalves (1,2), Charles Hall (3)#, David J. Kowbel (3), N. Louise Glass (3), Arnaldo Videira (1,2)
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Similarly to higher eukaryotes, filamentous fungi undergo programmed cell death upon exposure to chemical stresses. In *Neurospora crassa*, the protein kinase inhibitor staurosporine causes loss of cell viability, chromatin fragmentation, reactive oxygen species (ROS) production, glutathione depletion and induces ABC3, an ATP-binding cassette (ABC)-transporter that exports the drug to the extracellular space. We identified the gene encoded by NCU09974, termed CZT-1 (Cell death-activated Zinc cluster Transcription factor), as an important regulator of staurosporine-induced cell death both in laboratory strains as well as in wild isolates of *N. crassa*, underlining the occurrence of fungal natural drug resistance. Absence of *czt-1* leads to hypersensitivity to staurosporine, altered intracellular calcium dynamics and increased accumulation of ROS. Genomewide RNA sequencing of a Δ*czt-1* strain revealed that this transcription factor regulates the expression of a large number of genes, including components of the mitochondrial respiratory chain and genes related to the activity of the endoplasmic reticulum. Besides ABC3, CZT-1 also controls the expression of three other ABC-transporters, suggesting a role in multidrug resistance. Sequence homology analyses suggest that CZT-1 belongs to a novel and divergent subfamily of zinc cluster transcription factors. Because of the conservation between CZT-1 and uncharacterized proteins from human and crop pathogens it will be interesting to extend the characterization of these molecules.

2) The interplay between calcium, mitochondrial respiration and intracellular signaling during cell death

A. Pedro Gonçalves (1,2), J. Miguel Cordeiro (2), João Monteiro (1), N. Louise Glass (3), Nick D. Read (4), Arnaldo Videira (1,2) (1) IBMC - Instituto de Biologia Molecular e Celular - Universidade do Porto, Rua do Campo Alegre 823, 4150-180 Porto, Portugal (2) ICBAS - Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal (3) Plant and Microbial Biology Department, The University of California, Berkeley, 341 Koshland Hall, 94720, Berkeley, CA, USA (4) Manchester Fungal Infection Group, Institute of Inflammation and Repair, University of Manchester, Manchester M13 9NT, UK

Fungal cells respond to cell death stimuli with transient increases in the intracellular concentration of calcium (Ca2+) which work as signaling messages that determine cell survival. When exposed to the alkaloid cell death inducer staurosporine, *Neurospora crassa* cells trigger a signature response in the cytosolic levels of Ca2+ that involves extracellular uptake as well as release from storage organelles. A genetic and pharmacological approach revealed a novel Ca2+ uptake system in fungi that is involved in the Ca2+ response to staurosporine and composed by a transient receptor potential (TRP)-like channel. The recruitment of Ca2+ from intracellular storages is mediated by inositol-1,4,5-trisphosphate (IP3). We identified PLC-2, one of the four phospholipase C proteins in *N. crassa*, as a pivotal player in the regulation of staurosporine-induced cell death. Normal activity of the mitochondrial respiratory chain and the production of reactive oxygen species are required for the fungal response to the drug. At least two subunits of the mitochondrial complex I and a Ca2+-binding alternative NAD(P)H dehydrogenase seem to be involved in the development of the Ca2+ signature profile promoted by staurosporine. A genome-wide analysis of the response to the drug revealed distinct transcriptional programs in cells growing with different availabilities of extracellular Ca2+.

3) Understanding the molecular regulation of alternative oxidase in *Neurospora crassa*

Nishka Kishore, Kelly Adames and Frank E. Nargang. Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada. E-mail: kishore@ualberta.ca

When chemical inhibitors or mutations impair the standard mitochondrial electron transport chain (sETC) in *Neurospora crassa*, alternative oxidase (AOX) is induced. AOX transfers electrons directly from coenzyme Q to oxygen, thus bypassing the later steps of the sETC. In *N. crassa* AOX is encoded by the nuclear *aod-1* gene. Over the years, several genes playing a role in the expression and regulation of AOX have been identified using traditional genetic screens. More recently, the gene knockout library of *N. crassa* was screened for mutants unable to grow in the presence of antimycin A, an inhibitor of Complex III of the sETC. At least some such mutants would be expected to have deficiencies of AOX and might represent new regulatory mutants. Several AOX deficient mutants were identified including eight new mutants with severe AOX deficiencies. In an attempt to further characterize two of these strains, we have found that the AOX deficiency cannot be rescued by transformation with the wild-type gene thought to be affected in each strain. Subsequent analysis suggests that the strains carry secondary mutations that are responsible for the AOX deficiencies. We are currently attempting to identify the affected genes in these strains.

4) Efficient detection of unpaired DNA during meiosis requires a component of the homologous recombination machinery.

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Meiotic silencing is a process by which unpaired DNA between homologous chromosomes is detected and then silenced for the duration of meiosis. While the phenomenon of meiotic silencing is well recognized, the processes that detect unpaired DNA elements are poorly understood. Here we provide two lines of evidence suggesting that unpaired DNA detection in *Neurospora crassa* involves a physical search for DNA homology. First, we have found that a paralog of the *Saccharomyces cerevisiae* homologous recombination protein Rad54 is required for the efficient silencing of unpaired DNA. Rad54 is widely thought to participate in the repair of DNA breaks by facilitating the identification of homologous sequences, suggesting that it may also facilitate homologous sequence identification during unpaired DNA detection. Second, we have successfully masked unpaired DNA by arranging identical transgenes close to one another on a pair of homologous chromosomes. Importantly, unpaired DNA masking falls apart when we increase the distance between the transgenes, suggesting that a homology search does occur during the unpaired DNA detection stage of meiotic silencing, but that this search is spatially-constrained. These data support a model by which unpaired DNA detection occurs via a homology search pathway that is similar to that used in the repair of double strand breaks by homologous recombination.

5) Unraveling the molecular basis of heterologous gene expression in *Neurospora crassa*.

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Hydrolysis of plant cell wall polysaccharides into fermentable sugars will require a large quantity and wide variety of glycoside hydrolases. The production cost of these enzymes is significant and considered an economical bottle-neck in cellulosic biofuel production. Novel glycoside hydrolases, well-adjusted to be active under harsh conditions, are found to be secreted by bacteria that degrade plant cell walls in the stomach of cows or in thermophilic bacteria found to live in hot springs. Since these bacteria are not suitable as production organisms, alternatives are needed. Filamentous fungi are frequently used as production organisms for hydrolytic enzymes. As decomposers of organic matter, these organisms naturally secrete hydrolytic enzymes into the surrounding medium at high levels. Despite their ability to secrete large amounts of homologous protein, heterologous protein secretion is often very low. In this study, we have developed a strategy to unravel the molecular mechanisms underlying heterologous gene expression by producing several (heterologous) members of the same GH10 enzyme family in the model cellulose degrading fungus *Neurospora crassa*. Even though these GH10 proteins are structurally classified into the same family, a member that is endogenous to *N. crassa* is expressed higher and secreted more efficiently compared to a heterologous homolog. Our goal is to better understand the molecular mechanisms underlying this differentiated expression and assess whether these mechanisms are similar between related fungal species. So far, we expressed homologous- and heterologous GH10 family genes in *N. crassa* and identified both high- and poor expressing and secreting enzymes. We are currently comparing the transcriptome and the secretome of these recombinant strains to reveal differences in secretion-related pathways. To challenge our hypothesis that similar molecular mechanisms might be found in related fungal species, we are applying the same experimental strategy to the model fungus *Aspergillus nidulans*.

6) GFP recombination reporter analysis of Neurospora Δ*msh-2* octads reveals high frequency symmetric heteroduplex.

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Recombination generates heteroduplex (hDNA), mismatched parental DNA strands that can be used to infer the mechanisms involved. In an eight-spored fungus such as Neurospora, hDNA can be detected by marker segregation in octads. For example, aberrant 4+:4M segregation (such as ++++M+MMM) indicates symmetric hDNA, arising from Holliday junction migration, while 5+:3M is an indicator of asymmetric hDNA, a result of DNA synthesis initiated at a recombination hotspot. Since mismatch repair (MMR) often removes such evidence before sporogenesis, we have deleted *msh-2* in a cross in which GFP is inserted close to the recombination hotspot *cog*. Scanning the MMR-deficient octads for recombination events under a fluorescent microscope revealed the wide range of recombination outcomes at *his-3* normally hidden by the activity of *msh-2*. In contrast to recombination events in yeast, symmetric hDNA is common at *his-3* in the absence of MMR and occurs with equal frequency to asymmetric hDNA. Although much less frequent than either 5+:3M or aberrant 4+:4M, the frequency of 6+:2M octads is little changed by deletion of *msh-2*, suggesting an additional MMR mechanism that makes a substantial contribution when the *msh-2* pathway is inactivated. *msh-2* deletion increases *his-3* allelic recombination by a factor of 1.5-1.7 when the recombination initiator cog^+ is *in cis* to the closer *his-3* allele, but has no effect if the only copy of cog^+ is *in cis* to the more distant *his-3* allele. Since His^+ spores are more likely to result from asymmetric hDNA initiated at *cog* in the former and symmetric hDNA in the latter arrangement, we conclude that repair of asymmetric hDNA exhibits a strong bias in the direction of restoration rather than conversion, while symmetric hDNA may be repaired without bias.

7) Saturating the Neurospora genome for mutants *d*efective *i*n *m*ethylation (dim)

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Cytosine methylation, a fundamental form of epigenetic regulation, is found in many eukaryotes and plays a significant role in cancer and other diseases. Using the genetically tractable model organism *Neurospora crassa*, the Selker laboratory has identified genes that when mutated, cause the strains to be defective in methylation (*dim*). The process of DNA methylation in Neurospora has been shown to be dependent on DCDC, a five member complex that directs the histone methyltransferase DIM-5 to trimethylate Lysine 9 on histone H3 (H3K9me3). This mark is recognized by HP1, which directs DIM-2 to methylate DNA. In contrast, the HCHC complex employs HDA-1, CDP-2, HP1, and CHAP to deacetylate that same residue on H3. While we know a good deal about DNA methylation, it is still unclear whether we have identified all the genes involved in the process. Thus we continued our search for *dim* mutants, using a selection for reactivation of silenced drug resistance genes. Interestingly, we predominantly identified known *dim* genes, including *dim-5*, *dim-7*, *dim-8*, *dim-9*, *hpo*, *chap*, *cdp-2*, and *hda-1*. Using a sequencing-based approach, we identified mutations in these known *dim* genes, presumably responsible for the Dim^- phenotype. This *dim* mutant collection should be a useful resource to investigate the roles of these genes and their protein products in DNA methylation.

8) K^+ regulation in *Neurospora*: Three proteins do it all

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Although it is now almost axiomatic for metabolic regulatory systems to be complex, exceptions do exist, as—for example--regulation of potassium metabolism in *Neurospora crassa*. Only three proteins are needed to keep intracellular potassium ($[K^+]_i$) within the normal physiological range (150--200 mM): **i) Pma1p**, the plasma-membrane H^+-ATPase; this protein provides the driving voltage and therefore most of the energy for K^+ transport; **ii) Trk1p**, a stable, constitutive, channel-like plasma-membrane protein that is able to concentrate K^+ from extracellular concentrations below 50 µM, and **iii) Hak1p**, an MFS-type membrane protein which is less avid than Trk1p but is highly regulated. Specifically, Hak1p is suppressed by the presence of Trk1p and by elevated $[K^+]_o$, and is strongly up-regulated by stress: especially by carbon starvation. Both proteins also possess the important property of *sliding affinity*, when potassium starvation develops due to gradual extracellular depletion during growth. That is, as $[K^+]_o$ falls below 100 µM, Hap1p stabilizes net K^+ influx at ~ 0.05 mMoles/kg cell water * min; and Trk1p does somewhat better, stabilizing net influx at ~0.15 mMoles/kg cell water * min. despite the continuing decline of $[K^+]_o$. Such stabilized transport sustains several additional hours of exponential growth.

9) A role for VE-1 in light sensing and conidal development in *Neurospora crassa*

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Neurospora crassa perceives light through a light-dependent transcription factor complex, the WCC. In addition, the *N. crassa* genome contains genes for secondary photoreceptors: two phytochromes genes (*phy-1* and *phy-2*), one cryptochrome gene (*cry-1*), and one opsin gene (*nop-1*). In addition, the *N. crassa* genome contains a homolog of the *Aspergillus nidulans veA* gene, *ve-1*. In *A. nidulans* mutations in *veA* result in constitutive conidiation, and VeA forms a complex with blue and red photoreceptors. The *N. crassa ve-1* mutant has defects in aerial hyphal growth and increased conidiation.

We have characterized the light-dependent accumulation of carotenoids in strains with deletions in these genes. The threshold for photocarotenogenesis in the wild-type strain is 10 J/m^2, and this sensitivity is not altered in strains with mutations in *phy-1*. However, a reduction in the maximum accumulation of carotenoids and a small reduction in sensitivity to light was observed in *phy-2*, *nop-1* and *cry-1* mutants, suggesting that the corresponding proteins play a minor role in light sensing. A ten-fold reduction in sensitivity was observed in the *ve-1* mutant, an indication that VE-1 participates in the mechanism of photoreception in *N. crassa*.

We have characterized the expression of *ve-1* and the accumulation of VE-1 after illumination and during asexual development. We observed a minor increase in the accumulation of *ve-1* mRNA after light exposure in vegetative mycelia (30 min), that did not lead to changes in VE-1 accumulation. The mutation in *ve-1* results in decreased light-dependent accumulation of mRNA for several genes, including the carotenogenesis genes (*al-1, al-2, al-3, cao-2*), *wc-1*, *vvd*, and *frq*. We have characterized the cellular localization of VE-1 under different light conditions and we have observed that VE-1 is preferentialy located in the nucleus under all conditions, but VE-1 was also detected in cytoplasm. We observed the accumulation of *ve-1* mRNA in vegetative mycelia, and a reduction in mRNA accumulation after the induction of conidiation. VE-1 was not present during conidial development, but VE-1 was detected in vegetative mycelia. Our results suggest that the development of conidia requires a reduction in the amount of VE-1 in *N. crassa*.

10) Mechanism of quelling and siRNA production in *Neurospora*

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

11) Does sequence heterozygosity underlie suppression of meiotic silencing by wild Neurospora strains? Does Neurospora contain nucleus-limited genes?

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Bichpuri-1 *a* (B) and Spurger *A* (S) are among the four wild-isolated *N. crassa* strains identified whose crosses with the OR-derived testers *::Bml*^{r} and *::mei-3* showed suppression of meiotic silencing of the *bml* and *mei-3* genes, and typified the "Sad type" wild strains. Crosses of 68 other wild strains with the testers showed suppression of silencing in *mei-3*, but not *bml*, and typified the intermediate "Esm type". Only eight wild strains behaved like OR and showed silencing in crosses with both the testers. They were the "OR" type. A B x S cross followed by 10 generations of sibling crosses was used to create isogenic *mat A* and *mat a* strains in the B/S background, and to make new *mei-3* testers. If a tester- heterozygous cross in this background shows meiotic silencing, it would support our hypothesis that the Sad or Esm phenotype arises from heterozygosity for sequence polymorphism in the cross with the OR-derived testers. The polymorphisms might reduce pairing and silence meiotic silencing genes.
Additionally, we are introgressing *N. crassa* translocations into *N. tetrasperma* to produce self-fertile [(*T*) + (*N*)] strains that should generate [(*T*) + (*N*)] and [(*Dp*) + (*Df*)] progeny. If any [(*Dp*) + (*Df*)] is less self-fertile than the corresponding [(*T*) + (*N*)], it would provide evidence for "nucleus-limited" genes, i.e., where the wild-type allele (*WT*) fails to complement a null allele (*Δ*) in a [(*WT*) + (*Δ*)] heterokaryon. Such genes have not yet been found, but they are predicted by the putative nucleus-limited phenotype of the *N. crassa scon*^{c} mutant (Butler and Metzenberg 1972 J Bacteriol. 109:140-51), and the MatIS process in *Aspergillus nidulans* (Czaja *et al.* 2013 Genetics 193:1149-1162).

12) A novel protein links dynein complex to conventional kinesin

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In filamentous fungi, the kinesins and cytoplasmic dynein are "motor" molecules that are required for the proper growth and development of the mycelium. Great advances have been made in our understanding of these fascinating proteins and their multiple functions within growing hyphae. Their ability to transport cargoes to and from hyphal tips is one of the most studied functions; however, the mechanism of interaction between cytoplasmic dynein and kinesins remains elusive. In this study we employed genetic, molecular, and fluorescence microscopy techniques to isolate and analyze mutants affected in hyphal growth and the localization of cytoplasmic dynein in the model organism *Neurospora crassa*. Our efforts led to the identification of a novel protein involved in the interaction of cytoplasmic dynein with the conventional kinesin, NcKin. This protein is specific to the Ascomycetes and is highly conserved among the Pezizomycetes. It is required for proper hyphal growth and is transported to the hyphal tips through NcKin. Interestingly, this protein is required for maintaining a high concentration of dynein at growing hyphal tips of *N. crassa*. Our current focus is to understand the molecular mechanisms controlling the actions of this highly conserved novel protein. Our work should shed light on the evolution and the mechanisms of regulation of microtubule-dependent transport in filamentous fungi and other Eukaryotes.

13) Direct recognition of homology between double helices of DNA in *Neurospora crassa.*

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Chromosomal regions of identical or nearly identical DNA sequence can preferentially associate with one another in the apparent absence of DNA breakage and recombination. The archetype of such "break-independent pairing" is the genome-wide synapsis of homologous chromosomes in somatic nuclei of the fruit fly. Additional examples include transient associations of homologous loci in mammalian somatic nuclei, break-independent pairing of homologous chromosomes in meiosis, and repeat-directed DNA modifications in fungi. The molecular mechanism(s) underlying these pairing phenomena remain(s) an unsolved mystery of chromosome biology. Here we describe the basis for homology recognition during repeat-induced point mutation (RIP) discovered by Eric Selker and colleagues nearly 25 years ago in the fungus *Neurospora crassa* wherein DNA duplications are efficiently recognized and mutated, irrespective of their sequence, origin, or location in the genome. We show that RIP is triggered by short units of homology interspersed with a matching periodicity of 11 or 12 base pairs (bp) that slightly exceeds the 10.5-bp periodicity of relaxed B-DNA. The size of the minimal effective homology recognition unit is 3 bp. Participating DNA segments must be able to co-align along their lengths, and at least ~150 bp of sequence identity are required to initiate mutation. Additionally, RIP proceeds normally in the absence of MEI3, the only RAD51/DMC1 protein encoded in the *N. crassa* genome, demonstrating independence from the canonical homology recognition pathway. These and other results strongly suggest that intact double-stranded DNA molecules can engage in direct sequence-specific interactions *in vivo*, with a triplet of DNA base pairs as the likely fundamental recognition unit. A new perspective is thus provided from which to further analyze the break-independent recognition of homology that underlies RIP and, potentially, other processes where sequence-specific pairing of intact chromosomes is involved.

14) The functional characterization of the *Neurospora crassa* Hac-1 transcription factor reveals a crucial role for the Unfolded Protein Response in plant cell wall deconstruction

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High secretion capacity in filamentous fungi requires an extremely efficient system for protein synthesis, folding and transport. When the folding capacity of the endoplasmic reticulum (ER) is exceeded, a pathway known as the unfolded protein response (UPR) is triggered allowing cells to mitigate and cope with this stress. In yeast, this pathway relies on the transcription factor HAC1, which mediates the up-regulation of several genes required under these stressful conditions. In this work, we identify and characterize the *HAC1* orthologue in the filamentous fungus *Neurospora crassa*. We show that *hac-1* mRNA undergoes an ER stress-dependent unconventional splicing reaction, which in Neurospora removes a 23 nt intron, leading to a change in the open reading frame and the production of a functional transcription factor. By disrupting *hac-1*, we determined this gene to be crucial for activating UPR and for proper growth in the presence of ER stress-inducing chemical agents. Neurospora is naturally found growing on dead plant material and it has become a model organism for plant cell wall deconstruction studies. Notably, we found that Hac-1 is necessary for growth on cellulose or Avicel (crystalline cellulose). Further characterization of this phenomenon revealed that it is due, in part, to a drastic reduction in the levels of secreted proteins. Unexpectedly, however, we also observed that the expression of cellulolytic genes is partly impaired in the *hac-1* KO strain, which correlates with poor induction of key transcription factors (CLR) required for deconstruction of cellulose. Nevertheless, growth of *hac-1* deficient strains on xylan, cellobiose or glucose is not impaired, which can be partially explained by less challenging protein secretion demands. The characterization of this signaling pathway in Neurospora will help in the study of fungal plant cell wall deconstruction, highlighting UPR as relevant process that can be further manipulated with important biotechnological applications. FONDECYT1131030 and Millennium Nucleus for Fungal Integrative and Synthetic Biology (NC120043).

15) The intrinsically low fidelity of pre-mRNA splicing in *Neurospora*

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The spliceosome is a large RNA-protein complex that removes intron sequences from eukaryotic pre-mRNAs by the process of RNA splicing. Splicing is commonly thought to occur with a very low error rate to produce the correct reading frame in the spliced RNA and to avoid the production of potentially harmful proteins from mis-spliced RNAs. When we expressed the small non-protein-coding transcript of the mitochondrial VS plasmid in the nucleus of *Neurospora* we were surprised to find that it was efficiently spliced at one or more of eight 5' splice sites and ten 3' splice sites, which are present apparently by chance in the sequence. Further analyses of other plasmids and natural *Neurospora* nuclear genes indicates that the spliceosome is inherently much more promiscuous than is typically thought, to the extent that it might act as a genomic defense against foreign nucleic acids that have not been depleted of splice sites or evolved into functional introns. Evolutionary pressure to avoid being inactivated by RNA splicing may explain why plasmids are lacking in the nuclei of fungi but are commonly found in their mitochondria, which lack spliceosomes. We also explore the implications of these observations for models of the evolutionary origin of spliceosomal introns.

16) HAM11 is specific to germling fusion in *Neurospora crassa.*

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Fusion between cells is a necessary developmental process across the tree of life. In *Neurospora crassa*, fusion between germinating conidia (germlings) is a necessary step to establishing a robust colony. Two germlings in close proximity to each other will engage in a molecular conversation to facilitate growth towards each other, membrane fusion, and cytoplasmic mixing. The conversation is initiated by an unknown factor that leads to activation of a conserved MAP-kinase cascade. Once activated, the MAP-kinase, MAK2 oscillates at regular four-minute intervals between germling tip and nucleus. MAK2 putatively phosphorylates the transcription factor, PP-1, which regulates expression of several genes required for homing and fusion, including HAM11 (Hyphal Anastomosis Mutant 11). The phenotype of the deletion mutant, *Δham11* is specific to germlings. *Δham11* is fully fertile and capable of hyphal fusion within mature colonies. However, *Δham11* germlings fail to initiate chemotropism, oscillation of MAK2, and cell fusion when only *Δham11* germlings are present. Interestingly, a *Δham11* germling paired with a WT germling will successfully initiate chemotropism, oscillation of MAK2, and fusion. This indicates that *Δham11* is able to perceive the signal, but is unable to respond alone. We also constructed luciferase reporter strains that provide an additional quantitative readout for activation and regulation of the MAK2 pathway. Our data indicates that HAM11 is part of a positive feed back loop reinforcing the germling communication pathway.

17) MSN-1 is a transcription factor regulated by MAK-1 MAP kinase in *Neurospora crassa*.

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The maintenance of cell wall integrity (CWI) is required for the growth and development in filamentous fungus. We have previously reported that MAK-1 MAP kinase regulates the expression of CWI-related genes, *ncw-1* and *egl-1*, in *Neurospora crassa*. In addition, *mak-1* disruptant showed highly sensitivity to cell wall damaging agents, micafungin (MCFG) and polyoxin D (PD), which are beta-1,3-glucan synthase inhibitor and chitin synthase inhibitor, respectively. In *S. cerevisiae*, Rlm1 is a transcription factor in Mpk1 MAP kinase pathway (CWI pathway). However, transcription factor (s) regulated by MAK-1 still remains unclear in *N. crassa*. RLM-1, an ortholog of the yeast Rlm1, did not regulate the expression of *ncw-1* and *egl-1*. To explore transcription factors involved in CWI, we carried out the sensitivity test to MCFG and/or PD using disruptants library of transcription factor gene. The screening results showed that three disruptants were more sensitive to MCFG, and thirteen were to PD, than the wild-type strain. Among these, the only *msn-1* deletion strain (NCU02671) was more sensitive to both MCFG and PD than the wild-type strain. The upregulation of *ncw-1* and *egl-1* by micafungin treatment were almost completely abolished in *msn-1* disruptant. These results indicate that MSN-1 controls the induction of *ncw-1* and *egl-1*, and functions as MAK-1 downstream transcription factor in *N. crassa*.

18) Transcriptional regulation of the *erg* genes response to ergosterol biosynthesis inhibitors, azole and morpholine, in *N. crassa*

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Several classes of fungicides that inhibit the enzymes in ergosterol biosynthesis pathway have been developed. Azoles such as fluconazole and metconazole inhibit lanosterol 14α-demethylase ERG-8, and morpholines such as tridemorph and fenpropimorph inhibit both D14 reductase ERG-3 and C7-C8 isomerase ERG-1. It is well known that inhibition of ergosterol biosynthesis upregulates several *erg* genes in fungi. We analyzed the expression of 21 genes in ergosterol pathway when treated by fluconazole and fenpropimorph in *N. crassa*. Fluconazole induced the expression of *erg-8* and *erg-4*. In contrast, fenpropimorph induced *erg-1*, *erg-3*, *erg-9*, and *erg-10*, but did not *erg-8* and *erg-4*. These indicate that each fungicide upregulate each target enzyme gene. Mixed treatment of these fungicides upregulated the azole-induced gene, *erg-8* and *erg-4*, but did not the morpholine-induced genes. Deletion mutant of transcription factor *sah-2* was sensitive to fenpropimorph but not fluconazole. Furthermore SAH-2 regulated the upregulation of the morpholine-induced genes. These results suggest that *N. crassa* has at least two systems to detect enzymatic defects in ergosterol biosynthesis.

19) eRF1 and eIF4A3 mRNA stability in *Neurospora* is controlled by NMD, EJC, and CBP factors through 3'UTR introns.

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Eukaryotic mRNA stability can be controlled through the nonsense mediated mRNA decay (NMD) pathway, which recognizes premature termination codons. In higher eukaryotes, an intron downstream of a termination codon can trigger NMD through the function of the exon-junction complex (EJC). The nuclear cap binding complex (CBC) is also important for EJC-mediated NMD. We used metabolic labeling with 4-thiouracil (4TU) and a pulse-chase procedure to assess the roles of NMD, EJC and CBC factors in controlling mRNA stability in the filamentous fungus *Neurospora crassa*. The mRNAs for the EJC components eIF4A3 and Y14, and translation termination factor eRF1, each contain a 3'UTR intron and each was stabilized in mutants lacking NMD factors UPF1 or UPF2. The mRNA for *arg-2*, which contains an upstream open reading frame (uORF) that causes ribosomes that translate it to arrest during termination, was also stabilized in NMD mutants. Analyses of luciferase reporters demonstrated that intron-containing *eif4a3* and *erf1* 3'UTRs and the *arg-2* uORF, each conferred NMD-sensitivity to reporters. Intronless 3'UTRs and a mutated *arg-2* uORF that does not arrest ribosomes did not confer NMD-sensitivity. Viable *N. crassa* knockout mutants deficient in EJC or CBC components showed stabilization of *eif4a3*, *y14* and *erf1* mRNAs. The *Δy14* and *Δcbp80* strains showed stabilization of *luc* reporters with the intron-containing *eif4a3* 3'UTR but not intronless 3'UTRs. mRNAs with 3'UTR introns were selectively enriched in mRNPs that were affinity purified using monoclonal antibody directed against human Y14. These results demonstrate a role for *N. crassa* NMD, EJC and CBC factors in controlling the stability of mRNA with 3'UTR introns, and suggest roles for 3'UTR introns in post-transcriptional autoregulation of *eif4a3*, *y14* and *erf1*.

20) Circadian rhythms synchronize mitosis in *Neurospora crassa*

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

21) Circadian Regulation of Translation through the Eukaryotic Elongation Factor eEF-2 in *Neurospora crassa*.

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Circadian clocks provide an endogenous mechanism in diverse organisms to regulate daily rhythms in biological functions ranging from sleep-wake cycles to metabolism and gene expression. However the mechanisms that relay temporal information from circadian oscillators to the genes and processes that show rhythmic activity are not well understood. We found that the circadian clock in *Neurospora crassa* signals through rhythmic activation of conserved mitogen-activated protein kinase (MAPK) pathways, including the well-characterized osmosensing (OS) MAPK pathway. The clock regulates rhythms in the phosphorylation of the MAPK OS-2 (P-OS-2), such that OS-2 is phosphorylated and active during the day. P-OS-2 then interacts with other proteins, including transcription factors, kinases, and chromatin modification proteins, which lead to rhythmic activation or repression of target genes of the pathway. In yeast, Hog1 (a homolog of OS-2) has been shown to interact with Rck2 after osmotic stress (*i.e.*, salt shock) resulting in activation of Rck2. Active Rck2 kinase phosphorylates elongation factor 2 (EF-2) to inactivate it and repress translation [1]. In *N. crassa*, we have shown that P-OS-2 interacts with the serine/threonine protein kinase RCK-2, leading to rhythmic phosphorylation and activation of RCK-2. Clock-controlled RCK-2 in turn rhythmically phosphorylates eukaryotic elongation factor 2 (eEF-2). Currently, we are using ribosomal profiling to test the hypothesis that circadian clock control of translation, in part through the regulation of eEF-2 activity and translation elongation, functions to restrict protein synthesis to specific times of the day to optimize resources in anticipation of daily environmental stresses.

1. Teige, M., E. Scheikl, V. Reiser, H. Ruis and G. Ammerer (2001). "Rck2, a member of the calmodulin-protein kinase family, links protein synthesis to high osmolarity MAP kinase signaling in budding yeast." Proc Natl Acad Sci U S A **98**(10): 5625-5630.

22) Does the CENP-T-W-S-X tetramer link centromeres to kinetochores?

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The vertebrate centromeric proteins, CENP-T, -W, -S and -X, are able to form a CENP-T-W-S-X tetramer *in vitro* that binds DNA. Furthermore, the unstructured N-terminus of CENP-T interacts with the NDC80 complex at kinetochores. This suggests that CENP-T-W-S-X has a central role in linking centromeric DNA to kinetochores. Despite the appeal of this model, there is no evidence that this complex forms *in vivo*, no information on the DNA sequences it may bind at centromeres, and little understanding of how it interacts with nucleosomes. All four proteins are conserved in fungi, including Neurospora. Neurospora is an attractive model in which to study function of the CENP-T-W-S-X complex as its centromeric DNA is nearly completely assembled, allowing ChIP-seq reads to be mapped unambiguously. Here, we report on our investigations of the Neurospora CENP-T-W-S-X complex, including its interactions with centromeric DNA and canonical centromeric nucleosomes.

23) The role of a putative histone H4 variant in chromatin structure and function

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Nucleosome octamers condense, organize and regulate the eukaryotic genome. Canonical octamers consist of two copies of histones H3, H4, H2A and H2B. In recent years, histone variants have attracted much attention. Variants of H3 (replication-dependent H3.1, replication-independent H3.3, and the centromere-specific histone H3, CENP-A/CenH3) or H2A (H2AZ and macro-H2A) are deposited in specific chromosome regions or at specific times, and they recruit additional factors that together define chromosome territories. Current dogma states that there are no variants of histone H4 so that canonical H4 is present in every nucleosome incorporated in chromatin. *Neurospora crassa*, however, has three putative histone H4 genes. Two genes code for identical, canonical histone H4 proteins even though DNA sequences and gene structures are different. The third gene is highly divergent from this pair and has long been considered a pseudogene. Comparisons with the genomes of additional filamentous fungi revealed that the structure of this novel histone H4 variant, called H4v, is conserved. While there are changes to key residues in the histone fold domain, the major differences are in the extended N- and C-terminal tails, similar to what had been found for fungal CenH3 proteins. We showed that the *Neurospora* histone H4 variant is expressed and localized to the nucleus. ChIP-sequencing revealed H4v localization to specific regions of the genome. This finding contrasts to binding of both canonical H4 proteins, which were broadly distributed. In most specific regions, H4v appeared to replace canonical H4, as regions of enrichment corresponded to regions depleted of both canonical H4 proteins.

24) Control and Function of Two Fatty Acid Regulators, FAR-1 and FAR-2

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Neurospora crassa is an excellent model organism to describe transcription factor networks. We analyzed the behavior of two Fatty Acid Regulators, FAR-1 and FAR-2, transcription factors that modulate the response of Neurospora to the presence of fatty acids. We used ChIP-sequencing to find binding sites of FAR-1 and FAR-2 under light induction and various carbon sources (butyrate for short-chain fatty acids, oleate for long-chain fatty acids, and sucrose as control). Bioinformatic analyses revealed carbon source-specific differential binding of FAR-1 and FAR-2, though ~30% of all targets overlapped. RNA-sequencing in WT identified transcripts that changed independently of binding determined by ChIP-seq under any condition, thus separating indirect from direct targets of regulation by FARs. Studies in *far-1* and *far-2* deletion strains, as well as the *far-1 far-2* double mutant revealed redundant and exclusive regulatory circuits. Functions of FARs and their direct target genes were further examined by selected phenotypic assays, including linear growth rates on selected media, siderophore production, reaction to oxidative stress, and sexual development. We found reduced growth rates on Tween-containing medium, reduced siderophore production, reduced conidiation and increased vulnerability to oxidative stress in *far-1*, but not *far-2*. Linear growth rates were reduced in *far-2* in a carbon source-specific manner, and *far-2* was required for sexual development on butyrate medium. We identified novel groups of co-regulated genes not previously known as targets of FAR homologs in other fungi.

25) Localization and role of endocytosis associated proteins SLA-1 and MYO-1 in *Neurospora crassa*.

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Endocytosis in eukaryotic cells is a process by which plasma membrane and associated proteins are internalized. During receptor-mediated endocytosis several proteins participate in a coordinated pathway which involves binding membrane receptors, invagination, vesicle formation and scission. In this process actin plays an important role. The actin cytoskeleton is a key element for the establishment and maintenance of polar growth, characteristic of hyphal cells in filamentous fungi. In *Neurospora crassa* F-actin is present at the hyphal tips concentrated in the Spitzenkörper core while another actin form, actin patches, is found in the subapical region together with several protein components of the endocytic machinery. We found that MYO-1-GFP is not present in the hyphal tips but is present throughout the hyphal tube in two different structures: the subapical endocytic collar and developing septa. Here we characterized two mutants of *N. crassa* with deletions in proteins that play different roles during endocytosis in yeast, the adaptor protein SLA-1 and the actin binding protein MYO-1. Both mutants exhibited severe growth deficiency and marked morphological alterations including heavily crenulated hyphal tubes, abundant apical branching, near absence of aerial mycelium and conidiation. By visualizing actin with the Lifeact-GFP reporter, we found that the absence of *myo-1* or *sla-1* alters the actin cytoskeleton, producing periods of polarized and isotropic growth responsible for the crenulated morphology of the hyphae. These results suggest that endocytic proteins SLA-1 and MYO-1 play a major role in the growth of *N. crassa*, however a direct relationship between endocytosis and polarized growth remains to be established.

26) Genetic analysis of the components of the *ime-2* mediated signaling events during nonself recognition and programmed cell death PCD in *Neurospora crassa.*

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Recently, we revealed genetic and functional differences in meiotic initiation machinery between *Neurospora crassa* and *Saccharomyces cerevisiae*. While *N. crassa* is missing some meiotic genes identified in yeast, it has three homologs of the middle meiotic transcriptional regulator, Ndt80. None of the *NDT80* homologs are required for meiosis in *N. crassa*. One of the *NDT80* homologs, *vib-1* is essential for heterokaryon incompatibility (HI) in *N. crassa*, a nonself recognition mechanism in filamentous fungi. Mutations in *vib-1* suppress cell death caused by HI as well as secretion of the extracellular proteases during the nitrogen starvation. Furthermore, deletion of a *IME2* (a kinase involved in initiation of meiosis in *S. cerevisiae*) homolog in *N. crassa*, *ime-2*, does not affect sexual development, results in a significant elevation of secreted proteases in response to nitrogen starvation. Morever, a *Δvib-1 Δime-2* mutant restored wild-type levels of cell death during the HI and normal production of extracellular proteases; a deletion of *ime-2* suppressed these *vib-1* phenotypes. Based on the evidence, we hypothesize that IME-2 negatively regulates a cell death pathway that functions in parallel to the VIB-1 HI pathway and a protease secretion pathway positively regulated by VIB-1. We used a slightly modified yeast consensus sequence for Ime2 phosphorylation to scan (Scansite) the entire *N. crassa* genome for possible targets and obtained a list of 30 candidates including VIB-1. All targets were assessed for secretion of the extracellular proteases in absence of nitrogen. Strains containing deletions of 13 of the 30 genes identified in the screen were significantly affected in protease secretion. Mutations in these candidate genes will be tested for the ability to alleviate cell death and HI in the presence and absence of *ime-2* and *vib-1* hence assessing their role in the parallel HI/PCD pathway redundant with VIB-1.

27) The balance between exocytosis and endocytosis: mathematical predictions and experimental measurements.

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The existence and role of endocytosis in the growth of mycelial fungi have been subjects of great interest. Recent experimental evidence, albeit mainly qualitative, dissipated doubts about the very occurrence of endocytosis in mycelial fungi. It has been postulated that exocytosis in growing hyphal tips creates an excess of plasma membrane and thus the need for its removal, i.e. endocytosis. This excess would result from the greater number of vesicles required for cell wall extension and extracellular enzyme secretion over the amount necessary for plasma membrane extension. A good estimate of the membrane balance between exocytosis and endocytosis is difficult to calculate given the absence of reliable values for some parameters. Nevertheless, we have developed an interacting spreadsheet with values for accessible parameters such as growth rate, cell shape and size, wall thickness and vesicle size. The impact of other critical factors such as amount of wall generated by each exocytic discharge, relative contribution of macro- vs. microvesicles, proportion of pre-formed cell wall vs. polymer synthesized in situ, and vesicle load destined for extracellular secretion vs wall formation, is embodied in a single factor: "packing efficiency". For fast growing (35 µm min^{-1}) hyphae of *N. crassa* (10 µm diameter) with a wall 0.050 µm thick and secretory vesicles of 0.1 µm, a calculation of the number of vesicles necessary to make the cell wall based on a direct conversion of vesicle volume into wall volume (packing efficiency = 100%) shows 3 X more exocytic vesicles would be needed than those required to extend the plasma membrane. This assumption produces a rather unrealistic 66% excess of plasma membrane. Most likely only a fraction of this predicted vesicle population (in either numbers or wall generating potential) may be involved. An initial attempt was made to measure endocytosis experimentally by photobleaching the subapical endocytic collar of hyphae of Neurospora *crassa* (19.6 µm min^{-1}) tagged with the endocytic reporter fimbrin-GFP. The rate of appearance of fluorescent patches, each indicative of an endocytic event, was monitored by confocal microscopy. Assuming the diameter of endocytic vesicles to be 50 - 80 nm, we calculated that 4.0 - 10.3 % of the exocytosed membrane was endocytosed.

28) Database resources for Neurospora and Sordariomycetes genomics in FungiDB

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FungiDB (http://fungidb.org) is a freely available website and database system for comparative analyses of fungal genomics data. The FungiDB system is built on the EuPathDB (http://eupathdb.org) software system which supports resources for Plasmodium (PlasmoDB), Toxoplasma (ToxoDB), and Schistosoma (SchistoDB). Resources in FungiDB include a broad taxonomic sampling from Oomycetes to Dikarya, Chytrid, and Zygomycete fungi. The support of Sordariomycete species in FungiDB include genomes of *Neurospora crassa*, *N. discreta*, *N. tetrasperma*, *Sordaria macrospora*, *Trichoderma reesei*, Magnaporthe oryzae, *Fusarium oxysporum f. sp. lycopersici*, *F. verticillioides* (*Gibberella moniliformis*), and *F. graminearium* (*G. zeae*). Comparative analyses can be performed within the system as a series of queries including gene content, precomputed protein domains, gene ontology terms. Gene lists can also be transformed by orthology to compare gene content or map data from one species to another. Gene expression data from RNA-Seq is loaded where available for species (*N. crassa* currently) allowing for query building that include expression change or total amount. These strategy queries can be saved, shared, edited, and re-run upon updates to the genome databases allowing for transparent are reproducible analyses. Ongoing work will support SNP data from multiple strains of N. discrete and N. crassa that have been sequenced. Additional future work will incorporate phenotype data from *N. crassa* gene deletion collection and support additional RNA-Seq, ChIP-Seq, and proteomic data. Support for FungiDB has been provided by the Burroughs Welcome Fund, the Alfred P Sloan Foundation and through collaborative projects NIFA-USDA and NIH.

29) Comparative transcriptome analysis between two modes of mitochondrial-plasmid induced senescence in *Neurospora crassa*

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Senescence in *Neurospora crassa* is caused by dysfunctional mitochondria and is most commonly associated with the effects of mitochondrial plasmids. Variant forms of the Mauriceville retroplasmid (pM) cause senescence by integrating into the mitochondrial genome and disrupting essential mitochondrial genes or by over-replicating and interfering with protein synthesis. The transcriptomes of Mauriceville cultures undergoing senescence by these two distinct pathways were analyzed by RNA-sequencing. Four strains were analyzed: a Mauriceville natural isolate that shows wild-type growth (M), a pre-senescent culture, two transfers from senescence, associated with mtDNA alterations caused by plasmid integration (M-2), a derivative of M that contains a variant form of the plasmid (pMS) and undergoes senescence without alteration of mtDNA (MS), and a pre-senescent culture, two transfers from senescence, caused by plasmid over-replication that interferes with the synthesis of mitochondrial genes (MS-2). Nuclear-encoded genes involved in mitochondrial functions were consistently induced in both M and MS pre-senescent cultures (e.g. those involved in mt protein quality control), whereas pronounced differences in the expression profiles of other genes were detected in integrative and over-replicative forms of senescence. The MS culture showed greater induction in DNA damage response genes such as *uve-1*, a UV-induced endonuclease, and *mus-41*, an E3 ubiquitin ligase, as well as genes that respond to respiratory inhibitors (i.e. cyanide, uncouplers). MS cultures also induced genes involved in mitochondrial translation, supporting the hypothesis that plasmid over-replication interferes with mitochondrial protein synthesis. Interestingly, the expression of antioxidant genes among pre-senescent cultures was highly variable and the role reactive oxygen species play in both forms of senescence is unclear. Collectively, the data provide new insights in mitochondrial-nuclear communication in *Neurospora*.

30) Genomic sequencing of three Spore killer strains of *Neurospora intermedia* and *Neurospora sitophila*

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Meiotic drive is the phenomenon where a selfish genetic element will skew sexual transmission in its own favour, causing it to be transmitted to the next generation in excess of the expected 1:1 ratio. Spore killer is a meiotic drive element found in natural populations of Neurospora. In a cross between a strain carrying a Spore killer haplotype and a strain carrying a sensitive haplotype, half of the ascospores will die and the surviving spores will all show the killer phenotype in further crosses. The Spore killer haplotype consists of a 1-2 Mbp region of suppressed recombination on Linkage Group 3, where the genes that act in the killing are located. A complete identification of these genes has so far been prevented by the suppression of recombination. We have sequenced the genomes of three Neurospora strains carrying different killer elements (*N. sitophila* strain 6232 carrying Sk-1 and *N. intermedia* strains 7426 and 3194 carrying Sk-2 and Sk-3 respectively) with 75x coverage, using the Illumina MiSeq sequencing platform. We present here a description of the regions of suppressed recombination and a comparative analysis of the regions found in the different killer types and in sensitive strains of a number of Neurospora species.

31) Heat break of ascospore dormancy in *Neurospora crassa*

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Naturally, the model filamentous fungi Neurospora can sometimes be found under tree bark after a forest fire. Historically, people paid utmost attention to massive growth of this fungi when it spoiled large quality of bread at the French army bakeries in 1842 and when it appeared mysteriously throughout Tokyo city after the devastating earthquake and great fire of 1923. We now easily imagine the association of Neurospora with heat and fire since the heat-resistance ascospores is activated by exposed to heat. The dormancy and heat-resistance of Neurospora ascospores were investigated until mid-1970s, but further investigation has not been reported in almost 4 decades. By taking advantage of the Neurospora knockout collection, we identified proteins essential for heat break of ascospore dormancy in *Neurospora crassa*. Here, we will summarize and discuss our recent progress towards the identification of these proteins and their possible roles.

32) Speciation genomics of *Neurospora discreta*

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Differentiation of populations, adaptation of differentiated populations, and subsequent development of reproductive barriers are the processes that create the tree of life. Published study of Neurospora crassa discovered recently diverged populations containing genetic "islands" of differentiation involved in adaptation to temperature. To extend this finding, and to address its universality, we conducted a population genomic study of a species in the Neurospora discreta phylogenetic species complex with a latitudinal range from New Mexico to Alaska. We resequenced 49 isolates from New Mexico, California, Washington and Alaska, and 13 non-North American isolates. Phylogenomic analyses revealed two, nearly simultaneous divergences that define three clades: Alaska, California-Washington and New Mexico-Washington. The phylogeography of these clades is consistent with one ancestral lineage that diverged to a Rocky Mountain lineage and an Alaska lineage, and another lineage that simultaneously repopulated the Pacific Coast. Comparison of Southern and Northern populations from the California-Washington and New Mexico-Washington lineages revealed genetic "islands" of divergence. Analyses of ancient admixture indicated that some of the islands of most extreme divergence had possibly been introgressed from an unknown Neurospora species, and that other regions of the genomes of the three lineages may be of transpecific origin. Our work highlights the importance of interspecific acquisition of genomic variation as a motor for adaptation and evolution in Neurospora.

33) CPC-2 regulates developmental pathways in *Neurospora crassa* in association with heterotrimeric G proteins

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Cross Pathway Control-2 (CPC-2) is a conserved WD40 protein with similarity to RACK1 (receptor for activated C kinase) in mammals. It has 70% positional identity to the β-subunit of heterotrimeric G proteins. CPC-2 has been shown to be a truly multifunctional protein, with roles in female fertility and general amino acid control in *Neurospora crassa*, and cell cycle progression and meiotic development in other organisms. We are currently investigating the relationship between CPC-2 and heterotrimeric G proteins in *N. crassa* and how this might lead to modulation of growth and development. We are employing detailed phenotypic and biochemical characterization of gene deletion mutants and strains containing constitutively activated Gα alleles, with the goal of establishing the epistatic and physical relationships between components of the heterotrimeric G protein pathway and CPC-2.

34) The guanine nucleotide exchange factor RIC8 interacts with a regulator of MAPK signaling, STE50 in *Neurospora crassa*

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Heterotrimeric G proteins are essential components of signal transduction pathways that regulate environmental sensing, growth, and development in eukaryotes. RIC8 is a cystolic protein that can serve as a guanine nucleotide exchange factor (GEF) for G alpha proteins. A *ric8* homologue is present within the *Neurospora crassa* genome. We have identified a protein that interact with RIC8 via a yeast-two-hybrid cDNA library screen, STE-50, a regulator of MAPK signaling in fungi. We have made two strains; one containing the STE-50-FLAG fusion protein and the other containing both STE-50-FLAG and RIC8-V5 fusion proteins. We are optimizing the conditions to show protein-protein interaction with co-immunoprecipitation. *Neurospora* contains three different mitogen activated protein (MAP) kinase cascades. Two of the pathways contain Erk class MAP kinases (MAK-1 and MAK-2), while the third cascade has a terminal p38 MAP kinase (OS-2). We analyzed Δ*ste50* and Δ*ric8* single and double mutants for growth and developmental defects and for effects on the activity of the Erk class MAP kinases. Knockouts were analyzed for several growth and developmental phenotypes. Mutants lacking *ric8* or the MAPK genes, *mak-1* and *mak-2*, exhibited a "flat" phenotype on the agar surface, with short aerial hyphae and conidiation close to the agar surface. The morphology of Δ*ste50* and Δ*ste50* Δ*ric8* mutants resembled that of strains lacking *ric8*, *mak-1* or *mak-2*. Lastly, STE50 is composed of three characterized domains: an N-terminal Sterile-Alpha Motif (SAM) domain, a central serine/threonine rich region and a Ras Association (RA) domain at the C-terminus, we have identified that the N-terminus region of STE50 is essential in the interaction with RIC8.

35) Properties of unpaired regions for triggering or escaping meiotic silencing in *Neurospora crassa*.

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Neurospora crassa employs several defense mechanisms to protect its genome including Meiotic silencing by unpaired DNA. This mechanism functions during sexual cycle and is hypothesized to protect genome against the invasion of virus and endogenous transposable elements. Small RNAs are produced in response to the unpaired and function to silence these regions. We have sequenced smallRNAs from sexual tissue in a cross between the reference OR74A (FGSC 2489) and D60 (FGSC 8820). In addition we sequenced genome of D60 and compared it with the reference genome. Our bioinformatics analysis identified 32 regions uniquely presented in the OR74A genome which also showed high smallRNA production at 4 days post after fertilization. We predict these regions form unpaired loops during the sexual cycle and trigger meiotic silencing. SmallRNAs from these regions displayed the same features as ones from *sly-1*, a DNA transposable element in OR74A detected previously. The features include smRNAs produced from both strands of the unpaired region, 24bp~26bp long, peaking at 25bp, and uridine enrichment at the 5' end. Statistical analysis showed that the length of unpaired regions has a linear relationship with the smallRNA production. We also found 611 regions uniquely presented in OR74A genome but which lacked smallRNA production in sexual tissue. 92 of these regions encoded identified genes or hypothetical genes and 519 contained no genes. The genes within the unpaired regions which were not silenced had annotations or GO terms that indicated their role in the sexual cycle and included the mating type genes. Our results revealed the properties of unpaired regions that contribute to stimulation of or immunity to meiotic silencing.

36) High resolution characterization of reproductive compatibility in a genotyped experimental mating population of *Neurospora crassa*

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Positive correlations between geographic and genetic distance are commonly observed in natural populations. Yet, very few experimental studies have been performed to determine the role of reproductive isolation in producing such patterns. In this study, an experimental mating population of 11 mat A and 11 mat a *Neurospora crassa* strains was chosen from a collection of 24 mat A and 24 mat a strains from North America, the Caribbean, and Africa. These strains were previously genotyped via RNAseq (Ellison, Hall, & Kowbel, 2011). Genetic and geographic distance matrices of the 11x11 crosses were statistically significantly correlated (Mantel test). I performed reciprocal matings of all 121 strain pairings and collected data on the following reproductive traits: perithecial abundance, ascospore production, ascospore size distribution, ascospore color distribution (hyaline vs. pigmented), and ascospore germinability. In addition, for each of the 22 strains, conidial viability, size, and germination rate was determined and used as a measure of male fitness. Novel high throughput methods using flow cytometry were developed for this project and used to characterize the ascospores. To date, we have found the following: 1) The relationship between genetic distance and ascospore pigmentation (a measure of viability) indicates that intermediately related strain pairs produced more viable offspring than very closely or distantly related strain pairs. 2) Ascospore abundance was significantly positively correlated with ascospore size. 3) Reciprocal crosses showed that the female form of a strain was the dominant determinant of the reproductive success of crosses, regardless of male parentage. In conclusion, our findings show that inbreeding and outbreeding depression may play a role in shaping reproductive isolation between strains of *N. crassa*. Ongoing work seeks to identify the correlation between genetic variation in specific gene ontology groups and reproductive traits.

37) A method that exploits cell fusion to assay protein-protein interactions and co-localization in *Neurospora crassa*

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Neurospora crassa is a eukaryotic microbe that is an established model organism for the filamentous fungi. A gene knockout project is nearing completion and genome-wide analyses such as transcriptomics, proteomics and metabolomics are underway. However, a robust pipeline for assay of protein localization and protein-protein interactions is currently lacking. The objective of this study was to generate a platform that would allow high-throughput analysis of protein-protein interactions and protein co-localization for *N. crassa*. The method takes advantage of the tendency for *N. crassa* to undergo cell fusion during colony development and the ability to force heterokaryon formation by mixing cells with different auxotrophic mutations together on minimal medium. To implement this technique, we constructed recipient strains with mutations that result in a high frequency of homologous recombination of transforming DNA (Δ*mus-51* or Δ*mus-52*) and that eliminate repeat-induced point mutation (Δ*rid-1*), a pre-meiotic process that introduces mutations into duplicated genes during the sexual cycle. These recipients were transformed with vectors that would target V5-GFP or S-tag-RFP tagged fusion proteins to the *pan-2* or *inl* locus, respectively, creating pantothenate or inositol-requiring auxotrophs. Five sets of proteins that had been previously shown to interact were selected for this analysis: SAD-1/SAD-2, WC-1/WC-2, FRQ/FRH, OS-4/RRG-1 and GNB-1/GNG-1. In each case, one protein was targeted to *pan-2* and the other to *inl*. After crossing transformants to isolate homokaryons, strains containing the two different tagged proteins were mixed together on minimal medium to form heterokaryons. Western analysis using antibodies to the four tags demonstrated that all strains except SAD-2 expressed a tagged protein of the expected size. Fluorescence microscopy demonstrated co-localization of WC-1/WC-2 in the nucleus, FRQ/FRH in the cytoplasm, OS-4/RRG-1 in the cytoplasm, and GNB-1/GNG-1 in the cell membrane. Immunoprecipitation experiments indicated that the WC-1/WC-2, FRQ/FRH and OS-4/RRG-1 protein pairs interact *in vivo* in *N. crassa*. This high-throughput protein-protein direct interaction platform should be widely applicable to the filamentous fungi and beyond.

38) APS-1 and GRB are components of cytoplasmic and mitotic microtubule organizing centers. Olga Alicia Callejas Negrete[1], Rosa María Ramírez Cota[1], Pallavi Phatale[2], Michael Freitag[2], Robert W. Roberson[3], Salomón Bartnicki García[1] and Rosa R. Mouriño Pérez[1]. [1]Departamento de Microbiología. Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE). Ensenada, B.C., México. [2]Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR. USA. [3]School of Life Sciences. Arizona State University. Tempe, AZ. USA.

Pcp1 and Mto1 are two proteins responsible of the recruitment and organization of the γ-tubulin ring complex (γ-TuRC) to the inner and outer layer of the spindle pole bodies (SPBs) of *Schizosaccharomyces pombe*. GRB and APS-1 are the orthologous proteins in *Neurospora crassa*. We expected that GRB recruits γ-TuRC prior to mitosis for the proper formation of the mitotic spindle, and that APS-1 aids in polymerization of the cytoplasmic microtubules in SPBs as well as those in the cytoplasm. To test these hypotheses, we analyzed the role and dynamics of GRB and APS-1 by tagging them with GFP and/or mCherry. GRB was embedded in the nuclear envelope and co-localized in the SPB with γ-tubulin. GRB dynamics mirrored γ-tubulin behavior. We found GRB very closely associated with the centromere-specific H3, CenH3, during interphase. It also appeared that positioning of the γ-tubulin complex in the inner layer of the SPB is GRB-dependent. APS-1 is also present in the SPB where it co-localized with γ-tubulin. Additionally, APS-1 was found associated with cytoplasmic microtubules in a γ-tubulin-independent manner; it was not present in septa, as had been previously shown in *Aspergillus nidulans*. *Neurospora* GRB is essential but deletion of APS-1 was not lethal. We conclude that GRB assists in forming γ-tubulin-dependent MTOCs, but that APS-1 can organize microtubules with and without γ-tubulin.

39) Identification and characterization of key components of the WC-FLO, a novel molecular oscillator in *Neurospora crassa*

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All eukaryotes possess a circadian timing mechanism composed of at least a core circadian oscillator that uses proteins with opposing functions to form a negative feedback loop: positive-acting elements stimulate expression of the negative-acting elements, and the negative elements physically interact with the positive elements to inhibit their function. While the molecular details of the core oscillator are well described, new data suggests that the clock is comprised of multiple oscillators. The composition of the other oscillators, and the mechanisms by which multiple oscillators communicate with each other to generate robust rhythms in the organism, are not understood. In Neurospora, the core FRQ/WCC oscillator (FWO) is composed of the negative component FRQ/FRH and the positive component WCC. The FWO oscillator controls daily rhythms in gene expression and spore development. We recently discovered a set of genes, including *ccg-16* of unknown function, which are rhythmic in strains that lack FRQ, and thus a functional FWO, but which require the WCC for rhythmicity. These data indicated the existence of a second oscillator in Neurospora cells, called the WCC-FRQ-less oscillator (WC-FLO), which is coupled to the FWO through the WCC. We hypothesized that components of the WC-FLO physically interact with the WCC, similar to the interaction of FRQ with the WCC required negative feedback. To test this hypothesis, knockouts of genes encoding WCC interacting proteins are being examined for loss of *ccg-16:luciferase* reporter rhythms.

40) Identification and Functional analysis of New *Neurospora crassa* Nonself Recognition Loci

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Self/nonself recognition is a ubiquitous and essential function for many organisms. In filamentous fungi, self/nonself recognition is conferred by genetic differences at *het* (*het*erokaryon incompatibility) loci. The genes that mediate HI (heterokaryon incompatibility) exhibit characteristic evolutionary signatures, including balancing selection and trans-species polymorphisms. Recent analyses show that genes containing a HET domain are involved in HI, making HET domain genes good candidates for identifying new *het* loci. In this study, we utilized RNA-seq data from a population of 110 *Neurospora crassa* strains to look for HET domain genes that were highly polymorphic, have multiple alleles, and show balancing selection, and trans-species polymorphisms. Using this approach, we identified 19 of the 62 HET domain genes in *N. crassa* that fit the criteria for a *het* locus. Further, we showed that one of these HET domain genes, NCU09037, functions as a *het* locus.

41) The membrane transporter FLR1 confers tolerance of *Neurospora crassa* to the lignocellulytic fuel pretreatment byproduct furfural

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Ethanol bio-fuel derived from lignocellulosic biomass is a viable alternative to fossil-fuel-based transportation fuels. Pretreatment with a thermo and/or chemical process is necessary to make such sources available for enzymatic hydrolysis/fermentation but is accompanied by the production of compounds such as furaldehydes and phenolics that can inhibit efficient biomass utilization by fermentation. Profiling of the *Neurospora crassa* transcriptional response to furfural or 5-hydroxymethyl-2-furaldehyde (HMF) revealed changes in the expression of 2190 genes, determining that multiple processes, including oxidative stress, ribosomal biogenesis, amino acid synthesis, transport and RNA metabolism were affected. In a complementary approach, we also screened for strains that confer altered tolerance or furfural detoxification capabilities. Based on the proven functions of relevant genes from *Sacchromyces cerevisiae*, *Escherichia coli* and *Cupriavidus basilensis*, 25 *N. crassa* deletion mutants were assessed for growth in the presence of furfural (EC_{50}). A strain in which a gene encoding for the membrane transporter NCU05580 (designated *flr-1*) was deleted, conferred a ~30% increase in sensitivity to furfural (but not to other furans such as HMF and furfuryl alcohol). The mutant exhibited slow growth and was defective in aerial hyphae production. Overexpression of *flr-1* under a strong constitutive promoter (*cpc-1*$^{\Delta 2uorf}$) fully complemented the abnormal phenotype of ΔNCU05580. The overexpression strain grew 60% faster than the wild type in the presence of 20mM furfural (EC_{80}), confirming the functional link between *flr-1* and furfural sensitivity. In addition, we have isolated several furan-tolerant mutants following random insertion of the *cpc-1*$^{\Delta 2uorf}$ promoter cassette. Taken together, manipulation of genes involved in furan sensitivity/detoxification can provide a means for improving strains used for lignocellulose fermentation.

42) A cytogenetic approach to study calcium signaling genes of *Neurospora crassa*

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In *Neurospora crassa*, the calcium (Ca^{2+}) signaling pathway plays an important role in cellular communication and response to environmental stimuli. The Ca^{2+} signaling pathway is generally triggered by minute change in the intracellular free calcium concentration ($[Ca^{2+}]_2$), which in turn regulate activity of specific Ca^{2+}-sensors leading to transduction of specific cell signal. The change in $[Ca^{2+}]_2$ is mediated either by release of Ca^{2+} from intracellular stores or entry of Ca^{2+} from extracellular environment. However, in relation to second messenger molecules responsible for Ca^{2+} release from internal stores, the *N. crassa* Ca^{2+} signaling system differs significantly from its counterparts in plants and animal. We have been studying various Ca^{2+} signaling proteins such as CAMK-1, CAMK-2, CaM, NCS-1, PIK-1, CNA-1, and CNB-1, as well other proteins involved in cell signaling process such as NIK-2 and CRZ-1, using a cytogenetic approach. This approach is essentially based on *tcu-1* promoter driven regulated expression of the target Ca^{2+} signaling protein tagged with GFP or RFP. This system allows modulating expression of the target Ca^{2+} signaling protein by supplementing the medium with copper, thereby, facilitates studying the cell functions of essential genes. In addition, the GFP and RFP tags are used to determine the cellular localization and genetic interaction of the target Ca^{2+} signaling proteins. Thus, our study is aimed to gain deeper insight into the regulation and cell functions of Ca^{2+} signaling proteins in *N. crassa* using a cytogenetic approach.

43) BEM46 and auxin biosynthesis in *Neurospora crassa*

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The BEM46 protein is evolutionary conserved in eukaryotes (1). However, its function remains mostly elusive. We previously described the BEM46 protein to be targeted to the ER and being essential for ascospore germination in *Neurospora crassa* (2). As there is no *bem46* knock-out available from BROAD we established our own. While the Δ*bem46* mutant exhibits a lower ascospore germination than the wild type, it is still much higher than in the previously characterized over expressing and RNAi lines. Upon reinvestigation of the RNAi transformants we found strong evidence for accumulation of alternative spliced mRNA in the RNAi transformants. One alternative spliced mRNA is 0.5 and the other 1.2 kb in size, but both may encode small peptides of 123 and 40 amino acids, respectively. Both alternative spliced mRNAs were cloned and expressed in the wild type using the *cfp* promoter. Expression of either mRNA led to loss of ascospore germination, indicating that this phenotype is not due to down regulation or loss of the *bem46* mRNA, but caused by the alternative spliced mRNAs or rather their encoded peptides. The 123 amino acid peptide has a similar or even identical localization than the BEM46 protein. Using the *N. crassa* ortholog of the eisosomal protein PILA from *A. nidulans* we demonstrate partial co-localization of BEM46 with eisosomes (3).
Employing the yeast two-hybrid system, a single interaction partner, the anthranilate synthase component two (*trp*-1) was identified. This interaction was confirmed *in vivo* by a split-YFP approach. The Δ*trp*-1 exhibits a slightly reduced ascospore germination. Surprisingly, germinating conidiospores of the Δ*trp*-1 mutant produce ten fold higher amount of indoles compared to the wild type. A connection between BEM46 and tryptophan dependent auxine biosynthesis in *N. crassa* was observed. We describe the putative auxin biosynthetic pathway in the fungus using bioinformatical tools (3), and determined the transcription of the involved enzymes in different *bem46* wild type and mutant strains by qRT-PCR approaches. In addition to this the indole production of the strains in different developmental stages was also investigated.

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(2) MERCKER M, KOLLATH-LEIß K, ALLGAIER S, WEILAND N, KEMPKEN F (2009) The BEM46-like protein appears to be essential for hyphal development upon ascospore germination in *Neurospora crassa* and is targeted to the endoplasmic reticulum. *Curr Genet* 55:151-161

(3) KOLLAT-LEIß K, BÖNNIGER C, SARDAR P, KEMPKEN F (2013) Eisosomal localization and association of BEM46 with a trypthophan-derived auxin pathway in *N. crassa.* in preparation

44) Conserved RNA helicase FRH acts Nonenzymatically to Support the Intrinsically Disordered Neurospora clock protein FRQ. Jennifer M. Hurley[1], Luis F. Larrondo[2], Jennifer J. Loros[1,3], Jay C. Dunlap[1*] [1] Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH 03755, USA [2]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile [3] Department of Biochemistry, Geisel School of Medicine at Dartmouth, Hanover, NH 03755, USA

Protein conformation dictates a great deal of protein function. A class of naturally unstructured proteins, termed Intrinsically Disordered Proteins (IDPs), demonstrates that flexibility in structure can be as important mechanistically as rigid structure. At the core of the circadian transcription/translation feedback loop in *Neurospora crassa* is the protein Frequency (FRQ), shown here to share many characteristics of IDPs. FRQ in turn binds to Frequency Interacting RNA Helicase (FRH), whose clock function has been assumed to relate to its predicted helicase function. However, mutational analyses reveal that the helicase function of FRH is not essential for the clock, and a region of FRH distinct from the helicase region is essential for stabilizing FRQ against rapid degradation via pathway distinct from its typical ubiquitin-mediated turnover. These data lead to the hypothesis that FRQ is an IDP and that FRH acts nonenzymatically, stabilizing FRQ to enable proper clock circuitry/function.

45) Pre-exocytic vesicular organization: *Neurospora crassa* YPT-1 regulates vesicular traffic at the Golgi and at the Spitzenkörper.

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In eukaryotic cells secretion mechanisms require the coordinated action of small Rab GTPases, which interact with the membrane of vesicles and promote the association with other factors and the subsequent fusion of the vesicles with a target membrane. In contrast to other eukaryotic model systems, filamentous fungi contain the Spitzenkörper (Spk), a multi-vesicular complex found at the hyphal apex to which cargo-carrying vesicles arrive before being redirected to specific cell sites. The exact regulatory mechanisms utilized by hyphae to ensure the directionality of the secretory vesicles that reach the Spk are still unknown. Hence, we have analyzed the *N. crassa* Rab-GTPase YPT-1 (Rab1), a key regulator of the secretory pathway involved in ER-Golgi and late endosome-Golgi traffic steps in *Saccharomyces cerevisiae*. Laser scanning confocal microscopy of strains expressing fluorescently tagged versions of YPT-1 revealed its localization at the Spk and at Golgi equivalents. Co-expression of differently labeled YPT-1 and the post-Golgi Rab GTPases SEC-4 (Rab8) and YPT-3 (Rab11) showed that YPT-1 was confined at the microvesicular core of the Spk, while SEC-4 and YPT-3 localize in the Spk peripheral macrovesicular layer, suggesting that trafficking of macro and microvesicles of the Spk are regulated by distinct Rabs. Co-localization analysis of YPT-1 with the early Golgi markers USO-1 (p115) and VRG-4, and the late Golgi marker VPS-52 indicated the participation of this Rab at early and late Golgi vesicle trafficking steps. TIRFM (Total Internal Reflection Fluorescence Microscopy) revealed anterograde and retrograde movement of YPT-1 associated vesicles, sometimes decorating tubule-like structures at distal hyphal regions; and a significant flow of vesicles reaching apical and subapical regions. FRAP (Fluorescence Recovery After Photobleaching) analysis showed a fast recovery of GFP-YPT-1 fluorescence due to vesicles arrival at the Spk, confirming TIRFM observations. On sucrose density gradients, GFP-YPT-1-associated particles sedimented mainly in fractions with a density of 1.124-1.132 g/mL, which coincides with the density of microvesicles as previously shown. We propose that YPT-1 is an important component in the regulation of secretory vesicles traffic in filamentous fungi; its intracellular distribution and dynamics at Golgi structures and at the Spk core suggests that this Rab GTPase participates during both, early and late steps of protein secretion.

46) Connection between glycogen metabolism regulation, light and the circadian clock in *Neurospora crassa*

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In *Neurospora crassa*, a eukaryotic model for light responses and the circadian clock, genome-wide binding sites of the core clock component and blue-light photoreceptor WCC were identified using chromatin immunoprecipitation (ChIP) sequencing. The direct WCC targets were enriched for transcription factors (TFs), including TFs previously identified as regulators of glycogen metabolism. These data suggested that glycogen metabolism is indirectly regulated by light and the circadian clock. In support of this idea, we found that glycogen levels accumulate with a circadian rhythm. Furthermore, transcripts from *gsn* and *gpn*, encoding glycogen synthase and glycogen phosphorylase, respectively, are light induced and clock controlled. Using ChIP-PCR, we demonstrated that the transcription factor VOS-1 binds to the *gsn* and *gpn* promoters in the dark and in the light. The *vos-1*^{KO} mutant strain showed an impaired profile in glycogen accumulation during vegetative growth as compared to wild-type strains. These data support a role for VOS-1 in glycogen metabolism regulation. Experiments are currently underway to test if light and clock control of *gsn* and *gpn* are abolished in the *vos-1*^{KO} strain. Supported by FAPESP, CNPq and NIH P01 GM068087.

47) Transcriptional comparison of the filamentous fungus *Neurospora crassa* growing on three major monosaccharides D-glucose, D-xylose and L-arabinose.

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D-glucose, D-xylose and L-arabinose are three major monosaccharides in the plant cell wall. Complete utilization of all three sugars is still a bottleneck for second-generation cellulolytic bioethanol production, especially for L-arabinose. However, little is known about the gene expression profiles during L-arabinose utilization in fungi at genome-wide level. Using next-generation sequencing technology, we have analyzed the transcriptome of *N. crassa* grown on L-arabinose versus D-xylose, with D-glucose as the reference. We found that the gene expression profiles on L-arabinose were dramatically different from those on D-xylose. It appears that L-arabinose can rewire the fungal cell metabolic pathway widely and provoke the expression of many kinds of sugar transporters, hemicellulase genes and transcription factors. Three novel sugar transporters were identified and characterized for their substrates here, including one glucose transporter GLT-1 and two novel pentose transporters, XAT-1, XYT-1. One transcription factor associated with the regulation of hemicellulase genes, HCR-1 was also characterized in present study.

48) Neurospora importin-α (Nucleoporin-6) directs chromatin modifying complexes to sub-nuclear chromatin targets

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Please see Perkins Award lecture abstract

49) Fungal Nutritional ENCODE Project

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Conversion of lignocellulosic plant biomass to biofuels holds great potential for alleviating our reliance on fossil fuels. A major goal in this area of research is to engineer strains of fungi that can reliably secrete effective hydrolytic enzymes for deconstruction of plant biomass. To better engineer fungi, an understanding of how filamentous fungi respond to and degrade this compositionally diverse material is required. Using *Neurospora crassa* as a model, we have begun to characterize the transcriptional regulation and function of genes involved in carbon metabolism. We have collected and sequenced mRNA transcripts from *N. crassa* grown on a wide variety of specific plant cell wall components from mono and disaccharides to complex polysaccharide components, such as cellulose, hemicellulose and pectin. By comparative analyses of plant biomass deconstruction pathways, we can define novel hypotheses regarding function of unannotated genes, potential regulators of transcription of different subsets enzymes and signaling molecules that induce these regulatory circuits.

50) Function of the AOD2 and AOD5 transcription factors in the expression of AOX and gluconeogenesis genes in *Neurospora crassa*

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In *Neurospora crassa*, alternative oxidase (AOX) is encoded by the *aod-1* gene. AOX expression is induced when the standard electron transport chain functions poorly. The induction of AOX requires two transcription factors, AOD2 and AOD5, which are known to bind to the alternative oxidase induction motif (AIM) in the *aod-1* promoter region. To determine the intracellular location of AOD2 and AOD5 in both inducing and non-inducing conditions, we have constructed strains expressing only tagged versions of the two proteins. Following isolation of purified subcellular fractions, we show that AOD2 and AOD5 are found in the nucleus under all conditions tested. In addition, chromatin immunoprecipitation followed by quantitative PCR demonstrates that AOD2 and AOD5 are constitutively bound to the AIM sequence. This suggests that the bound heterodimer may activate transcription of *aod-1* in response to signals arising from poorly functioning mitochondria. In addition, the orthologues of AOD2 and AOD5 have been shown to be required for expression of genes encoding enzymes involved in gluconeogenesis in other fungi and we have now shown that AOD2 and AOD5 are required for growth in various non-fermentable carbon sources. We are currently examining the relationship between binding of AOD2 and AOD5 at various promoters and the expression of genes in response to growth in different carbon sources and AOX inducing conditions.

51) A homology based search for Neurospora *rec* genes.

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rec-1$^+$, *rec-2*$^+$ and *rec-3*$^+$ are dominant *trans*-acting genes that suppress meiotic recombination in specific regions of the *Neurospora crassa* genome. For example, up to 1% of progeny from a *rec-2* by *rec-2* cross experience recombination in *his-3* but only 0.005% when one or both parents carries *rec-2*$^+$. *rec-2*$^+$ resides in a 10 kb stretch of DNA absent from *rec-2* strains, which have instead a 2.8 kb stretch of unique DNA. Several pieces of evidence show that meiotic silencing at this large insertion is responsible for the dominance of *rec-2*$^+$. If meiotic silencing is blocked by *sad-1*, recombination at *his-3* is increased substantially in *rec-2*$^+$ by *rec-2* heterozygotes, a *rec-2* deletion behaves like *rec-2*$^+$ as a dominant suppressor of recombination and, finally, inserting *rec-2* at the same location in a mating pair yields a high recombination frequency at *his-3*. Indeed, meiotic silencing is responsible for the apparent dominance of all three *rec*$^+$ genes. In the absence of meiotic silencing, recombination frequencies at *his-1* in *rec-1*$^+$/*rec-1* heterozygotes and at *am* in *rec-3*$^+$/*rec-3* heterozygotes are indistinguishable from those of *rec-1* and *rec-3* homozygotes respectively. Thus, the products of *rec-1*, *rec-2* and *rec-3* act to promote recombination in specific regions of the genome and are probably all genes located within indels that are regulated by meiotic silencing. We have sequenced the genome of a *rec-1* strain by PacBio technology to search for large indels in the appropriate genomic region. Of three candidate indels, a 4.7kb insertion unique to the *rec-1* strain has homology to the predicted *rec-2* gene.

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