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Program and Abstracts, Neurospora 2012

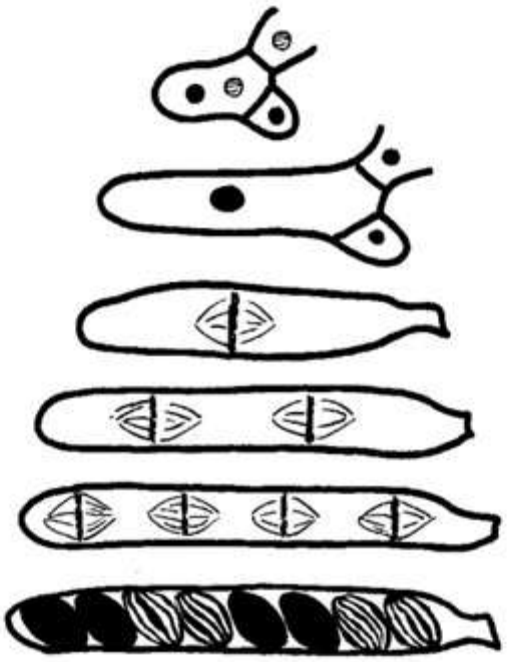
Abstract

Program and Abstracts from the Neurospora 2012 meeting at Asilomar, March 8 - 11.

PROGRAM

and

ABSTRACTS



NEUROSPORA 2012

March 8 - 11
Asilomar Conference Center

Pacific Grove
California

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Neurospora 2012

March 8 - 11

Asilomar Conference Center

Pacific Grove
California

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Reception sponsor

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Brief Schedule

DAY	MORNING	AFTERNOON	EVENING
Thursday		Arrival	Dinner
March 8		Registration	Mixer (Kiln)
Friday	Breakfast	Lunch	Dinner
March 9	Plenary Session I	Plenary Session II	Poster Session I
Saturday	Breakfast	Lunch	Banquet
March 10	Plenary Session III	Plenary Session IV	Poster Session II
Sunday	Breakfast	Lunch	
March 11	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 on Saturday evening.

Schedule of Activities

Thursday, March 8

16.00 – 18.00 Registration, Administration
18.00 Dinner, Crocker
19.00 – 22.00 Mixer (Kiln), **Sponsored by Intrexon Corporation**

Friday, March 9

7.30 – 8.30 Breakfast, Crocker

Morning Cell Biology and Morphogenesis Fred Farr Forum (Chair: Meritxell Riquelme)

8.30 – 8.40 Welcome and announcements (NPC)
8.40 – 9.00 **Michael Plamann** Dynein heavy chain mutations cause multiple mislocalization phenotypes
9.00 – 9.20 **Steve Free** Neurospora cell wall synthesis and functions
9.20 – 9.40 **Leonora Martínez-Núñez** Localization of the β (1-3) endoglucanases **EGLC-1** and **EGLC-2** and their role in the morphogenesis of *Neurospora crassa*
9.40 – 10.00 **Rosa Mourino-Perez** Timeline of proteins involved in septum formation in *Neurospora crassa*

10.00 – 10.30 Coffee break

10.30 – 11.10 **Greg Jedd** Beadle & Tatum Award Lecture Bioinformatics identifies septal pore associated proteins in *Neurospora crassa*
11.10 – 11.30 **Lynn Epstein** The *Neurospora crassa* mutant NcdeltaEgt-1 identifies an ergothioneine biosynthetic gene and demonstrates that ergothioneine enhances conidial survival and protects against peroxide toxicity during conidial germination
11.30 – 11.50 **Frank Nargang** Components and Function of the Neurospora TOB complex

12.00 – 14.00 Lunch, Crocker

Business Meeting and Presentation of Awards

Afternoon Gene Regulation Fred Farr Forum (Chair: Jason Stajich)

14.30 – 14.50 **Luis Corrochano** Interactions between light and development in the regulation of *Neurospora* gene expression
14.50 – 15.10 **Fernanda Freitas** Functional and structural characterization of N. crassa proteins identified in DNA-protein complexes
15.10 – 15.30 **Michael Rountree** The control and function of Histone H3 Lysine 27 trimethylation
15.30 – 15.50 **Michael Freitag** Centromere foundation proteins of *Neurospora*

15.50 – 16.20 Coffee break

16.20 – 16.40 **Kyle Pomraning** A dynamin-like protein affects both RIP and premeiotic recombination
16.40 – 17.00 **Eugene Gladyshev** Biochemical properties of NcRVT protein encoded by a reverse transcriptase-like gene from *Neurospora crassa*
17.00 – 17.20 **David Kowbel** Antisense transcription in the fungus *Neurospora crassa*
17.20 – 17.40 **Yi Liu** Small RNA biogenesis pathways in *Neurospora*

Evening

18.00 Dinner, Crocker
19.00 – 22.00 Poster session, Kiln

Saturday, March 10

Morning Signaling and Development Fred Farr Forum (Chair: André Fleißner)

7.30 – 8.30 Breakfast, Crocker

8.30 – 8.50 **Stefanie Pöggeler** A homolog of the mammalian STRIPAK complex controls sexual development in *Sordaria macrospora*
8.50 – 9.10 **André Fleißner** Structural features of sterols influence cell signaling and fusion
9.10 – 9.30 **Javier Palma-Guerrero** *lfd-1*: a novel gene required for membrane merge during cell fusion in *Neurospora crassa*
9.30 – 9.50 **Maria Célia Bertolini** Extracellular pH and glycogen metabolism regulation in *Neurospora crassa*. New insights into the pH signaling pathway

9.50 – 10.20 Coffee break

10.20 – 10.40 **Oded Yarden** Phosphorylation of COT1 at S189 is required for early polar growth and is involved in regulation of micro/macro-conidiation in association with MOB2A/B
10.40 – 11.00 **Jacqueline Servin** Global analysis of serine-threonine protein kinase genes
11.00 – 11.20 **Barry Bowmann** Calcium ATPases in the ER and Golgi are required for normal morphological development in *Neurospora crassa*.
11.20 – 11.40 **Nick Read** Calcium signaling in *Neurospora crassa*

12.00 – 13.00 Lunch, Crocker

Afternoon Light and circadian clock Fred Farr Forum (Chair: Luis Larrondo)

14.30 – 14.50 **Deb Bell-Pedersen** Direct Transcriptional Control of the OS MAPK Pathway by the Circadian Clock
14.50 – 15.10 **Jennifer Hurley** Light inducible system for tunable protein expression in *Neurospora crassa*
15.10 – 15.30 **Christian Heintzen** Global light- and temperature responses in a clockless strain of *Neurospora*
15.30 – 15.50 **Patricia Lakin-Thomas** Potential components of the FRQ-less oscillator

15.50 – 16.20 Coffee break

16.20 – 16.40 **Martha Merrow** Temperature compensation of circadian entrainment in *Neurospora* and human cells
16.40 – 17.00 **Chandrashekara Mallappa** Roles for CSP-1 in light and circadian clock-regulated gene expression
17.00 – 17.20 **Luis Larrondo** Transcriptional regulators and time-of-day-specific gene expression in the *Neurospora* circadian system: a spatiotemporal approach
17.20 – 17.40 **Michael Brunner** Light-activated and clock-controlled transcription activation by the white collar complex

Evening

18.00 – 19.30 Banquet, Crocker
Banquet Speaker: **Jennifer Loros**

19.30 – 22.00 Poster session (Kiln)

Sunday, March 11

Morning Genomics, Evolution and Technology Fred Farr Forum (Chair: Charles Hall)

7.30 – 8.30 Breakfast, Crocker

8.30 – 8.50 **Scott Baker** Associating genes with phenotypes in *Neurospora* mutant strains
8.50 – 9.10 **Charles Hall** Perkins Award Recipient The development of genetics and genomics for analysis of complex traits in the model filamentous fungus, *Neurospora crassa*
9.10 – 9.30 **Chris Ellison** Perkins Award Recipient Harnessing natural variation to identify gene regulatory networks in *Neurospora crassa*
9.30 – 9.50 **Elizabeth Znameroski** Induction of lignocellulose degrading enzymes in *Neurospora crassa* by cellodextrins

9.50 – 10.20 Coffee break

10.20 – 10.40 **David Catcheside** Insights into recombination using gfp and next generation sequencing
10.40 – 11.00 **Jason Stajich** Comparative genomics of *Neurospora* and other fungi with FungiDB
11.00 – 11.20 **Jeremy Zucker** Genome-scale reconstruction and validation of *Neurospora crassa* metabolism
11.20 – 11.40 **Jay Dunlap et al.** The *Neurospora* Functional Genomics Program Project

11.00 Checkout

12.00 – 13.00 Lunch, Crocker (or box lunches)

Invited Talk Abstracts

Dynein heavy chain mutations cause multiple mislocalization phenotypes
Robert Schnittker, Senthilkumar Sivaguraunathan, David Razafsky, Stephen King, and Michael Plamann School of Biological Sciences, University of Missouri-Kansas City, MO 64110 plamannm@umkc.edu

Cytoplasmic dynein transports cargoes that are crucial for a variety of cellular functions. We utilized the ascomycete fungus *Neurospora crass*a in a series of genetic, cell biological and biochemical analyses to study dynein function and regulation. Thirty-four dynein heavy chain mutations were isolated using a genetic screen and characterized by performing a series of localization studies. Our studies revealed that dynein can mislocalize as long linear tracks, comet tails, aggregates, and dispersed signals in mutant strains and can alter microtubule organization and nuclear distribution to varying degrees. Biochemical analyses of dynein from one of the mutant strains revealed a potential link between in vitro biochemical properties and intracellular function of dynein. We propose a model in which dynein normally cycles to the hyphal tip and from there to distal regions as it performs its transport functions. The multiple mislocalization phenotypes we observed may represent the entrapment of dynein at different stages of the dynein transport cycle.

Neurospora cell wall biosynthesis and functions.
Stephen J. Free, Abhiram Maddi, and Asuma Tanaka, SUNY University at Buffalo, Dept. of Biological Sciences, Buffalo, NY 14260 free@buffalo.edu

The cell wall is a vital structure for the growth and survival of filamentous fungi. The wall consists of a chitin/β-1,3-glucan matrix into which integral cell wall proteins are covalently incorporated. The chitin and glucans are synthesized on plasma membrane-associated chitin and glucan synthases. The cell wall proteins are synthesized on ER-associated ribosomes and pass through the canonical secretory pathway before being secreted into the cell wall space. The chitin, glucans and cell wall proteins are cross-linked together to generate the cell wall structure. We have demonstrated that the N-linked galactomannans are required for the incorporation of cell wall proteins into the cell wall chitin/glucan matrix. These galactomannans are generated by the post-translational modification of cell wall proteins as they pass through the secretory pathway. We have also identified two GPI-anchored cell wall α-1,6-mannanases, DFG5 and DCW1, which are required for the incorporation of the cell wall proteins into the cell wall. We present a model in which DFG5 and DCW1 function to cross-link the galactomannans into the cell wall, and thereby incorporate cell wall proteins into the cell wall matrix. We also report that an α-1,3-glucan synthase functions in a cell wall biosynthesis during the development of aerial hyphae and conidia. We show that α-1,3-glucan is found in the cell walls of developing conidia, and that mutants lacking the α-1,3-glucan synthases are defective in aerial hyphae and conidia development. This demonstrates that differences in cell wall glucans, as well as differences in cell wall proteins, contribute to cell type-specificity.

Localization of the β (1-3) endoglucanases EGLC-1 and EGLC-2 and their role in the morphogenesis of *Neurospora crassa*.
Martinez-Nunez, Leonora; Riquelme, Meritxell Centro de Investigacion Cientifica y Educacion Superior de Ensenada, Baja California, Mexico.

The unitary model of cell wall growth suggests that the polarized extension of hyphae in filamentous fungi is the combined result of the synthesis and discharge of new cell wall polymers, the action of hydrolytic enzymes that provide plasticity to the wall and turgor pressure to drive cell expansion. There is limited information on enzymes capable of hydrolyzing cell wall polymers and that could be contributing to plasticize the cell wall. EGLC-1 and EGLC-2 are putative β (1-3) endoglucanases in *Neurospora crassa*, with potential binding sites for a glucosyl phosphatidylinositol group (GPI), which would allow them to get anchored into the plasma membrane. Using fusion PCR and cloning plasmid, two recombinant vectors were constructed to tag each of the putative endoglucanases with GFP. In one vector, the gfp sequence was inserted within the eglc-1 encoding sequence, just after the signal peptide encoding sequence. For the eglc-2 sequence, the gfp sequence was inserted before the GPI-binding site. These plasmids were expressed in *N. crassa* strain FGSC # 9717 and prototrophic strains expressing EGLC-1-GFP or EGLC-2-GFP were analyzed by confocal laser scanning microscopy to monitor the location of the GFP-tagged proteins. Both proteins were localized in the hyphal apical plasma membrane and in septa. The fluorescence patterns observed at each of these sites were different for each protein. These results show that lytic activity of enzymes such as endoglucanases EGLC-1 and EGLC-2 in *N. crassa* are present in fungal morphogenesis, where they probably play a role in cell wall remodeling, as postulated by the unitary model of cell wall growth.

Timeline of proteins involved in septum formation in *Neurospora crassa*

Delgado-Alvarez, Diego L. [1], Olga A. Callejas-Negrete[1], Arianne Ramirez-Del Villar[1], Stephan Seiler[2], Salomon Bartnicki-Garcia[1], Rosa R. Mourino- Perez[1] [1]Departamento de Microbiologia. CICESE, Mexico. [2]University of Goettingen, Germany.

The cellular machinery responsible for cytokinesis and septum formation is well conserved in the eukaryotes. These processes share three basic steps: selection of the division plane, assembly of an actin contractile ring (CAR) and the constriction of the CAR coupled with the invagination of plasma membrane at these sites. To maintain an orderly sequence of these steps, spatial cues and temporal controls must play a crucial role. To study temporal distribution, we performed live-cell imaging of *Neurospora crassa* strains carrying GFP fusions involved in different stages of septum formation. For site selection, we used the landmark protein Bud4. To monitor actin ring formation, we followed the starting Rho4 module (Rho4, Bud3 and Rgf3), responsible for the activation of formin (Bni), which in turn promotes actin polymerization. We imaged the actin cytoskeleton by means of five actin binding proteins: TPM, coronin, FIM, Arp3 and Lifeact. We measured the times at which the proteins appeared in relation to the internalization of plasma membrane labeled with FM4-64. We were able to construct a timeline of the proteins involved in septum formation. For the most part the sequence was the same as in other organisms. Surprisingly, we found that actin cables are formed at future septation sites much earlier than previously thought.

Bioinformatics identifies septal pore associated proteins in *Neurospora crassa*

Gregory Jedd, Temasek Life Sciences Laboratory, and Department of Biological Sciences, National University of Singapore, Singapore 117604

Like animals and plants, multicellular fungal hyphae possess cell-to-cell channels that allow intercellular cooperation, and communication. Using a combination of mass spectrometry of Neurospora Woronin body associated proteins, and a bioinformatics approach that identifies related proteins based on composition and character, we have identified 17 septal pore associated (SPA) proteins that localize in rings around the pore, and in pore-centered foci. SPA proteins are not homologous at the primary sequence level, but share overall physical properties with intrinsically disordered proteins. Some SPA proteins form aggregates at the septal pore, and in vitro assembly assays suggest self-assembly through a novel non-amyloidal mechanism involving mainly random coil structural moieties. SPA loss-of-function phenotypes include excessive septation, septal pore degeneration, and uncontrolled Woronin body activation. These data identify a new family of disordered proteins that control cell-to-cell communication, and diverse aspects of septal homeostasis.

The *Neurospora crassa* mutant NcdeltaEgt-1 identifies an ergothioneine biosynthetic gene and demonstrates that ergothioneine enhances conidial survival and protects against peroxide toxicity during conidial germination.

Lynn Epstein[1], Marco H. Bello[1], Viviana Barrera-Perez[1], and Dexter Morin[2]. [1]Department of Plant Pathology and [2],Department of Molecular Biosciences, University of California, Davis, CA 95616, USA. lepstein@ucdavis.edu

Ergothioneine (EGT) is a histidine derivative with sulfur on the imidazole ring and a trimethylated amine; it has been postulated to have an antioxidant function. We used the EGT monobromobimane derivative to identify EGT by LC/MS and for quantification. EGT concentrations are significantly (α=0.05) higher (5X) in wild type conidia than in mycelia. The knockout in gene NCU04343 does not produce EGT. An *in silico* analysis suggests that 1) the gene, named NcEgt-1, was acquired early in the mycota lineage as a fusion of two adjacent prokaryotic genes, that was then lost in the Saccharomycotina, and that 2) NcEgt-1 catalyzes the first two steps of EGT biosynthesis from histidine to hercynine to hercynylcysteine sulfoxide. Comparisons of the wild type with the knock-out indicate that endogenous EGT 1) helps protect conidia during the quiescent period between conidiogenesis and germination, 2) has a role in conidial longevity, 3) quenches peroxide *in vivo* and 4) helps protect conidia during the germination process from peroxide but not from superoxide or Cu^{2+}. The data will be discussed in relation to the translocation of glutathione from vacuoles to the cytoplasm before germ tube emergence.

Components and function of the *Neurospora crassa* TOB complex.
Frank E. Nargang, Sebastian W.K. Lackey, and Jeremy G. Wideman. Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9. Email: frank.nargang@ualberta.ca.

Various proteins of the mitochondrial outer membrane, including all beta-barrel proteins, are inserted into the membrane by the TOB complex (topogenesis of beta-barrel proteins), also known as the SAM complex (sorting and assembly machinery). The major protein of the complex is Tob55. We have found that Tob55 exists in various complexes that can be purified from the outer membrane. The core complex consists of Tob55, Tob38, and Tob37 whereas the holo complex also includes the Mdm10 protein. The three core complex components are essential for viability. Deficiency of any holo-complex protein results in inefficient beta-barrel protein assembly. Mdm10 is a multifunctional protein that has also been identified as a component of the ERMES complex (endoplasmic reticulum-mitochondrial encounter structure) that has been recently identified in *Saccharomyces cerevisiae*. The complex is thought to be important for lipid and calcium exchange between the two organelles. Other components of the ERMES complex include Mdm12 and Mmm1. We have shown that deficiency of these proteins also decreases beta-barrel assembly into the outer membrane. Mutants lacking any of the ERMES complex members are also characterized by enlarged mitochondria. We are currently investigating the roles of different functional domains in the proteins of the TOB and ERMES complexes.

Interactions between light and development in the regulation of *Neurospora* gene expression
Carmen Ruger-Herreros, Luis M. Corrochano, Universidad de Sevilla, Sevilla, Spain. corrochano@us.es

Several environmental cues, including blue light, promote conidiation, a developmental pathway that leads to conidiophores and the production of conidia. The activation by light of key regulatory genes may explain the activation by light of conidiation. Several mutants have been isolated that are blocked at different stages of conidiation. Mutation in *fluffyoid* (*fld*) or *fluffy* (*fl*) block conidiation during formation of minor or major constrictions, respectively. The FL protein is a 792-amino acid polypeptide with a Zn2Cys6 binuclear zinc cluster from the Gal4p family. Blue light activates *fl*, and light regulation operates through the transient binding of the White Collar Complex (WCC) to the promoter of *fl*. We proposed earlier that FLD regulates *fl* expression by promoting its repression in the dark. We have identified *fld* after sequencing in the *fld* mutant candidate genes located in the vicinity of the *fld* locus. The FLD protein contains 676 amino acids with a Zn2Cys6 binuclear zinc cluster in the amino-end. The gene *fld* is not induced by light, but is missregulated in the absence of *fl* suggesting an interaction between FLD and FL. We have observed the activation by light of a set of *Neurospora* genes in the absence of *fl* suggesting that FL inhibits the WCC. FL, FLD, and the WCC may compete directly or indirectly for regulatory binding sites in some promoters resulting in light and/or developmental regulation.

Functional and structural characterization of N. crassa proteins identified in DNA-protein complexes.
[1]Fernanda Z Freitas, [2]Angelo J Magro, [1]Henrique C dePaoli, [2]Marcos RM Fontes, [1]Maria C Bertolini. [1]IQ-UNESP, Araraquara, SP, Brazil. [2]IB-UNESP, Botucatu, SP, Brazil

Eukaryotic gene expression is regulated by combining DNA-protein(s) interactions and simultaneous changes in chromatin structure. Attempts to identify protein(s) binding the *gsn* promoter STRE motif returned five transcriptional regulators candidates. Among them, the ORFs NCU03482 and NCU06679 products are noteworthy. Analysis of their polypeptide sequences revealed protein domains involved in transcription regulation. The NCU03482 product (RUVBL1-like helicase) belongs to the AAA/Tip49 family involved with chromatin remodeling and transcription regulation. The NCU06679 product (HAT type-B-subunit-2), features five WD-40 domains and comprise a family of eukaryotic proteins implicated in transcription regulation. Their three-dimensional structures were determined based on the protein structures of, respectively, human RUVBL1 (PDB 2C9O) and *Drosophila* Nurf55 (PDB 2XYI). Both have NLS but no DNA-binding sites. The recombinant RUVBL1-like was able to form a DNA-protein complex with the STRE *gsn* promoter fragment suggesting that it might oligomerize to exert its activity. Our search also reveals that *N. crassa* RUVBL1 and Nurf55, together with the other candidates, have orthologues in MLL1/MLL, BAF53, Sin3A,and NuA4 chromatin modification complexes, thus forming a multiprotein-complex that might modulate the chromatin dynamics at the *gsn* locus controlling *gsn* transcript levels.

Control and function of histone H3 lysine 27 trimethylation in Neurospora

Michael R. Rountree[1], Kirsty S.F. Jamieson[1], Zachary A. Lewis[2], Jason E. Stajich[3] and Eric U. Selker[1]. [1] Institute of Molecular Biology, University of Oregon, Eugene, OR 97403. [2] Department of Microbiology, University of Georgia, Athens, GA 30602. [3] Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521

Histones carry substantial information in the form of reversible covalent modifications that play important roles in genetic and epigenetic process, including gene expression, recombination, repair and DNA methylation. Trimethylation of lysine 27 of histone H3 (H3K27me3) is the basis of the epigenetically-inherited repression/"cellular memory" by the Polycomb Group (PcG) proteins, first discovered in Drosophila. Although not fully understood, H3K27me3 plays roles in higher eukaryotes in homeotic gene regulation, X-chromosome inactivation, imprinting and cell proliferation. While budding and fission yeast lack H3K27me3, Neurospora possesses this modification and offers a great model to study it. H3K27me3 occupies ~7% of the Neurospora genome showing particular concentration near telomeres. Our studies of the control and function of H3K27me3 in Neurospora will be presented.

Centromere foundation proteins of Neurospora

Kristina M. Smith, Pallavi Phatale, Jonathan M. Galazka, Sarah Ferrer, Alec J. Peters, Lanelle Connolly and Michael Freitag. Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, USA.

Centromeric DNA, the centromere-specific histone H3 variant (CenH3), and centromeric DNA binding proteins form the foundation for attachment of kinetochore protein complex assembly. Correct assembly and proper maintenance of these large and cell cycle-regulated complexes is essential for attachment of spindle microtubules, which transport chromosomes into daughter nuclei during nuclear division. Over the past decade little information has emerged on centromere and kinetochore organization in filamentous fungi, even though these protein complexes are essential, and to date, only centromeres of *Neurospora crassa* have been studied in any detail. This was enabled by early groundbreaking studies on the underlying centromeric DNA structure [1, 2] and the availability of an arsenal of genetic, biochemical and cytological tools to study centromere proteins and centromere DNA composition. We analyzed the almost complete Neurospora genome for the presence of satellite or other near-repetitive sequences [3], confirming earlier studies [2] that predicted Neurospora centromeric DNA to be composed of relics of transposable elements. To learn more about centromere assembly and maintenance, we subjected *Neurospora crassa* to ChIP-sequencing with tagged CenH3, CEN-C and CEN-T, as well as antibodies against histone modifications thought to be required for centromere function [3]. Our findings suggest that centromere maintenance in Neurospora is qualitatively different from that in fission yeast, where expression of small RNA and subsequent heterochromatin formation is required for the assembly but not maintenance of centromeres. To better understand centromere assembly we are dissecting protein interactions between different centromere foundation proteins, namely CenH3, CEN-B, CEN-C and CEN-T, by genetic and biochemical means.

[1] M. Centola and J. Carbon, 1994, Mol. Cell. Biol. 14: 1510-1519.
[2] E. B. Cambareri *et al.*, 1998, Mol. Cell. Biol. 18: 5465-5477.
[3] K. M. Smith *et al.*, 2011, Mol. Cell. Biol. 31: 2528-2542.

A dynamin-like protein affects both RIP and premeiotic recombination

Kyle R. Pomraning[1], Ann Kobsa[2], Eric U. Selker[2] and Michael Freitag[1] [1]Program for Molecular and Cellular Biology and Department of Biochemistry & Biophysics, Oregon State University, Corvallis, OR, USA; [2]Institute of Molecular Biology, University of Oregon, Eugene, OR, USA.

Repeat-induced point mutation (RIP) and premeiotic recombination affect gene-sized duplications in many filamentous fungi. RIP causes G:C to T:A transition mutations while premeiotic recombination can result in loss of repeated DNA segments [1]. Both processes occur after fertilization but prior to meiosis and can be very efficient, in some cases mutating and/or deleting the duplication in essentially every nucleus. At least in *Neurospora crassa*, RIP has countered the expansion of gene and transposon families [2], suggesting that genome streamlining and protection from transposition events may yield long-term benefits to Neurospora populations. We employ genetic approaches to elucidate the mechanism of premeiotic recombination and RIP. Here we report the successful identification of semi-dominant mutations that affect both of these processes by using UV mutagenesis, followed by a screen for reduced RIP of linked duplications of *hph* and *pan-2*. Classical genetic mapping and complementation tests revealed that a mutation in the histone H3 gene, $hH3^{dim\text{-}4}$, is responsible for greatly reduced RIP of one mutant. We identified two additional mutations by bulk segregant analysis and high-throughput Illumina sequencing. Single point mutations were found in the same gene, encoding a novel dynamin-like long GTPase, albeit in different conserved domains. Both premeiotic recombination and RIP frequencies are affected, supporting the idea that these processes are mechanistically linked. To investigate this further, we are screening the Neurospora single gene deletion collection for mutants that show RIP defects, starting with deletion mutants that are known or expected to affect recombination pathways. [1] Galagan, J., and Selker, E.U. (2004). Trends in Genetics 20, 417-423. [2] Selker, E.U. (1990). Annu. Rev. Genet. 24, 579-613.

Biochemical properties of NcRVT protein encoded by a reverse transcriptase-like gene from *Neurospora crassa*

Eugene Gladyshev (1), Irina Arkhipova (2), Nancy Kleckner (1) (1) Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138 and (2) Josephine Bay Paul Center, Marine Biological Laboratory, Woods Hole, MA 02543

NcRVT belongs to a recently discovered class of reverse transcriptase-related cellular genes of fungi, animals, plants and bacteria. NcRVT protein can be purified from vegetative mycelia of wild-type Neurospora strains grown in the presence of 0.1 ug/ml blasticidin, an antibiotic that blocks protein synthesis. An optional ammonium fractionation step may be used to concentrate NcRVT protein prior to centrifugation in a sucrose density gradient. Further purification is achieved by ion-exchange chromatography on DEAE Sepharose, where the protein can be eluted in a nearly pure form. In the presence of manganese, purified NcRVT protein has a potent terminal transferase activity with a pronounced preference for ribo- over deoxyribonucleoside triphosphates. Site-directed mutagenesis of a catalytic aspartate residue confirmed its essential role in this activity, ruling out the presence of any other co-purified polymerases. NcRVT cannot initiate polymerization de novo, instead it extends 12-14 nt RNAs that appear to be non- covalently associated with the protein. NcRVT does not readily accept exogenous primers or common primer-template combinations, however extension of co-purified tRNA, ribosomal RNA, and small nucleolar RNA fragments has been observed. Experiments are currently in progress to determine the nature of the associated RNAs as well as of NcRVT extension products in vivo.

Antisense transcription in the fungus *Neurospora crassa.*

David Kowbel[1], James Craig[1], Charles Hall[1], and N. Louise Glass[1]. [1]Department of Plant and Microbial Biology, University of California, Berkeley CA, 94720-3102 USA.

Antisense transcription is important for gene regulation in eukaryotes. We assessed the amount of sense and antisense transcription by sequencing the polyadenylated ends of mRNA isolated from the filamentous fungus *Neurospora crassa* and mapped to the *Neurospora crassa* genome. Sense and antisense transcripts orientation are resolved by visualization of the polyadenylated ends on the Integrative Genome Viewer (IGV) from the Broad Institute. Most of the antisense ends map to the 3'UTR and are due to expression from the overlapping 3'UTR of adjacent genes. Excluding these regions, antisense expression from over 200 genes accounted for approximately 2% of all mRNAs in the Neurospora genome. Multiple sites may also be utilized for each gene resulting in alternative transcript ends or partial transcripts. The inclusion of coverage files from RNAseq experiments and *Neurospora crassa* ESTS from NCBI mapped to the IGV revealed that antisense transcripts may also arise from non-coding RNA genes.

Small RNA biogenesis pathways in *Neurospora*

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Argonaute proteins are required for the biogenesis of some small RNAs, including the PIWI-interacting RNAs and some microRNAs. How Argonautes mediate the production of sRNAs independent of their slicer activity is not known. We previously showed that the maturation of the *Neurospora* miRNA-like sRNA, milR-1, requires the Argonaute protein QDE-2, Dicer, and QIP. Here, we reconstitute this Argonaute-dependent sRNA biogenesis pathway *in vitro* and discover that the exosome, a major eukaryotic RNA processing complex, is also required for milR-1 production. Our results demonstrate that QDE-2 mediates milR-1 maturation by recruiting the exosome and QIP and by determining the size of milR-1. The exonuclease QIP first unwinds the pre-milR-1 duplex and then mediates 3' to 5' trimming of the pre-milRNA together with exosome. Together, our results establish the biochemical mechanism of an Argonaute-dependent small RNA biogenesis pathway and the critical roles of the exosome in small RNA processing.

A homolog of the mammalian STRIPAK complex controls sexual development in *Sordaria macrospora*

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The filamentous ascomycete *Sordaria macrospora* is used as a model to study fruiting body development. The fruiting bodies of *S. macrospora* are composed of many different cell types and their development is a complex cellular differentiation process controlled by many developmentally regulated genes. We generated numerous mutants of *S. macrospora* which are blocked at various stages of fruiting-body development. Molecular genetics procedures have been applied to isolate and characterize genes that regulate this developmental process. Among the genes isolated by complementing the mutants was the *pro11* gene, encoding a conserved WD40 repeat protein. PRO11 shows significant homology to vertebrate proteins of the striatin-family. Members of this family are highly conserved eukaryotic multimodular proteins and are central components of the striatin-interacting phosphatase and kinase (STRIPAK) complex. Using tandem affinity purification, yeast two-hybrid, co-immunoprecipitation and mutant analyses, we found that previously identified developmental proteins are additional subunits of a STRIPAK complex in *S. macrospora*. This includes the putative kinase activator SmMOB3 and the striatin interacting protein PRO22, together with the scaffolding subunit of protein phosphatase 2A (PP2A). Analysis of truncated gene products identified domains essential for direct protein-protein interactions within this complex. Complementation analysis using knockout mutants revealed a crucial role of the STRIPAK complex during sexual development. We propose that the developmental switch from vegetative growth to sexual propagation structures relies on the integrity of the multimodular STRIPAK complex.

Specific Structural Features of Sterols Affect Growth and Development of *Neurospora crassa*

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Ergosterol is the main membrane sterol of fungi. Deficiencies in ergosterol biosynthesis result in pleiotropic defects in growth and development of *Neurospora crassa*. Phenotypic comparison of mutants affected at different steps of sterol biosynthesis revealed that the accumulation of different ergosterol precursors causes specific developmental defects. For example, deletion of *erg-2*, which encodes an enzyme mediating the last step in the pathway, strongly impairs both vegetative tropic hyphal interactions related to hyphal fusion and subsequent cell merger.

In wild type, fusion of conidial germ tubes requires the coordinated dynamic recruitment of the MAP kinase MAK-2 and the cytoplasmic protein SO to the tips of interacting cells. In Δ*erg-2* fusion germlings, both MAK-2 and SO mislocalize, suggesting that the sterol composition of the plasma membrane strongly influences the assembly and function of membrane associated signaling complexes.

In contrast to Δ*erg-2*, mutant strains deficient in ERG-10a and ERG-10b, two enzymes with redundant function that act upstream of ERG-2, are not affected in cell-cell communication. However, Δ*erg10a/b* fusion germling pairs frequently arrest at the stage of plasma membrane merger, similar to mutants deficient in the PRM1 protein, a potential plasma membrane fusogen.

By relating the sterol composition and fusion competence of several additional *erg* mutants, we find that the accumulation of sterol intermediates specifically impairs distinct steps of germling fusion. While the presence of two double bonds in the sterol side chain provokes Δ*erg-2*-like deficiencies, the absence of a double bond in the sterol ring system causes Δ*Prm1*-like defects. Thus, simply the presence or absence of individual double bonds within the sterol molecule can have profound effects on fungal growth and development.

lfd-1: a novel gene required for membrane merger during cell fusion in _Neurospora crassa_.
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Cell–cell fusion is essential for a variety of developmental steps in many eukaryotic organisms. In *Neurospora crassa* cell fusion occurs during all stages of growth and reproduction, specifically between germlings and hyphae during vegetative growth and at multiple steps during sexual reproduction. The process of cell fusion in *N. crassa*, similar to the process in other organisms, follows three consecutive steps: 1) "precontact", involves the attraction between cells directing their growth towards each other, 2) Adhesion and cell wall breakdown, 3) plasma membrane merger and fusion pore formation. Although the molecular mechanisms associated with intracellular membrane fusion are well characterized, the molecular mechanisms of plasma membrane merger between cells are poorly understood. Only one gene codifying a protein involved in this last step of cell fusion has been previously identified in *N. crassa*: *Prm-1*. Mutations in *Prm-1* show a 50% reduction in both vegetative and sexual cell fusion. Here we describe a novel gene, *late fusion defect-1 (lfd-1)*, which is also involved in late cell fusion events in *N. crassa*. Strains containing mutations in this novel gene are affected in either plasma membrane merger or in events immediately preceding it. Our data suggest that *lfd-1* is part of the cell fusion machinery, acting complementary, but independently, of PRM-1.

Extracellular pH and glycogen metabolism regulation in _Neurospora crassa_. New insights into the pH signaling pathway
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Glycogen is a polysaccharide widely distributed in microorganisms and animal cells and its metabolism is under intricate regulation. Its accumulation in a specific situation results from the balance between glycogen synthase and glycogen phosphorylase activities that control synthesis and degradation, respectively. These enzymes are highly regulated at transcriptional and post-translational levels. The existence of a DNA motif for the *Aspergillus nidulans* pH responsive transcription factor PacC in the promoter of the gene encoding glycogen synthase (*gsn*) in *Neurospora crassa* prompted us to investigate whether this protein regulates glycogen accumulation. Transcription factors such as PacC in *A. nidulans* and Rim101p in *Saccharomyces cerevisiae* play a role in the signaling pathway that mediates adaptation to ambient pH by inducing the expression of alkaline genes and repressing acidic genes. We show that *pacC* is over-expressed and *gsn* is down-regulated in wild-type *N. crassa* at pH 7.8 and this result coincides with low glycogen accumulation. In the *N. crassa* $pacC^{KO}$ strain the glycogen levels and *gsn* expression at alkaline pH were, respectively, similar to and higher than the wild-type strain at normal pH (5.8). These results characterize *gsn* as an acidic gene and suggest a regulatory role for PACC in *gsn* expression. The truncated recombinant protein, containing the DNA-binding domain specifically bound to a *gsn* DNA fragment containing the PacC motif. DNA-protein complexes were observed with extracts from cells grown at normal and alkaline pH and were confirmed by ChIP analysis. The PACC present in these extracts showed equal molecular mass, indicating that the protein is already processed at normal pH, in contrast to *A. nidulans.* Together, these results show that the pH signaling pathway controls glycogen accumulation by regulating *gsn* expression and suggest the existence of a different mechanism for PACC activation in *N. crassa*.

Phosphorylation of COT1 at S189 is required for early polar growth and is involved in regulation of micro/macro-conidiation in association with MOB2A/B
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NDR kinases require the interaction with MOB proteins for activity and function in the regulation of growth and development in a variety of organisms. Two NDR kinases, DBF2 and COT1, have been identified in *Neurospora crassa*. DBF2 is a member of the septation initiation network and COT1 is involved in the regulation of polar cell morphology. The N-terminal region of COT1 is sufficient for the formation of the COT1-MOB2A/B heterodimer and MOB2A/B have been suggested to have overlapping functions within the COT1 pathway. Using MS analysis we have identified a novel phosphorylation site within the COT1 N-terminal region at serine189. Site directed mutagenesis and gene replacement were used to form *cot-1*^{S189A} and *cot-1*^{S189E} strains, mimicking non-phosphorylated and constitutively phosphorylated forms of COT1. As conidial germination rate in *cot-1*^{S189A} was reduced by over 60%, but phosphorylation state of S189 had no effect on hyphal elongation, we concluded that phosphorylation of S189 is important during early phases of polar growth. In the presence of a *cot-1*^{S189A} allele, hypermicroconidiation was found to occur in a □*mob-2b* background. In addition, when *cot-1*^{S189E} was introduced into a □*mob-2a* background, a macroconidiation defect present in □*mob-2a* was completely suppressed. Taken together, our results indicate that S189 plays a role in conidiation and germination and that the former may be mediated by MOB2A/B. Furthermore, even though MOB2A/B may have overlapping functions in hyphal tip extension and branching, they have distinct COT1-dependent roles in regulation of micro and macroconidiation.

Global analysis of serine-threonine protein kinase genes in *Neurospora crassa*

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The *Neurospora crassa* genome knockout project funded by an NIH Program Project has produced over 8,000 unique strains representing systematic single gene deletions. Earlier work focused on the analyses of knockout strains corresponding to annotated transcription factors in *N. crassa*. Here, we analyzed another class of proteins, serine-threonine (S/T) protein kinases, which are crucial to eukaryotic signaling pathways. We were able to isolate viable mutants for 77 of the 86 kinase genes included in this study. Of these, 57% exhibited at least one growth or developmental phenotype; 40% possessed a defect in more than one trait. Serine-threonine kinase knockouts were subjected to chemical screening using a panel of eight chemical treatments, with 25 mutants exhibiting sensitivity or resistance to at least one chemical. This brought the total percentage of S/T mutants with phenotypes in our study to 71%. The *apg-1* gene encodes a S/T kinase required for autophagy in other organisms. The *apg-1* knockout mutant possessed the greatest number of phenotypes, including defects in asexual and sexual growth and development and altered sensitivity to five chemical treatments. We showed that NCU02245/*stk-19* is required for chemotropic interactions between female and male cells during mating. Finally, we demonstrated allelism between the S/T kinase gene NCU00406 and velvet (*vel*), which encodes a p21-activated protein kinase (PAK) gene important for asexual and sexual growth and development in *Neurospora*.

Calcium ATPases in the ER and Golgi are required for normal morphological development in *Neurospora crassa*.

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Calcium is essential for growth, but becomes toxic at cytosolic concentrations above ~0.2 uM. Polar growth in filamentous fungi has been hypothesized to depend upon a calcium gradient at the hyphal tip. We have investigated the role of five transport proteins in maintaining calcium homeostasis in *N. crassa*. CAX, is a Ca^{2+}/H^{+} exchange protein that moves calcium into vacuoles. The other four are Ca^{2+}-ATPases. NCA-1 is in the ER, NCA-2 and NCA-3 are in the plasma membrane. New data with GFP-tagged protein showed the PMR Ca^{2+}-ATPase resides in a Golgi compartment. Deletion of the *pmr* gene, but not the other genes, dramatically changes the morphology of hyphae, resulting in a highly branched, aconidial mycelium. The Dpmr strain will not grow if calcium or manganese are omitted from Vogel's medium, unlike the wild type which grows at normal rates. Addition of a high concentration of Mn^{+2} (1 mM), partially, but not completely, suppresses the morphological defects. This may be due to the role of Mn as a required cofactor for glycosylating enzymes in the Golgi. In the double mutant strain Dpmr Dnca-2, cellular calcium is elevated nearly ~40-fold, compared to Dpmr, and the hyper-branching phenotype of Dpmr is suppressed. By contrast the Dpmr Dnca-1 strain, which accumulates less calcium than the wild type, has a more extreme hyper-branching phenotype, resulting in tight, spherical colonies on Vogel's medium. The data indicate that the calcium Ca^{2+}-ATPases in the ER and Golgi are essential for normal morphological development and play an important role in controlling intracellular levels of calcium.

Calcium signaling and homeostasis during colony initiation in *Neurospora crassa*

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During colony formation in *Neurospora crassa*, germinating conidia form germ tubes that are involved in colony establishment, and conidial anastomosis tubes (CATs) that generate fused networks of conidial germlings. Calcium signalling and homeostasis are important for numerous processes in filamentous fungi but their roles in colony formation are little understood. In order to study this, we have used a combination of pharmacological treatments, screening of 57 mutants compromised in different components of the calcium signalling/homeostatic machinery, calcium measurement with the genetically encoded calcium sensitive probe aequorin, and live-cell imaging of calmodulin labelled with GFP. Macroconidia form CATs in the absence of external calcium but they do not fuse indicating that external calcium is required for CAT chemotropism. CAT fusion was found to be more sensitive than germ tube formation to the L-type calcium channel blockers verapamil, diltiazem and KP4. However, our results indicate that verapamil and diltiazem (but not KP4) probably also interact with other targets that perturb calcium homeostasis. A deletion mutant of the L-type calcium channel, CCH-1, exhibited reduced CAT fusion and a double deletion mutant of the two calcium channel proteins CCH-1 and MID-1 exhibited an even greater reduction in CAT fusion. Other deletion mutants exhibiting reduced CAT fusion were NCU07966.2 (a calcium ATPase), HAM-3 (a calmodulin-binding striatin) and NCU01266.2 (a phospholipase C). The primary intracellular calcium receptor, calmodulin (CaM), regulates the activity of numerous target proteins. Two CaM antagonists (calmidazolium and trifluoperazine) were found to selectively inhibit CAT fusion. GFP labelled CaM localized as dynamic spots associated with the plasma membrane and within the cytoplasm. In germ tubes, Cam also localized at developing septa (CATs lack septa). However, CATs that were undergoing chemoattraction towards each other showed a distinctly different pattern of localization at their growing tips; CAT tips exhibited a very pronounced accumulation of Cam whilst germ tube tips did not. Various antifungal proteins and peptides have been found to either directly or indirectly target a range of components of the calcium signalling/homeostatic machinery of *N. crassa*.

Circadian clock regulation of MAPK pathway activation in Neurospora
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About 20% of Neurospora genes are under control of the circadian clock system at the level of transcript accumulation, and the bulk of the clock-controlled mRNAs have peak accumulation in the late night to early morning. These data suggested the existence of global mechanisms for rhythmic control of gene expression. Consistent with this idea, we found that the Neurospora OS MAPK pathway, a phosphorelay signal transduction pathway that responds to changes in osmotic stress, functions as an output pathway from the FRQ/WCC oscillator. ChIP-seq with known oscillator proteins revealed that phosophorelay/MAPK pathway components are direct targets of the White Colar Complex (WCC), providing a direct connection between the clock and the output pathway. Activation of the p38 MAPK pathway by the FRQ/WCC oscillator culminates in rhythmic MAPK activity, which through time-of-day-specific activation of downstream effector molecules, controls rhythms in several target clock-controlled genes. In addition, MAPK activity controls key regulators of translation, suggesting a novel mechanism for circadian control of translation.

Light inducible system for tunable protein expression in *Neurospora crassa*
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Fungi are able to perform extensive post-translational modification needed in the complex world of eukaryotic organisms, which makes them excellent tools to study a variety of eukaryotic processes as well as good candidates for protein production systems for hard to express proteins from more complex organisms. While there are several fungal protein expression systems in place, more could be done to exploit *Neurospora crassa*. Though some expression promoters in *Neurospora* have been studied, such as *ccg-1* and *qa-2*, these promoters are either leaky or do not induce protein expression at high levels. In order to create an *in vivo* protein expression in *Neurospora* with high expression and tightly regulation, we have harnessed the *vvd* promoter. By following the expression of genes under the regulation of the *vvd* promoter, we were able to demonstrate that, compared to a wild type *Neurospora* strain; there is a 1 to 2-fold higher level of expression of mRNA driven by the *vvd* promoter in a *vvd* knockout strain. Up to three fold more protein is expressed when driven by the *vvd* promoter than when driven by the *ccg-1* promoter. When exposed to measured amounts of light, mRNA expression shows a graded response under the *vvd* promoter and rapidly returns to basal levels when returned to the dark, demonstrating that the *vvd* promoter is a highly tunable and regulatable system for protein expression in *Neurospora crassa*. This work was supported by grants from the National Institutes of Health to J.M.H. (Grant F32 GM096574), J.J.L. (Grant R01 GM08336) and J.C.D (Grants GM34985 and P01GM68087)

Global light and temperature responses in a clock-less strain of Neurospora
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The Neurospora clock follows a blueprint shared by pro- and eukaryotic organisms where clock gene products produce molecular rhythmicity by engaging in positive and negative feedback interactions. The Neurospora FRQ-WHITE-COLLAR circadian oscillator (FWO) imposes rhythmicity onto processes such as asexual spore development.
However, in its absence other timers, often referred to as FRQ-less oscillators (FLO) can control rhythms in spore development. To identify molecular components of such timers and of genes under control of photoreceptors other than the WHITE-COLLAR COMPLEX (WCC) we created a strain in which all key components of the FWO were deleted.
Comparison of deep sequencing data obtained form a DFWO and a wild- type strain grown under different conditions of light and temperature should allow us to identify novel pathways that feed in to circadian and/or non-circadian oscillators.

FRQ-less rhythms in *Neurospora*
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In the circadian system of *Neurospora*, the feedback loop involving the FRQ, WC-1 and WC-2 gene products is an important component. However, rhythms in conidiation and in biochemistry can be seen under many different conditions when this FRQ/WCC feedback loop is not functioning. We are interested in those rhythms that can be observed in FRQ-less strains, and in the one or more FRQ-less oscillator(s) (FLOs) that must drive those rhythms. Our work is aimed at determining how many FLOs there may be and identifying the mechanism of rhythmicity. We are working with several previously-identified clock mutations, primarily *prd-1* and *prd-2*, and a new mutation tentatively named UV90, which affect rhythmicity in FRQ-less strains and are good candidates for components of the FLO. All three of these mutations affect several different FRQ-less rhythms, and also affect rhythmicity in FRQ-sufficient strains. Our data support a model in which a single FRQ-less oscillator and the FRQ/WCC feedback loop mutually interact to produce the complete circadian system.

Temperature compensation of circadian entrainment in Neurospora and human cells
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Temperature compensation is one of several properties that are shared by all circadian clocks. It generally refers to the robustness of the free running period despite substantial changes in temperature. However, systems can be over or under compensated, and although they change less than might be expected based on test tube reactions, they do change substantially as the temperature moves higher or lower. Furthermore, we know that small changes in period can lead to large changes in entrained phase. We therefore systematically investigated entrainment over a broad range of temperatures for cellular clocks derived from poikilotherms (Neurospora) and homeotherms (human tissue culture cells).

Roles for CSP-1 in Light and Circadian Clock-Regulated Gene Expression
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The *csp-1* gene encodes a transcription factor. It is induced by blue light (Chen et al. EMBO J. 2009) and is also regulated by the circadian clock (Lambreghts et al. GENETICS 2007). Both the gene and the CSP-1 are expressed with peaks in morning, and using ChIP-sequencing we find CSP-1 to bind to many regions of the genome and to influence the expression of both light- and clock-controlled genes. RNA-seq analysis suggests that CSP-1 acts mainly as a repressor. CSP-1 acts as a second order clock regulator, serving to transduce clock regulation of gene expression from the core circadian oscillator to a bank of output clock-controlled genes (ccgs) as verified by ccg-luciferase gene fusions. CSP-1 is thus required for rhythmic or proper phase of expression of its target genes.

Towards a four-dimensional understanding of transcriptional networks in *Neurospora crassa*.
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It has been suggested that ~20% of the Neurospora-transcriptome may be under circadian control. Although a major mechanism relaying this temporal information from the circadian oscillator through the output-pathways might be at the transcriptional level, there is scarce information regarding the elements that could be exerting such a control. We are using several codon-optimized luciferase transcriptional and translational reporters to monitor time-of-day-specific gene expression and to identify key elements mediating this process. Thus, we have identified transcription factors -such as SUB-1- that affect the expression of known and novel *ccgs*, some of which also seem to be transcriptional regulators providing, therefore, access to a group of third-tier *ccgs*. In addition, we are characterizing several bZIP-coding genes -that exhibit rhythmic expression- as potential nodes of circadian control. These studies are being complemented with a Protein-Binding Microarray approach, in order to explore the networks and transcriptional cascades that could be involved in the control of the circadian output pathways. Finally, we are exploring the spatial differences observed in the temporal control of gene expression. Funding: FONDECYT1090513

The global repressor CSP1 links metabolism and the circadian clock of *Neurospora crassa*
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The WCC of the circadian clock of *Neurospora* activates morning-specific expression of the transcription repressor CSP1. Newly synthesized CSP1 forms a transient complex with the co-repressors RCM1 and RCO1. CSP1 is rapidly hyperphosphorylated and degraded via and the ubiquitin ligase UBR1. Many genes controlled by CSP1 are rhythmically expressed and peak in the evening. A negative feedback of CSP1 on its own transcription attenuates CSP1-mediated repression of target genes. CSP1 binds to the promoter of the *wc-1* gene and represses transcription of *wc1*. Over-expression of CSP1 results in reduced WC1 expression and lengthening of the circadian period. CSP1 is regulated by metabolic cues and links the circadian clock and metabolism.

Associating genes with phenotypes in *Neurospora* mutant strains
Scott E. Baker, Aric Weist, Mike Plamann, Igor Grigoriev, Joel Martin, Wendy Schackwitz, Michael Freitag and Kevin McCluskey. US DOE and FGSC

Next generation sequencing technologies have the potential to accelerate the pace of associating phenotypes with genotypes. The rich history of classical genetic analysis combined with a high quality reference genome sequence and community supported strain archive in the form of the Fungal Genetics Stock Center, make Neurospora crassa an ideal system to demonstrate the power of next generation sequencing methods. We have "re-sequenced" several mutant strains of N. crassa to generate a rich database of single nucleotide variants (SNVs), small insertion/deletions against which individual mutant strains can be compared. In addition, we used the high quality N. crassa genetic map to constrain the chromosomal region examined enabling us to quickly identify "unique" mutations in open reading frames and associate candidate mutations with phenotypes. Additional insight is provided from multi-strain comparisons of nuclear and mitochondrial genomes.

The development of genetics and genomics for analysis of complex traits in the model filamentous fungus, *Neurospora crassa*.
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We have developed a set of strains and tools that will facilitate the rapid identification of genes and regulatory networks contributing to quantifiable traits in the filamentous ascomycete *N. crassa*. In all organisms combinations of genes acting at multiple sites in the genome control many traits. Previous studies that map genes by linkage to phenotype have suffered from poor resolution. RNA-seq from wild *N. crassa* isolates gives us both sequence and expression data. RNA-sequencing on 112 wild isolates from a Louisiana (LA) population of *N. crassa* has been completed. Relative expression levels have been determined for genes from each isolate and each isolate has been genotyped at approximately 74,000 high-frequency SNP positions. Within the LA population a whole-genome association study has been conducted to associate transcripts with SNPs affecting their expression both in *cis* and in *trans*. SNPs that are potential master regulators of transcription have been identified within the LA population. Several statistically significant transcript sets with common putative regulators that fall into defined functional categories have been identified.
Current work is ongoing to test for linkage between SNPs and several phenotypes of interest including germling communication and hyphal architecture.

Harnessing natural variation in gene expression levels to infer function of unknown genes in *Neurospora crassa*
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More than half of *Neurospora crassa* predicted genes are of unknown function. We have used natural variation in gene expression levels across 48 wild strains of *N. crassa* to identify candidate regulatory modules containing genes of known and unknown function. To illustrate the utility of these coexpression clusters for inferring function of unknown genes, we use them in combination with experimental characterization of deletion mutants to identify the functional role and downstream targets of a previously uncharacterized C2H2 zinc finger transcription factor. We additionally implicate two other genes of unknown function, both conserved among filamentous fungi, as playing a role in hyphal architecture and nitrogen catabolite repression, respectively.

Induction of lignocellulose degrading enzymes in *Neurospora crassa* by cellodextrins.
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N. crassa colonizes burnt grasslands and metabolizes both cellulose and hemicellulose. When switched from a favored carbon source to cellulose, *N. crassa* dramatically upregulates expression and secretion of a wide variety of genes encoding lignocellulolytic enzymes. The means by which *N. crassa* and other filamentous fungi sense the presence of cellulose in the environment remains unclear. Here, we show that a *N. crassa* mutant carrying deletions of two genes encoding extracellular beta-glucosidase enzymes and one intracellular beta-glucosidase enzyme (d3BG) lacks beta-glucosidase activity, but efficiently induces cellulase gene expression and cellulolytic activity in the presence of cellobiose. These data indicate that cellobiose, or a modified cellobiose, functions as an inducer of lignocellulolytic gene expression and activity in *N. crassa*. We have also identified two cellodextrin transporters involved in sensing cellulose. A *N. crassa* mutant carrying deletions for both transporters is unable to induce cellulase gene expression in response to crystalline cellulose. Furthermore, a mutant lacking beta-glucosidase enzymes and transporters (d3BGdT) does not induce cellulase gene expression in response to cellobiose. We are currently characterizing the transport kinetics of each transporter in the d3BG background with the goal of understanding how transport of cellodextrins influences cellulose sensing and induction of cellulase expression.

Insights into recombination using gfp and next generation sequencing.

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Work with Neurospora has made significant contributions to understanding the mechanisms of genetic recombination. Mary Mitchell used Neurospora to provide the first definitive proof that the exchange of information between chromosomes involves gene conversion as well as crossing over. More recently, evidence that recombination may involve copy events that alternate between homologous chromosomes was first found in Neurospora and only more recently in other organisms.
The outcome of recombination events provides clues to the mechanisms involved. The best evidence comes from determining the genotype of all the products of recombination, the whole octad of spores arising from a single meiosis, because each spore carries information originally located in single DNA strands of the zygote in which the exchange events occurred. Such data is hard won as there are few loci where convenient scoring of the genotype is available and most depend on laborious testing of the ability of each individual spore from an octad to grow on specific media. Moreover, most studies of recombination have focussed on loci where recombination is relatively frequent. However, there is evidence that events at *am*, a locus that rarely undergoes recombination, involve proportionally more gene conversion and less crossing over, suggesting that recombination pathways may differ at such loci. Those clues are based on interpretation of "chromatid data" obtained by mapping SNPs carried by selectable recombinants between mutations that confer nutritional requirements. In these cases, as the whole octad is not available, distinguishing between conversion and crossing over is equivocal. Thus, the available data are constrained to a few loci and limited number of octads.
To overcome these limitations, we have developed a fluorescence-based recombination reporter system and genome sequencing of whole octads. The gfp methodology has been used to obtain octad data for recombination at three loci, to examine the effect of a knockout mutation of the mismatch repair gene *msh-2* and to show that recombination at *his-3* occurs with normal timing even when the normal mechanism for initiation of recombination is blocked by a *spo-11* knockout.

Comparative genomics of Neurospora and other fungi with FungiDB

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FungiDB (http://FungiDB.org) is a functional genomic database and website tool for fungal genomes to enable data mining and analyses of the pan-fungal genomic resources. The resource was developed in partnership with the Eukaryotic Pathogen Bioinformatic resource center (http://EuPathDB.org). Built with the similar infrastructure and user interface of EuPathDB, FungiDB allows for sophisticated and integrated searches to be performed using an intuitive graphical system. The current release contains sequence and annotation for species spanning the Ascomycota, Basidiomycota, and Zygomycota fungi. The functional genomics data include gene expression data from microarray and RNA-Seq, yeast two hybrid interaction data, and variation data in the form of SNPs and indels across several groups of fungi with multiple resequenced strains. A user interface to the precomputed orthology and paralogy of complete gene sets from the supported fungal genomes along with key metazoan, plant, microbial eukaryotes, and bacteria enable phylogenetic profiling across the tree of life. *Neurospora crassa* genomic and functional data have been integrated into the database permitting comp The data-mining interface also permits the ability to make inferences using functional data in one species transformed by orthology into another species, providing a powerful resource for *in silico* experimentation. FungiDB is supported by the Burroughs Wellcome Fund.

Genome-scale reconstruction and validation of *Neurospora crassa* metabolism.

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Despite *Neurospora crassa*s rich history as a model organism, systematic efforts to computationally predict its phenotypes have so far been lacking. Here, we present the first genome-scale metabolic model of N. crassa. This model is based on the integration of an extensively curated *Neurospora*-specific knowledge base (*NeurosporaCyc*) and a novel optimization-based method called Fast Automated Reconstruction of Metabolism (FARM). To assess the model accuracy, we first performed an in silico knockout of each gene in the model and predicted growth/nogrowth on Vogels media using the technique of Flux Balance Analysis (FBA). We then attempted to rescue the essential genes by adding supplemental nutrients to the in silico media. Our gene essentiality predictions are highly consistent with experiment on 285 gene knockouts (sensitivity=100%, specificity=96%) and 43 supplemental rescue phenotypes (93% accuracy). To expand the range of phenotypes the model is capable of predicting, we also measured the ability of wild-type Neurospora to grow on 384 different carbon, nitrogen, sulfur, and phosphorous sources using Biolog phenotype arrays. Our model, phenotype data, and the supporting *NeurosporaCyc* website should provide a valuable resource for the Neurospora scientific community.

The Neurospora Functional Genomics Project

The Program Project Consortium at Dartmouth Medical School, Yale, Broad Institute, Boston University, Texas A&M, University of Missouri- Kansas City, FGSC, Oregon State University, Oregon Health Sciences University, University of Oregon, UC Irvine

The central goal of the three interdependent efforts in this Program Project builds upon successful functional genomics, annotation, and expression analyses of *Neurospora crassa*. A primary goal will be to understand how *N. crassa* transitions from mycelial growth to complete asexual spore development. To do so we will focus on two key triggers of asexual development: light and desiccation. Project #1 will complete the Neurospora gene knockouts and extend the systematic disruption of genes to *Aspergillus*. We will also develop strains and tools for Projects #2 and #3, which aim to describe and reconstruct the cascading regulatory programs that underlie *N. crassa's* developmental response, from the level of chromatin structure through the gene regulatory network. To date, our work has focused on light regulation. Project #3 will use ChIP-seq mapping of histone modifications, transcription factor binding sites, sites of DNA methylation and nucleosome occupancy with corresponding transcriptome measurements to generate a deep description of genome and epigenome dynamics. Project #2, in an informatic-intensive systems biology approach, will integrate these data and use computational modeling to develop predictive models of the interconnected light and asexual development gene regulatory networks.

Poster Abstracts

Cell Signaling and Development

1) HYM1 functions as dual scaffold for NDR and MAP kinase pathways in *Neurospora crassa* to coordinate hyphal polarity with cell communication and sexual development

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HYM1/MO25-type proteins function as general co-activator proteins of germinal center kinases (GCKs), which activate nuclear-DBF2-related (NDR) kinase pathways and thereby regulate cellular morphogenesis and proliferation. Here we show that, in addition to a scaffold function of HYM1 for the POD6-COT1 GCK-NDR kinase complex in *N. crassa*, HYM1 is also critical for the NRC1-STE7-MAK2 MAP kinase pathway, which regulates vegetative cell-cell communication and sexual development. HYM1 interacts with all three kinases of the MAK2 MAP kinase cascade and co-localizes with MAK2 at the apex of growing cells. Deletion of *hym-1* phenocopies all defects observed for MAK2 pathway mutants by abolishing MAK2 activity. A NRC1-STE7 fusion protein reconstitutes MAK2 signaling, while constitutive activation of the individual MAPKKK and MAPKK proteins of the MAK2 pathway does not. These data identify HYM1 as novel scaffold for the NRC1-STE7-MAK2 pathway and establish HYM1 as central player for coordinating NDR and MAP kinase signaling during cell polarity, cell communication and sexual development.

2) MAP Kinases and Protoperithecial Morphogenesis in *Neurospora crassa*

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Multicellular development in fungi is fundamentally different from that of animals or plants. In filamentous fungi, multicellular structures are formed by aggregation and adhesion of hyphae, followed by septation and specialization of hyphal compartments within the aggregate. The perithecium, a flask-shaped sexual fruitbody produced by *Neurospora crassa*, provides a model system in which to study fungal multicellular development. This study presents an intricate description of the early stages of protoperithecial morphogenesis in the *N. crassa* wild type and details the development of gene-deletion mutants of all nine mitogen-activated protein (MAP) kinases conserved in *N. crassa*. It confirms that all three MAP kinase cascades are required for sexual development. However, only the pheromone response and cell wall integrity pathways, but not the osmoregulatory pathway, are essential for hyphal cell fusion. Evidence of cell fusion-related processes, regulated through MAP kinase signaling, including: extracellular matrix deposition; hyphal attachment and enveloping, have been identified as novel features important for the construction of fertilizable protoperithecia. KML and AL both contributed equally to this work.

3) Genetic interactions between G alpha and G beta subunits of heterotrimeric G proteins in *Neurospora crassa*

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Heterotrimeric G proteins consist of alpha, beta, and gamma subunits. Regulation is accomplished through the alternation between binding of GDP (inactive form) and GTP (active form) on the alpha subunit and dissociation of the alpha subunit and beta-gamma dimer. *Neurospora crassa* has three G alpha subunits (GNA-1, GNA-2 and GNA- 3), one G beta (GNB-1), and one G gamma (GNG-1). In this study, we analyze genetic epistasis and physical interactions between GNB-1 and the three *Neurospora* G alpha subunits. Using a biochemical approach, all three G alpha proteins could be coimmunoprecipitated in complexes that include GNG-1 and GNB-1. Using a genetic approach, strains lacking *gnb-1* and one G alpha gene as well as *gnb-1* mutants carrying constitutively activated, GTPase-deficient G alpha alleles are currently being analyzed for phenotypes.

Cell Biology and Morphogenesis

4) Evidence for the impact of reactive oxygen species on branch density homeostasis in *Neurospora crassa*.
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In preliminary screens, several functions, most notably, genes involved in the control of reactive oxygen species (ROS), were identified as playing a role in the process of growth rate compensation of branch density. The maintenance of branch density under growth at various temperatures was examined in a selection of mutants in genes known to be important in the control of ROS. In all ROS control mutants tested, growth was shown to branch tighter when grown at higher temperatures (which result in faster growth rates) and looser when grown at lower temperatures (which results in slower growth rates). This can be contrasted with wild-type Neurospora which branches at the same density under both conditions. We also tested the impact of environmental agents which lower the concentration of ROS on branching. In tests on wild type Neurospora, water soluble anti-oxidants (reducing agents), Ascorbic Acid and Glutathione produced unusual branching patterns. While normal branching shows a gamma distribution with a single peak, hypha exposed to Ascorbic Acid or Glutathione display a distribution of branching with two clear maxima. They show an increase in both very closely spaced branching as well as an increase in more distantly spaced branching.

5) Regulation of the BUD3-BUD4 landmark complex during septum formation by the NDR kinases DBF2 and COT1
Yvonne Heilig, Anne Dettmann and Stephan Seiler. Institute for Microbiology and Genetics, University of Gottingen, Germany

Cytokinesis is essential for cell proliferation, yet the mechanisms for determining the cell division plane are only poorly understood. Our data indicate that the anillin BUD4 marks septum placement by organizing the RHO4-BUD3-BUD4 GTPase module and that this complex is controlled through two NDR kinase signaling cascades, the septation initiation network (SIN) and the morphogenesis network (MOR). By using a combination of live cell imaging, genetic and biochemical approaches, we show that COT1 and DBF2 localize to the constricting septum and are regulated by the two specific upstream germinal center (GC) kinases POD6 and NCU04096 that phosphorylate the respective NDR kinase at their C-terminal hydrobhobic motif. A third GC kinase, MST1, functions as generic regulator of both NDR kinases. *cot-1* and *dbf-2* mutants display opposite septation defects. Epistasis analysis of *sin, mor* and *bud* double mutants places the SIN upstream of the MOR, which in turn inhibits BUD function. We demonstrate that COT1, but not DBF2, binds to and phosphorylates BUD3 and BUD4. Mutational analysis of BUD3 identifies Ser798, located within an amphiphatic helix of BUD3 that seems phosphorylated by COT1. Localization of this amphiphatic helix at septa is only possible in its nonphosphorylated form. In summary, our data suggest a preliminary model, in which the MOR kinase COT1 phosphorylates BUD3 and BUD4 and that this phosphorylation inhibits cortical localization and function of the BUD complex.

6) The *Neurospora crassa* RHO1 and RHO2 GTPase modules share partially overlapping functions in the regulation of cell wall integrity and hyphal polarity
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Rho-type GTPases are key regulators that control cellular morphogenesis, but their functions during hyphal growth in filamentous fungi is only beginning to emerge. RHO1 is essential for viability. By generating conditional *rho-1* mutants, we have dissected the function of RHO1 in cell polarization and maintenance of cell wall integrity in *Neurospora crassa*. Moreover, we identified NCU00668/RGF1 as unique exchange factor for the activation of RHO1, whose activity seems controlled by an intra-molecular interaction of its DEP and GEF domains that blocks the activation of the GTPase. The RHO1-RGF1 module controls actin organization and the cell wall integrity MAK1 MAP kinase pathway through the direct interaction of active RHO1 with the formin BNI1 and PKC1, respectively, and functions as regulatory subunit of the glucan synthase. *N. crassa* possesses a second GTPase, RHO2, that is highly homologous to RH1. RHO2 is of minor importance for growth and viability and does not interact with BNI1. However, conditional *rho-1;rho-2* double mutants display strong synthetic growth and cell polarity defects. We show that RHO-2 does not affect glucan synthase activity, but interacts with PKC1 and regulates the activity of the MAK1 MAP kinase pathway.

7) Functional characterization and cellular dynamics of the CDC-42 - RAC - CDC-24 module in *Neurospora crassa*
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Rho-type GTPases are key regulators that control eukaryotic cell polarity, but their role in fungal morphogenesis is only beginning to emerge. *Neurospora crassa rac* and *cdc-42* deletion mutants generate highly compact colonies with severe morphological defects. Double mutants carrying conditional and loss of function alleles of *rac* and *cdc-42* are lethal, indicating that both GTPases share at least one common essential function. The defects of the GTPase mutants are phenocopied by deletion and conditional alleles of the guanine exchange factor (GEF) *cdc-24*, and *in vitro* GDP-GTP exchange assays identify CDC-24 as specific GEF for both CDC-42 and RAC. *In vivo* confocal microscopy shows that this module is organized as membrane-associated cap that covers the hyphal apex. However, the specific localization patterns of the three proteins are distinct, indicating different functions of RAC and CDC-42 within the hyphal tip. These localizations, together with the distinct cellular defects of *rac* and *cdc-42* mutants, suggest that CDC-42 is more important for polarity establishment, while the primary function of RAC may be maintaining polarity. In summary, this study identifies CDC-24 as essential regulator for RAC and CDC-42 that have common and distinct functions during polarity establishment and maintenance of cell polarity in *N. crassa*.

8) High-Throughput Evaluation of the Effect of Chitosan, Amphotericin B and Benomyl on *Neurospora Crassa*.
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Chitosan is a natural polymer derived from chitin with fungicidal activity. This polymer permeabilises the fungal membrane in an energy dependent manner. The biological activity of chitosan is related with its molecular weight and deacetylation degree. We evaluated spectrophotometrically (in microtitter plates) the effect of chitosan, amphotericin B and benomyl in germination and growth of *Neurospora crassa* (#2489) (Nc). The method followed M-38A protocol for filamentous fungi (Clinical Laboratory Institute Standards). Using culture media of diverse nutrient content some precipitated chitosan and were discarded. We identified by OD (490, 595 nm) lag, exponential and stationary phases for Nc and established 10^6 conidia/ml as working inoculum for antifungal evaluation. OD readings correlated with biomass quantified by thermogravimetry (TG-DTA) and a microbalance. We have established an indirect method for detecting fungus growth validated by quantitative direct methods. Using this method with 70KDa chitosan we have established an $IC_{50\text{-}70}$ of ca. 0.01mg.ml^{-1} for Nc 48h after inoculation. This method could be used in chemogenomics and massive sequencing studies to analyze the mode of action of chitosan and other antifungals.

9) The *Neurospora crassa* mutant NcdeltaEgt-1 identifies an ergothioneine biosynthetic gene and demonstrates that ergothioneine enhances conidial survival and protects against peroxide toxicity during conidial germination.
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Ergothioneine (EGT) is a histidine derivative with sulfur on the imidazole ring and a trimethylated amine; it has been postulated to have an antioxidant function. We used the EGT monobromobimane derivative to identify EGT by LC/MS and for quantification. EGT concentrations are significantly (α=0.05) higher (5X) in wild type conidia than in mycelia. The knockout in gene NCU04343 does not produce EGT. An *in silico* analysis suggests that 1) the gene, named NcEgt-1, was acquired early in the mycota lineage as a fusion of two adjacent prokaryotic genes, that was then lost in the Saccharomycotina, and that 2) NcEgt-1 catalyzes the first two steps of EGT biosynthesis from histidine to hercynine to hercynylcysteine sulfoxide. Comparisons of the wild type with the knock-out indicate that endogenous EGT 1) helps protect conidia during the quiescent period between conidiogenesis and germination, 2) has a role in conidial longevity, 3) quenches peroxide *in vivo* and 4) helps protect conidia during the germination process from peroxide but not from superoxide or Cu^{2+}. The data will be discussed in relation to the translocation of glutathione from vacuoles to the cytoplasm before germ tube emergence.

10) Localization of the β (1-3) endoglucanases EGLC-1 and EGLC-2 and their role in the morphogenesis of *Neurospora crassa*.
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The unitary model of cell wall growth suggests that the polarized extension of hyphae in filamentous fungi is the combined result of the synthesis and discharge of new cell wall polymers, the action of hydrolytic enzymes that provide plasticity to the wall and turgor pressure to drive cell expansion. There is limited information on enzymes capable of hydrolyzing cell wall polymers and that could be contributing to plasticize the cell wall. EGLC-1 and EGLC-2 are putative β (1-3) endoglucanases in *Neurospora crassa*, with potential binding sites for a glucosyl phosphatidylinositol group (GPI), which would allow them to get anchored into the plasma membrane. Using fusion PCR and cloning plasmid, two recombinant vectors were constructed to tag each of the putative endoglucanases with GFP. In one vector, the gfp sequence was inserted within the eglc-1 encoding sequence, just after the signal peptide encoding sequence. For the eglc-2 sequence, the gfp sequence was inserted before the GPI-binding site. These plasmids were expressed in *N. crassa* strain FGSC # 9717 and prototrophic strains expressing EGLC-1-GFP or EGLC-2-GFP were analyzed by confocal laser scanning microscopy to monitor the location of the GFP-tagged proteins. Both proteins were localized in the hyphal apical plasma membrane and in septa. The fluorescence patterns observed at each of these sites were different for each protein. These results show that lytic activity of enzymes such as endoglucanases EGLC-1 and EGLC-2 in *N. crassa* are present in fungal morphogenesis, where they probably play a role in cell wall remodeling, as postulated by the unitary model of cell wall growth.

11) Dynein heavy chain mutations cause multiple mislocalization phenotypes
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Cytoplasmic dynein transports cargoes that are crucial for a variety of cellular functions. We utilized the ascomycete fungus *Neurospora crass*a in a series of genetic, cell biological and biochemical analyses to study dynein function and regulation. Thirty-four dynein heavy chain mutations were isolated using a genetic screen and characterized by performing a series of localization studies. Our studies revealed that dynein can mislocalize as long linear tracks, comet tails, aggregates, and dispersed signals in mutant strains and can alter microtubule organization and nuclear distribution to varying degrees. Biochemical analyses of dynein from one of the mutant strains revealed a potential link between in vitro biochemical properties and intracellular function of dynein. We propose a model in which dynein normally cycles to the hyphal tip and from there to distal regions as it performs its transport functions. The multiple mislocalization phenotypes we observed may represent the entrapment of dynein at different stages of the dynein transport cycle.

12) The pericentrin GRB during cell cycle in *Neurospora crassa*
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Pericentrin proteins are responsible of the recruitment of the gamma-tubulin complex to the inner plate of the Spindle Pole Body (SPB), and are important to ensure the correct mitotic spindle assembly. The positioning of the gamma- tubulin complex on the outside of the SPB plate is carried out by other important proteins in the positioning of the nuclei in fungi. In this work we describe the localization and dynamics of GRB homolog of Pcp-1 of *Schizosaccharomyces pombe*, in the filamentous fungus *Neurospora crassa*. GRB was tagged with the fluorescent proteins GFP and mCHFP, and it was observed as bright spots on the nuclei surface and associated with gamma-tubulin at the SPBs, during all the stages of the life cycle of *N. crassa*. GRB is embedded in the nuclei membrane, and also associated with the cytoplasmic Mts and with the spindle during mitosis. GRB is sometimes found on two opposite ends of the nucleus before the spindle is formed. GRB is localized closed to the centromeric histone CenH3 during interphase. The mutation of *grb* gene showed this protein to be essential in *N. crassa* and it is part of the SPBs and the mitosis machinery.

13) Timeline of proteins involved in septum formation in *Neurospora crassa*

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The cellular machinery responsible for cytokinesis and septum formation is well conserved in the eukaryotes. These processes share three basic steps: selection of the division plane, assembly of an actin contractile ring (CAR) and the constriction of the CAR coupled with the invagination of plasma membrane at these sites. To maintain an orderly sequence of these steps, spatial cues and temporal controls must play a crucial role. To study temporal distribution, we performed live-cell imaging of *Neurospora crassa* strains carrying GFP fusions involved in different stages of septum formation. For site selection, we used the landmark protein Bud4. To monitor actin ring formation, we followed the starting Rho4 module (Rho4, Bud3 and Rgf3), responsible for the activation of formin (Bni), which in turn promotes actin polymerization. We imaged the actin cytoskeleton by means of five actin binding proteins: TPM, coronin, FIM, Arp3 and Lifeact. We measured the times at which the proteins appeared in relation to the internalization of plasma membrane labeled with FM4-64. We were able to construct a timeline of the proteins involved in septum formation. For the most part the sequence was the same as in other organisms. Surprisingly, we found that actin cables are formed at future septation sites much earlier than previously thought.

14) Microtubules dynamics in the +Tip MTB-3 mutant of *Neurospora crassa*

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Microtubules (Mts) plus-end constitutes a platform for the accumulation of structurally and functionally diverse group of proteins, collectively called as MT plus-end tracking proteins (+TIPs). +TIPs control Mts dynamics and link Mts to various cellular structures. MTB-3 is the homolog of EB1, a member of highly conserved End-binding family of proteins. To address the effect of the lack of MTB-3 in *N. crassa* cells, we examined a deletion mutant of *mtb-3* gene and also tagged Mts with GFP to assess their dynamics and dynamic instability in the mutant compared with a wild type strain (WT). The elongation rate of the *mtb-3* mutant was 3.4 m min^{-1} and the WT was 3.9 m min^{-1}, there was no strong phenotype of the *mtb-3* mutant, just an increase in the rate of the appearance of two lateral branches simultaneously. Microtubules in the *mtb-3* mutant were fewer and thinner than in the WT, less organized and also there were more short Mts fragments. In the apex, just a few Mts reached the apical dome. During Mts dynamic instability, polymerization and depolymerization rate were both significantly decreased in the *mtb-3* mutant by 21% and 28% respectively compared to the WT. Nuclear positioning and dynamics was also affected in the in the *mtb-3* mutant, nuclear exclusion zone was larger than the WT, and also nuclei shape and movement was disturb. In conclusion, the lack of MTB-3 slightly affects growth and phenotype of *N. crassa* hyphae, but it has a strong effect in Mts dynamics, dynamic instability and also in the function of Mts of transport and organelle positioning.

15) Coronin, a modulator of endocytosis and tip morphogenesis in *Neurospora crassa*.

Ramon O. Echauri Espinosa,Olga A. Callejas-Negrete, Robert W. Roberson, Salomon Bartnicki-Garcia and Rosa R. Mourino-Perez

Filamentous fungi are ideal models to study molecular processes that determine polarized growth. Actin has been shown to play important roles in the regulation of polarized hyphal tip growth, including Spitzenkrper (Spk) functions. The actin binding protein coronin plays a major role in organizing the actin cytoskeleton in fungal cells. To determine the role of coronin in polarized tip growth and Spk function, we have studied a coronin gene deletion mutant (*Δcor-1*) in the filamentous model fungus *Neurospora crassa*. Disruption of *cor-1* resulted in delayed establishment of cell polarity during spore germination, reduced hyphal tip growth rates, distorted hyphal morphology, and a branching rate fivefold higher than in the WT strain. The Spk in *Δcor-1* hyphae was reduced in size, altered in its morphology and unstable in its structure, and exhibited meandering movement. Furthermore, uptake of FM4-64 dye was delayed in *Δcor-1* cells suggesting a disruption in endocytosis. In summary we found, that although *cor-1* is not an essential gene in *N. crassa* its deletion negatively influenced key cellular functions required for hyphal morphogenesis in general and the organization of the polarized tip growth apparatus in particular. These observations underpin the multiple roles of actin during cell polarity establishment and maintenance, and suggest that coronin is a key regulator of F-actin remodeling in *N. crassa*, including the turnover of actin patches during endocytosis.

16) The Role of Chitin Synthases in Hyphal Morphogenesis of *Neurospora crassa*

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The chitin is one of the mainly components of the cell wall in filamentous fungi and is synthesizing by chitin synthases(CHS). The filamentous fungus *Neurospora crassa* has one representative for each of the seven CHS classes described. Previous studies have shown that in *N. crassa*, CHS-1, CHS-3 and CHS-6, are concentrated at the core of the Spitzenkrper (Spk) and in forming septa. In this study we have endogenously tagged *chs-2*, *chs-4*, *chs-5* and *chs-7* with *gfp* to study their distribution in living hyphae of *N. crassa*. CHS-5 and CHS-7 both have a myosin motor like domain at their amino termini. All CHS-2, CHS-4, CHS-5 and CHS-7, were found in nascent septa. As the septum ring developed, CHS-2-GFP moved centripetally until it localized exclusively around the septal pore. CHS-5-GFP was localized also in the core of the Spk. We observed a partial colocalization of CHS-1-mCherry and CHS-5-GFP in the Spk. Total internal reflection fluorescence microscopy (TIRFM) analysis revealed putative chitosomes containing CHS-5-GFP moving along wavy tracks, presumably actin cables. Our results suggest that there are different populations of chitosomes, each containing a class of CHS. Mutants with single gene deletions of *chs-1*, *chs-3*, *chs-5*, *chs-6*, or *chs-7* grew slightly slower than the parental strain (FGSC#9718); only *Ächs-6* displayed a marked reduction in growth. In *Ächs-5*, *Ächs-7* and in a double mutant (*Ächs-1* x *Ächs-3*) strains produced less aerial hyphae and conidia but were more decreased in a double mutant. Currently, we are conducting Co-IP assays to identified putative proteins that are interacting with CHS.

Gene Regulation

17) Antisense transcription in the fungus *Neurospora crassa.*

David Kowbel[1], James Craig[1], Charles Hall[1], and N. Louise Glass[1]. [1]Department of Plant and Microbial Biology, University of California, Berkeley CA, 94720-3102 USA.

Antisense transcription is important for gene regulation in eukaryotes. We assessed the amount of sense and antisense transcription by sequencing the polyadenylated ends of mRNA isolated from the filamentous fungus *Neurospora crassa* and mapped to the *Neurospora crassa* genome. Sense and antisense transcripts orientation are resolved by visualization of the polyadenylated ends on the Integrative Genome Viewer (IGV) from the Broad Institute. Most of the antisense ends map to the 3'UTR and are due to expression from the overlapping 3'UTR of adjacent genes. Excluding these regions, antisense expression from over 200 genes accounted for approximately 2% of all mRNAs in the Neurospora genome. Multiple sites may also be utilized for each gene resulting in alternative transcript ends or partial transcripts. The inclusion of coverage files from RNAseq experiments and *Neurospora crassa* ESTS from NCBI mapped to the IGV revealed that antisense transcripts may also arise from non-coding RNA genes.

18) Mapping the regulatory network of lignocellulose degradation using *Neurospora crassa* as a model organism.

James Craig[1], Sam Coradetti[1], Yi Xiong[1], and N. Louise Glass[1]. [1] Department of Plant and Microbial Biology, University of California, Berkeley CA, 94720-3102 USA.

We use *Neurospora crassa* as a model organism to further our understanding of transcriptional networks controlling the expression of lignocellulose degrading enzymes in filamentous fungi. Utilizing next generation RNA sequencing, a cellulose specific regulon was identified by comparing global expression profiles of *Neurospora* grown on cellulose versus no-carbon media. A phenotypic screen of the *Neurospora* transcription factor deletion collection uncovered two novel genes (provisionally named cellulose degradation regulator 1 (*cdr-1* and *cdr-2*) that had specific and severe growth defects on crystalline cellulose (avicel). RNAseq profiling of these mutants indicated that the transcription factors are required for expression of all the major cellulase genes in *N. crassa*. The regulons of *cdr-1* and *cdr-2* also show significant overlap with each other as well as the wild-type cellulose-specific regulon. The *cdr-1* and *cdr-2* regulons were subsequently verified and refined with ChIP-seq. Finally, both genes are well conserved across filamentous fungi and may play similar key roles in these species

19) Functional and structural characterization of N. crassa proteins identified in DNA-protein complexes.
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Eukaryotic gene expression is regulated by combining DNA-protein(s) interactions and simultaneous changes in chromatin structure. Attempts to identify protein(s) binding the *gsn* promoter STRE motif returned five transcriptional regulators candidates. Among them, the ORFs NCU03482 and NCU06679 products are noteworthy. Analysis of their polypeptide sequences revealed protein domains involved in transcription regulation. The NCU03482 product (RUVBL1-like helicase) belongs to the AAA/Tip49 family involved with chromatin remodeling and transcription regulation. The NCU06679 product (HAT type-B-subunit-2), features five WD-40 domains and comprise a family of eukaryotic proteins implicated in transcription regulation. Their three-dimensional structures were determined based on the protein structures of, respectively, human RUVBL1 (PDB 2C9O) and *Drosophila* Nurf55 (PDB 2XYI). Both have NLS but no DNA-binding sites. The recombinant RUVBL1-like was able to form a DNA-protein complex with the STRE *gsn* promoter fragment suggesting that it might oligomerize to exert its activity. Our search also reveals that *N. crassa* RUVBL1 and Nurf55, together with the other candidates, have orthologues in MLL1/MLL, BAF53, Sin3A,and NuA4 chromatin modification complexes, thus forming a multiprotein-complex that might modulate the chromatin dynamics at the *gsn* locus controlling *gsn* transcript levels.

20) A dynamin-like protein affects both RIP and premeiotic recombination
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Repeat-induced point mutation (RIP) and premeiotic recombination affect gene-sized duplications in many filamentous fungi. RIP causes G:C to T:A transition mutations while premeiotic recombination can result in loss of repeated DNA segments [1]. Both processes occur after fertilization but prior to meiosis and can be very efficient, in some cases mutating and/or deleting the duplication in essentially every nucleus. At least in *Neurospora crassa*, RIP has countered the expansion of gene and transposon families [2], suggesting that genome streamlining and protection from transposition events may yield long-term benefits to Neurospora populations. We employ genetic approaches to elucidate the mechanism of premeiotic recombination and RIP. Here we report the successful identification of semi-dominant mutations that affect both of these processes by using UV mutagenesis, followed by a screen for reduced RIP of linked duplications of *hph* and *pan-2*. Classical genetic mapping and complementation tests revealed that a mutation in the histone H3 gene, $hH3^{dim\text{-}4}$, is responsible for greatly reduced RIP of one mutant. We identified two additional mutations by bulk segregant analysis and high-throughput Illumina sequencing. Single point mutations were found in the same gene, encoding a novel dynamin-like long GTPase, albeit in different conserved domains. Both premeiotic recombination and RIP frequencies are affected, supporting the idea that these processes are mechanistically linked. To investigate this further, we are screening the Neurospora single gene deletion collection for mutants that show RIP defects, starting with deletion mutants that are known or expected to affect recombination pathways. [1] Galagan, J., and Selker, E.U. (2004). Trends in Genetics 20, 417-423. [2] Selker, E.U. (1990). Annu. Rev. Genet. 24, 579-613.

21) The centromere-specific H3, CenH3, has multiple domains required for centromere localization and retention
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Centromere and kinetochore assembly and inheritance are dependent on signature proteins whose functions appear highly conserved between most eukaryotes. Comparison of available sequence data reveals that certain domains of these proteins can be highly divergent, even between different strains within one taxon. These observations predict the existence of both conserved and divergent protein interactions during centromere and kinetochore assembly. The “centromere identifier”, the centromere-specific histone H3 (CenH3) forms the platform for centromere assembly and is one such bipartite protein, as it contains a hypervariable N-terminal region and a highly conserved histone fold domain (HFD). We showed that C-terminally tagged *Podospora anserine* CenH3 (PaCenH3-GFP) substitutes for Neurospora CenH3 (NcCenH3) in mitosis and meiosis. Replacement of NcCenH3 with *Fusarium graminearum* CenH3 (FgCenH3) supported only mitosis in Neurospora, and tagging at the C-terminus resulted in defects in meiosis. Domain swapping experiments of the N-terminus of FgCenH3 with the HFD of PaCenH3 allows mitosis and meiosis, but chimeras with N-terminal NcCenH3 or PaCenH3 combined with the HFD domain of FgCenH3 were infertile or barren. Results from domain-swapping experiments suggest that only a few amino acids within the HFD are crucial during meiosis. We propose that these differences play an important role during the assembly and inheritance of regional centromeres, and we will report results of further dissection of CenH3 from four different filamentous fungi.

22) Characterization of two Fatty Acid Regulator transcription factors in *Neurospora crassa*
Erin L. Bredeweg[1], Fei Yang[2], Kristina M. Smith[1], Rigzin Dekhang[2], Jillian M. Emerson[3], Jay C. Dunlap[3], Deborah Bell-Pedersen[2], Matthew S. Sachs[2] and Michael Freitag[1] [1]Program for Molecular and Cellular Biology, Department of Biochemistry and Biophysics, and Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR, USA; [2]Department of Biology and Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX, USA; [3]Department of Genetics, Dartmouth Medical School, Hanover, NH, USA.

Two transcription factors, Fatty Acid Regulator FAR-1 (NCU08000) and FAR-2 (NCU03643) have been identified as homologues of *Nectria haematococca* "cutinase transcription factors alpha and beta", and *Aspergillus nidulans* FarA and FarB, respectively. Genome-wide analyses of binding sites obtained by ChIP-Seq and phenotyping reveal FAR-1 as an important regulator of beta-oxidation enzymes when Neurospora is grown on media with long-chain fatty acids, or is subjected to oxidative stress. FAR-2, which shows better conservation amongst *Pezizomycotina*, regulates the metabolism of short-chain fatty acids. Though FAR-1 and FAR-2 exhibit a number of overlapping target binding sites by ChIP-seq, and bind to similar consensus binding sites thus supporting the shared function of these transcription factors, phenotyping highlights their distinct characteristics. Such differences in regulation by FAR-1 and FAR-2 are reflected by different linear growth rates on various carbon sources, e.g. oleate, where *Δfar-1* grows slowly, and butyrate, where *Δfar-2* grows slowly. A *Δfar-1* strain also exhibits growth and conidiation defects on hydrogen peroxide, defects not observed in *Δfar-2* strains, but amplified by growth on oleate. Further characterization of these transcription factors and how they regulate their target genes will better define how Neurospora responds to different environmental carbon sources.

Light and Circadian Clock

23) Temperature compensation of circadian entrainment in Neurospora and human cells
Martha Merrow, Margien Raven, Sanne Roessingh, Annaleen Hulshof, Serge Daan and Maria Olmedo The University of Groningen, Groningen, The Netherlands m.merrow@rug.nl

Temperature compensation is one of several properties that are shared by all circadian clocks. It generally refers to the robustness of the free running period despite substantial changes in temperature. However, systems can be over or under compensated, and although they change less than might be expected based on test tube reactions, they do change substantially as the temperature moves higher or lower. Furthermore, we know that small changes in period can lead to large changes in entrained phase. We therefore systematically investigated entrainment over a broad range of temperatures for cellular clocks derived from poikilotherms (Neurospora) and homeotherms (human tissue culture cells).

24) Light inducible system for tunable protein expression in *Neurospora crassa*
Jennifer M Hurley[1], Chen-Hui Chen[1], Jennifer J Loros[2], and Jay C Dunlap[1]. [1]Department of Genetics [2]Department of Biochemistry Dartmouth Medical School, Hanover, NH 03755, USA

Fungi are able to perform extensive post-translational modification needed in the complex world of eukaryotic organisms, which makes them excellent tools to study a variety of eukaryotic processes as well as good candidates for protein production systems for hard to express proteins from more complex organisms. While there are several fungal protein expression systems in place, more could be done to exploit *Neurospora crassa*. Though some expression promoters in *Neurospora* have been studied, such as *ccg-1* and *qa-2*, these promoters are either leaky or do not induce protein expression at high levels. In order to create an *in vivo* protein expression in *Neurospora* with high expression and tightly regulation, we have harnessed the *vvd* promoter. By following the expression of genes under the regulation of the *vvd* promoter, we were able to demonstrate that, compared to a wild type *Neurospora* strain; there is a 1 to 2-fold higher level of expression of mRNA driven by the *vvd* promoter in a *vvd* knockout strain. Up to three fold more protein is expressed when driven by the *vvd* promoter than when driven by the *ccg-1* promoter. When exposed to measured amounts of light, mRNA expression shows a graded response under the *vvd* promoter and rapidly returns to basal levels when returned to the dark, demonstrating that the *vvd* promoter is a highly tunable and regulatable system for protein expression in *Neurospora crassa*. This work was supported by grants from the National Institutes of Health to J.M.H. (Grant F32 GM096574), J.J.L. (Grant R01 GM08336) and J.C.D (Grants GM34985 and P01GM68087)

25) Photosensing in Neurospora by a non-oscillatory feedback module of the circadian clock: a mathematical model.
Elan Gin [1], Axel Diernfellner [2], Michael Brunner [2], Thomas Hoefer [1]. [1] Division of Theoretical Systems Biology, German Cancer Research Center, Heidelberg, Germany. [2] University of Heidelberg, Biochemistry Center, Heidelberg, Germany.

In *Neurospora crassa*, the blue-light photoreceptors, WHITE COLLAR-1 and WHITE COLLAR-2 proteins, are central to light sensing. Light activates rhw WHITE COLLAR (WCC) complex, inducing transcription of light-induced genes. In the presence of continuous light, Neurospora's light responses are transient and light-regulated processes such as gene expression, are down-regulated, a phenomenon called photoadaptation. The molecular mechanisms of photoadaptation are not well understood, but it is known that the interconnected feedback loops by the transcription factors WHITE COLLAR-1 and WHITE COLLAR-2 and their negative regulators FREQUENCY (FRQ) and VIVID (VVD) are important to the photoadaptation response. Through a combination of experimental work and mathematical modelling, we developed a mathematical model of the interaction between the WCC and VVD using time-resolved experiments. While VVD is known to play a role in adaptation of light responses, we show that VVD, through heterodimerization with the WCC, is also involved in masking the effects of lower light intensities and in maintaining sensitivity to light. Our results suggest that the replenishment of the light-activatable pool of the WCC directly via the heterodimerization path is responsible for the subsequent light responses.

26) Frequency demultiplication unravels a robust circadian clock undergoing entrainment.
Christian Hong[1], Antonio Pregueiro[2], Luis Larrondo[3], Jennifer Loros[2], and Jay Dunlap[2] [1] University of Cincinnati College of Medicine, Cincinnati, OH USA. [2] Dartmouth Medical School, Hanover, NH, USA. [3] Pontificia Universidad Catolica de Chile, Santiago, Chile.

Entrainment to environmental cycles is a defining property of circadian rhythms, and entrainment of these rhythms by cycles that repeat twice or more often per day is also a characteristic of circadian rhythms. Although first reported nearly a century ago, this property of subharmonic entrainment, known as frequency demultiplication, has not been intensively studied and is poorly understood in molecular terms. To better understand this phenomenon, we employed a simple three variable mathematical model to simulate molecular profiles of core clock components under high frequency external cues, and tested predictions using the well-understood circadian system that controls developmental patterning in *N. crassa*. In a core feedback loop of this circadian oscillator, the protein product of the *frq* gene (FRQ) feeds back to depress the activity of its activator, WCC. In addition to this self-regulation, expression of *frq* and FRQ is known to increase with temperature. Simulations predict sustained bimodal oscillations of *frq* mRNA and FRQ under 12-h temperature cycles. We validated this prediction with bioluminescence assay tracking *frq* gene expressions under 12-h temperature cycles of 22˚C:27˚C, and indicate robustness of the circadian clock.

27) Conservation and divergence of the circadian clock in the *Neurospora discreta* species complex
Craig Wager, Katarina Slobodenko, Joseph Kawash, and Kwangwon Lee. Department of Biology, Rutgers University, Camden, NJ 08102

The 24-hour biological rhythm (circadian rhythm) has been attributed as a fitness trait in multiple organisms. To address the ecological significance of the circadian rhythm in fungi, we studied species within the *Neurospora discreta* species complex. Sequence comparisons of the coding regions of the core clock homologs of the sequenced *N. discreta* strain revealed high sequence similarity with *N. crassa*. In the type species of the clade, *N. discreta sensu stricto* exhibits a robust circadian rhythm with a temperature compensated free-running period of about 21 hours. In contrast, the North American population of the globally distributed strains assigned to the *N. discreta phylogenetic species 4 subgroup b* (PS4b) have diverged from this robust overt rhythmicity. Under the standard entrainment regime, North American PS4b strains are not fully entrained; 1) exhibit substantially decreased amplitude in the entrained and free-running overt rhythm, 2) the rhythm of PS4b was suppressed under circumstances where rhythmicity was observed in *N. crassa*, and 3) respond differently to temperature and light-dark entrainment regimens. To understand the molecular variation of the oscillator underlying these divergent behaviors of the overt rhythm, we analyzed the expression of the key clock protein FREQUENCY and found lower abundance under constant light and lower free-running amplitude in *N. discreta* PS4b strains. Together, these data suggest that the observed diversion overt rhythms among *N. discreta* species reflect the variations within the circadian oscillators. Based on our findings, we concluded that the divergent circadian behaviors identified in PS4b are the result of local adaptation.

28) Transcriptional regulators and time-of-day-specific gene expression in the Neurospora circadian system: a spatiotemporal approach.

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It has been suggested that ~20% of the Neurospora-transcriptome may be under circadian control. Although a major mechanism relaying this temporal information from the circadian oscillator through the output-pathways might be at the transcriptional level, there is scarce information regarding the elements that could be exerting such a control. We are using several codon-optimized luciferase transcriptional and translational reporters to monitor time-of-day-specific gene expression and to identify key elements mediating this process. Thus, we have identified transcription factors - such as SUB-1- that affect the expression of known and novel *ccgs*, some of which also seem to be transcriptional regulators providing, therefore, access to a group of third-tier *ccgs*. In addition, we are characterizing several bZIP-coding genes -that exhibit rhythmic expression- as potential nodes of circadian control. These studies are being complemented with a Protein-Binding Microarray approach, in order to explore the networks and transcriptional cascades that could be involved in the control of the circadian output pathways. Finally, we are exploring the spatial differences observed in the temporal control of gene expression. Funding: FONDECYT1090513

29) 1H NMR Metabolite Analysis of Light Inducible Genes in *Neurospora crassa*

Nathan M. Richardson[1], Nicole Knabe[2], Jay C. Dunlap[2], Jennifer J. Loros[2], Scott E. Baker[3] and Kathleen McAteer[4]. 1.Washington State University, WA. 2.Department of Genetics, Dartmouth Medical School, NH. 3.Pacific Northwest National Laboratory, WA. 4.Washington State University Tri-Cities, WA

Nuclear magnetic resonance spectroscopy (NMR) is a valuable tool used to identify and quantify metabolites in complex chemical mixtures. Using the model fungal organism, *Neurospora crassa*, metabolites were analyzed from a wild type strain to evaluate biosynthetic products over the time-course of a light induction experiment. The organism was isolated from light for a period of 48 hours and then exposed to light for the following 2 hours. Samples were generated from time points of 0, 15, 30, 60, 120, and 240 minutes following light induction. Intracellular aqueous and lipid fractions were generated. Each of these samples was analyzed by NMR spectroscopy to determine how light induction influences levels of different metabolites over time. We are working to correlate levels of metabolites from our 1H NMR experiment with previously published transcriptome data (Chen *et al.*, The EMBO Journal (2009) 28:1029 - 1042). Our comparative analysis lays the foundation for future research related to light influenced metabolite synthesis.

Evolution and Genomics

30) The development of genetics and genomics for analysis of complex traits in the model filamentous fungus, *Neurospora crassa*.

Charles Hall*, Christopher E. Ellison*, David Kowbel*, Juliet Welch*, Rachel B. Brem**, John W. Taylor*, N. Louise Glass*. Departments of *Plant & Microbial Biology and **Molecular and Cell Biology, University of California, Berkeley, CA 94720-3102, USA.

We have developed a set of strains and tools that will facilitate the rapid identification of genes and regulatory networks contributing to quantifiable traits in the filamentous ascomycete *Neurospora crassa*. In all organisms combinations of genes acting at multiple sites in the genome control many traits. Previous studies that map genes by linkage to phenotype have suffered from poor resolution. RNA-seq from wild *N. crassa* isolates gives us both sequence and expression data. RNA-sequencing on 112 wild isolates from a Louisiana (LA) population of *N. crassa* has been completed. Relative expression levels have been determined for genes from each isolate and each isolate has been genotyped at approximately 74,000 high-frequency SNP positions. Within the LA population a whole-genome association study has been conducted to associate transcripts with SNPs affecting their expression both in cis and in trans. SNPs that are potential master regulators of transcription have been identified within the LA population. Several statistically significant transcript sets with common putative regulators that fall into defined functional categories have been identified. Current work is ongoing to test for linkage between SNPs and several phenotypes of interest including germling communication and hyphal architecture.

31) Genome-scale reconstruction and validation of *Neurospora crassa* metabolism.
Jeremy Zucker [1,2], Jonathan Dreyfuss[2], Heather Hood[4], Matthew Sachs[3], Joseph Sturino [3], James Galagan[2] [1]Broad Institute of MIT and Harvard. [2]Boston University. [3]Texas A & M University [4]Oregon Health & Sciences University

Despite *Neurospora crassa*s rich history as a model organism, systematic efforts to computationally predict its phenotypes have so far been lacking. Here, we present the first genome-scale metabolic model of N. crassa. This model is based on the integration of an extensively curated *Neurospora*-specific knowledge base (*NeurosporaCyc*) and a novel optimization-based method called Fast Automated Reconstruction of Metabolism (FARM). To assess the model accuracy, we first performed an in silico knockout of each gene in the model and predicted growth/nogrowth on Vogels media using the technique of Flux Balance Analysis (FBA). We then attempted to rescue the essential genes by adding supplemental nutrients to the in silico media. Our gene essentiality predictions are highly consistent with experiment on 285 gene knockouts (sensitivity=100%, specificity=96%) and 43 supplemental rescue phenotypes (93% accuracy). To expand the range of phenotypes the model is capable of predicting, we also measured the ability of wild-type Neurospora to grow on 384 different carbon, nitrogen, sulfur, and phosphorous sources using Biolog phenotype arrays. Our model, phenotype data, and the supporting *NeurosporaCyc* website should provide a valuable resource for the Neurospora scientific community.

32) New ways of looking at meiotic recombination in Neurospora.
Fred Bowring[1], Jane Yeadon[1], Kyle R. Pomraning[2], Kristina M. Smith[2], Michael Freitag[2] and David Catcheside[1]. [1]School of Biological Sciences, Flinders University, Adelaide, Australia; [2]Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR, USA.

The methods routinely used to study Neurospora recombination have some limitations. Firstly, while they are relatively easily obtained, chromatid data can be ambiguous. Secondly, although it is a simple matter to determine if a particular mutation alters the frequency of recombination, identifying any effect on the timing of recombination is problematic. Finally, it has not been practicable to study recombination at more than a few loci in a given cross. Here we report the results from a fluorescence-based recombination reporter system and full octad sequencing that together remove these limitations. High quality recombination data from ordered octads can be obtained by simply scanning rosettes from a cross heterozygous for different GFP alleles. Furthermore, a cross between two mutant GFP alleles can be used to determine the timing of recombination. In this type of cross recombination can yield a wild]type GFP so it is possible to ascertain at what stage in the developmental sequence nuclei first fluoresce. In order to do a genome-wide audit of recombination we are currently sequencing the genomes of ordered octads from wild-type and from a mutant in which the heteroduplex DNA generated during recombination is preserved.

33) Insight from whole genome and gene specific sequence in classical mutant strains.
Aric E. Wiest, Alex J. McCarthy, Rob R. Schnittker, Scott E. Baker* and Kevin McCluskey. Fungal Genetics Stock Center, University of Missouri- Kansas City, School of Biological Sciences. *US DOE Pacific Northwest National Laboratory, Richland Washington.

While whole genome sequencing has proven to be useful for the identification of individual mutations, the insight provided by comparisons between the reference genome and novel genomes provides unique information in a variety of areas. For example, the number and characteristics of insertions and deletions reveals presumably neutral variation among different laboratory strains. The ability of strains to tolerate the numerous nuclear and mitochondrial frameshift mutations caused by indels is surprising, as is the number of nonsense mutations in some strains. Additional analysis includes the distribution of a newly discovered 4-base repeat that is manifest as an insertion or deletion with regard to the reference genome. Similarly, characterization of known and genetically mapped mutations reinforces our understanding of the limits of genetic mapping. Ongoing work continues the themes of gene and mutation characterization.

Other Topics

34) The use of filamentous fungi to convert solid waste into consumable products.
Alex Zapata, Elizabeth Phillippi, Blair Mitchell, Jacob Yablonowski, Jonathan Schoer & Michael Watters. Valparaiso University, Valparaiso Indiana.

Here we report on the use of Neurospora to reduce human solid waste while converting it into fungal biomass which has the potential to be used as a dietary supplement. Although this project was proposed to address problems inherent to long-term space flight: food storage & waste management, it holds potential benefit in diverse situations including livestock confinement operations. Typically portrayed as an environmental hazard and source of undesirable odor, solid waste represents an untapped resource. Filamentous fungi are natural decomposers with the ability to use this resource and reduce its environmental impact. We examined fungal growth and composition to determine the conditions which maximize the rate of conversion of waste into fungal biomass. We compared the effect of the length of incubation, method of aeration, available surface area, and presence of supplemental salts on fungal growth and

nutritional composition. Rates of conversion and nutritional content were highly variable, however rates as high as 75% (3.75g of fungus produced from 5g of solid waste), with fungal protein content up to 50%, were obtained. Additionally fungal growth reduced the characteristic odor of the media. Some of the primary chemicals responsible for the odor of solid waste are indole & a range of short chain fatty acids. We present data that the fungal mass is consuming these chemicals from the media to fuel its own metabolism and thus acting to eliminate the normally associated odor.

35) Induction of lignocellulose degrading enzymes in *Neurospora crassa* by cellodextrins.

Elizabeth A. Znameroski, Samuel Coradetti, Christine Roche, Jordan Tsai, Anthony Iavarone, N. Louise Glass and Jamie H. D. Cate. Energy Biosciences Institute, University of California, Berkeley, CA.

N. crassa colonizes burnt grasslands and metabolizes both cellulose and hemicellulose. When switched from a favored carbon source to cellulose, *N. crassa* dramatically upregulates expression and secretion of a wide variety of genes encoding lignocellulolytic enzymes. The means by which *N. crassa* and other filamentous fungi sense the presence of cellulose in the environment remains unclear. Here, we show that a *N. crassa* mutant carrying deletions of two genes encoding extracellular beta-glucosidase enzymes and one intracellular beta-glucosidase enzyme (d3BG) lacks beta-glucosidase activity, but efficiently induces cellulase gene expression and cellulolytic activity in the presence of cellobiose. These data indicate that cellobiose, or a modified cellobiose, functions as an inducer of lignocellulolytic gene expression and activity in *N. crassa*. We have also identified two cellodextrin transporters involved in sensing cellulose. A *N. crassa* mutant carrying deletions for both transporters is unable to induce cellulase gene expression in response to crystalline cellulose. Furthermore, a mutant lacking beta-glucosidase enzymes and transporters (d3BGdT) does not induce cellulase gene expression in response to cellobiose. We are currently characterizing the transport kinetics of each transporter in the d3BG background with the goal of understanding how transport of cellodextrins influences cellulose sensing and induction of cellulase expression.

36) Biochemical properties of NcRVT protein encoded by a reverse transcriptase-like gene from *Neurospora crassa*

Eugene Gladyshev (1), Irina Arkhipova (2), Nancy Kleckner (1) (1) Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138 and (2) Josephine Bay Paul Center, Marine Biological Laboratory, Woods Hole, MA 02543

NcRVT belongs to a recently discovered class of reverse transcriptase-related cellular genes of fungi, animals, plants and bacteria. NcRVT protein can be purified from vegetative mycelia of wild-type Neurospora strains grown in the presence of 0.1 ug/ml blasticidin, an antibiotic that blocks protein synthesis. An optional ammonium fractionation step may be used to concentrate NcRVT protein prior to centrifugation in a sucrose density gradient. Further purification is achieved by ion-exchange chromatography on DEAE Sepharose, where the protein can be eluted in a nearly pure form. In the presence of manganese, purified NcRVT protein has a potent terminal transferase activity with a pronounced preference for ribo- over deoxyribonucleoside triphosphates. Site-directed mutagenesis of a catalytic aspartate residue confirmed its essential role in this activity, ruling out the presence of any other co-purified polymerases. NcRVT cannot initiate polymerization de novo, instead it extends 12-14 nt RNAs that appear to be non- covalently associated with the protein. NcRVT does not readily accept exogenous primers or common primer-template combinations, however extension of co-purified tRNA, ribosomal RNA, and small nucleolar RNA fragments has been observed. Experiments are currently in progress to determine the nature of the associated RNAs as well as of NcRVT extension products in vivo.

37) Vectorial supply of vesicles directs thigmotropism of Neurospora

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Thigmotropism is the ability of an organism to exhibit an orientation response to a mechanical stimulus. We have quantified this thigmotropic response of *Neurospora crassa* to microfabricated slides with ridges of defined height and topography. We show that mutants that lack the formin Bni1 and the Rho-GTPase Cdc42, (activator of Bni1), have an attenuated thigmotropic response. In contrast, null mutants that lacked cell end-marker protein, Tea1, and KipA, the kinesin responsible for its localisation, had significantly increased thigmotropism. These results indicate that vesicle delivery to the hyphal tip via the actin cytoskeleton is critical for thigmotropism. Disruption of actin in the region of the hyphal tip which contacts obstacles such as ridges on microfabricated slides may lead to a bias in vesicle delivery to one area of the tip and therefore a change in hyphal growth orientation. This mechanism may differ to that reported in *Candida albicans* in so far as it does not seem to be dependent on the mechanosensitive calcium channel protein Mid1. The *N. crassa Δ-mid1* mutant was not affected in its thigmotropic response. Our findings suggest that thigmotropism in *C. albicans* and *N. crassa* are similar in being dependent on the regulation of the vectorial supply of secretory vesicles, but different in the extent to which this process is dependent on local calcium-ion gradients.

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