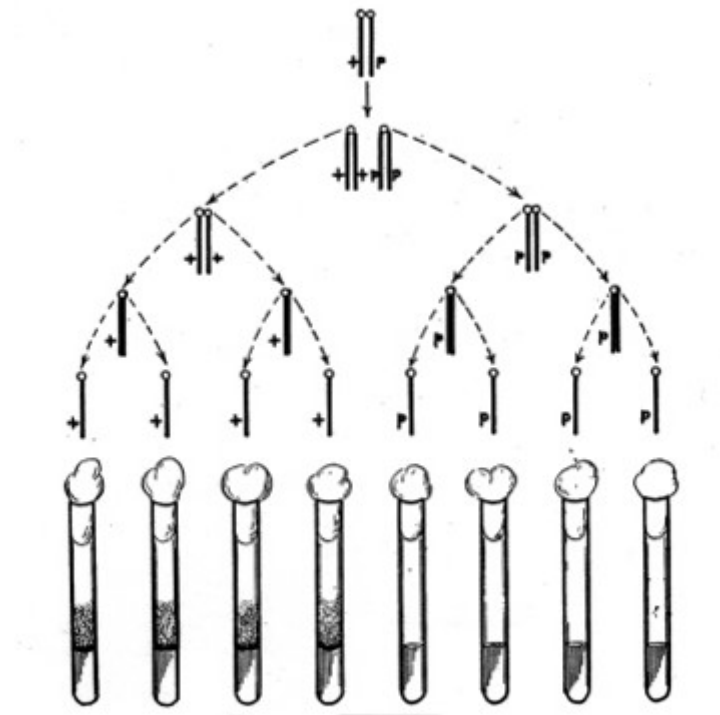


Neurospora 2016

March 10-13, 2016
Asilomar Conference Center
Pacific Grove
California



Program & Abstracts

In the 1920's and 1930's researchers were looking for organisms in which to test hypotheses regarding the new field of genetics. *Neurospora* was selected for use because it grew rapidly on chemically defined medium and reproduced in the laboratory. It has a haploid genome and the production and isolation of morphological or biochemical mutants is straight-forward.

Four figures were designed and commissioned by George Beadle and were published in at least two places:

American Scientist 34:31-53 (1946) 'Genes and the chemistry of the organism,' and

Fortschritte der Chemie organischer Naturstoffe 5:300-330 (1948) 'Some recent developments in chemical genetics'.

Beadle and Tatum won the 1958 Nobel prize for their demonstration of the One-gene, One-enzyme hypothesis and they shared the prize with Joshua Lederberg who had demonstrated transduction in *Salmonella*.

The drawings show, among other things, the process of isolation of auxotrophic mutants, the screening of auxotrophic mutants, the segregation of mutants, and the chromosome events associated with this segregation.

The original drawings are held at the Fungal Genetics Stock Center.

Neurospora 2016

March 10-13

Asilomar Conference Center
Pacific Grove
California

Scientific Organizers

Barry Bowman

Dept. Molecular Cell & Developmental Biology
University of California - Santa Cruz

Jason E. Stajich

Dept. Plant Pathology & Microbiology
University of California - Riverside

Neurospora Policy Committee

N. Louise Glass

Dept of Plant and Microbial Biology
University of California - Berkeley

Luis F. Larrondo

Dept Genética Molecular y Micro
Pontificia Universidad Católica de Chile

Barry Bowman

Molecular Cell & Developmental Biology
University of California - Santa Cruz

Jason E. Stajich

Dept. Plant Pathology & Microbiology
University of California - Riverside

Brief Schedule

	Morning	Afternoon	Evening
Thursday March 10		Arrival Registration	Dinner Mixer (Heather)
Friday March 11	Breakfast Plenary Session I Cell Biology and Morphogenesis	Lunch Plenary Session II Metabolism, Signaling and Development	Dinner Forum: Neurospora Community Building Poster Session
Saturday March 12	Breakfast Plenary Session III Gene Expression and Epigenetics	Lunch Plenary Session IV Genomics, Evolution, and Tools	Banquet Speaker: Matthew Sachs Poster Session
Sunday March 13	Breakfast Plenary Session V Circadian Clocks and Environmental Sensing	Lunch Departure	

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

Neurospora 2016 Scientific Program

Friday March 11th

7:30 - 8:30 Breakfast, Crocker Dining Hall

Morning (Heather) Cell Biology & Morphogenesis

Chairs: Barry Bowman / Asen Daskalov

8:30-8:40 Welcome & Announcements

- | | | |
|-------------|---------------------------|--|
| 8:40-9:00 | David Catcheside | A snapshot of genome-wide meiotic recombination in <i>Neurospora</i> . |
| 9:00-9:20 | Oded Yarden | The <i>Neurospora crassa</i> COT1 kinase – a regulator of polar growth: Interactions with type 2A phosphatase and the RNA-binding protein GUL1 |
| 9:20-9:40 | Frank Kempken | The BEM46 protein in <i>N. crassa</i> : Eisosomal localization and its link to tryptophan and auxin biosynthesis |
| 9:40-10:00 | Michael Freitag | The Kinetochore Interaction Network (KIN) of <i>Neurospora</i> |
| 10-10:30 | Coffee Break | |
| 10:30-10:50 | Barry J Bowman | Organelle Biogenesis in <i>Neurospora</i> : Characterization of a Novel Prevacuolar Compartment |
| 10:50-11:10 | Meritxell Riquelme | The Spitzenkörper of <i>Neurospora crassa</i> : a sublime valve for vesicle flow control |
| 11:10-11:30 | André Fleißner | Signal exchange and integration during vegetative cell fusion in <i>Neurospora crassa</i> |
| 11:30-11:50 | Rosa Mourino | Is hyphal morphogenesis impacted by endocytosis? |

12:10-13:00 Lunch, Crocker Dining Hall

Afternoon Metabolism, Signaling, & Development

Chairs: Kristina Smith / Kevin McCluskey

- | | | |
|-------------|----------------------------|--|
| 14:30-14:50 | Lori Huberman | Identifying carbohydrate sensing pathways in <i>Neurospora crassa</i> |
| 14:50-15:10 | Jeffrey Townsend | Two light receptors, the fast-evolving phytochrome PHY-2 and the oxygen-sensitive opsin NOP-1, modulate sexual development by perception of light in <i>Neurospora</i> |
| 15:10-15:30 | Steven Free | Melanin Biosynthesis in the Perithecium |
| 15:30-15:50 | Katherine Borkovich | Global analysis of predicted G protein coupled receptor genes |
| 15:50-16:20 | Coffee Break | |
| 16:20-16:40 | Chien-Hung Yu | A code within the code: codon usage affects translational dynamics to regulate protein folding |

16:40-17:00	Arit Ghosh	Regulation of cross pathway control by CPC-2 via the bZIP transcription factor CPC-1 in <i>Neurospora crassa</i>
17:00-17:20	Darae Jun	Investigating the mechanism of cellulase translocation through the secretory pathway of <i>Neurospora crassa</i>
17:20-17:40	Marcus Roper	Measuring individual size in <i>Neurospora</i>

Evening

18:00-19:00	Dinner, Crocker Dining Hall
19:30-20:30	Special interactive session: Building Neurospora collaborations (Heather)
20:30-22:00	Poster session

Saturday March 12th

7:30 - 8:30	Breakfast, Crocker Dining Hall
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Morning Gene Expression and Epigenetics

Chairs: Jennifer Hurley / Louise Glass

8:30-8:50	Luis M. Corrochano	Localization and stability of the regulator VE-1 during conidiation in <i>Neurospora crassa</i>
8:50-9:10	Shinji Honda	Shelterin is required for telomeric integrity in <i>Neurospora crassa</i>
9:10-9:30	Zachary Lewis	<i>Neurospora mus-30</i> encodes an LSH/DDM1-type chromatin remodeling enzyme required for genome maintenance
9:30-9:50	Kirsty Jamieson	Elements of constitutive heterochromatin determine the distribution of facultative heterochromatin
9:50-10:20	Coffee Break	
10:20-10:40	Thomas Hammond	Investigating unpaired DNA detection during meiosis in <i>Neurospora crassa</i> with ectopic fragments of the Round Spore gene.
10:40-11:00	Jesper Svedberg	The effects of meiotic drive on genome architecture in Spore killer strains of <i>Neurospora</i>
11:00-11:20	Durgadas P. Kasbekar	<i>Neurospora</i> crosses with hybrid translocation strains uncover a novel meiotic drive that targets homokaryotic progeny derived from alternate segregation
11:20-11:40	Jason Stajich	A Novel DNA transposon is recognized and silenced by Meiotic Silencing by small RNAs

12:00-13:00	Lunch, Crocker Dining Hall
	Neurospora Policy Committee Business meeting (NPC members and those interested sit at one table in Crocker)

Afternoon Evolution, Genomics, and New Tools

Chairs: Zachary Lewis / Jason Stajich

14:30-14:50	Christopher Hann-Soden	Untold Diversity in North American <i>Neurospora</i> Species Suggests Intrinsic Speciation
14:50-15:10	Adriana Romero-Olivares	<i>Neurospora discreta</i> as a model to assess adaptation of soil fungi to warming

15:10-15:30	Kevin McCluskey	New resources, research, and progress at the Fungal Genetics Stock Center
15:30-15:50	Luis F Larrondo	Neurospora meets Synthetic Biology: optogenetic tools to manipulate gene expression for scientific and artistic purposes
15:50-16:20		Coffee Break
16:20 - 16:40	Clifford Slayman	A Classical Transport Mutant, Whole Genome Sequencing, and the Shocking Truth About Hygromycin
16:40-17:20	(6, five-minute talks, chosen from submissions on Friday)	New, Cool, Tools for Neurospora research
17:20 - 18:00	Moderated by organizers	Synthesis of workshop & discussion

Evening

18:00	Banquet, Crocker Dining Hall
19:30-20:30	Banquet Speaker (Heather): Matthew Sachs , Texas A&M University
20:45 - 22:00	Post-banquet Social / Poster Session

Sunday, March 13th

7:30 - 8:30	Breakfast, Crocker Dining Hall
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Morning Circadian Clocks & Environmental Sensing

Chairs: Luis Larrondo / Luis Corrochano

9:00-9:20	Deborah Bell-Pedersen	Circadian Clock Regulation of mRNA Translation: eEF2 and the Ribosome Code
9:20-9:40	Kwangwon Lee	Local adaptation by losing circadian control of asexual development in <i>Neurospora discreta</i> .
9:40-10:00	Jennifer Hurley	Circadian control of global proteomic output in <i>Neurospora crassa</i> .
10 - 10:30		Coffee Break
10:30-10:50	William Belden	Facultative Heterochromatin at the clock gene frequency
10:50-11:10	Jennifer Loros	Structure/function analysis of WC-1 reveals mechanisms of White Collar Complex differential activation of light-regulated versus clock-regulated genes
11:10-11:30	Chris Hong	Phase Response Analysis of the Circadian Clock in <i>Neurospora crassa</i>
11:30-11:50	Brad Bartholomai	<i>period-1</i> encodes an ATP-dependent RNA helicase that influences nutritional compensation of the <i>Neurospora</i> circadian clock
12-1:00 PM	Lunch, Crocker Dining Hall / Box Lunches	
	Departure	

Abstracts

A snapshot of genome-wide meiotic recombination in *Neurospora*.

Fred Bowring¹, Bertrand Llorente², Marie-Claude Marsolier-Kergoat³, Scott E. Baker⁴, Kevin McCluskey⁵, Michael Freitag⁶, Jane Yeadon¹, and David Catcheside¹. ¹ School of Biological Sciences, Flinders University, PO Box 2100, Adelaide, South Australia, 5001. ² CNRS, Inserm, Aix-Marseille Université, Institut Paoli-Calmettes 27, Bd Leï Roure CS 30059, 13273 Marseille Cedex 09, France. ³ IBITECS, CEA/Saclay, France, and Musée de l'Homme, Paris, France. ⁴ Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington, USA. ⁵ Kansas State University, 1712 Claflin Rd 4744 Throckmorton Ctr. Manhattan, KS 66506, USA. ⁶ Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331-7303, USA.

Genetic diversity in laboratory stocks coupled with features of its reproductive biology make *Neurospora* an excellent model organism for the study of meiotic recombination. At the end of the *Neurospora* sexual phase, a complete set of the products of a single meiosis (an octad) is contained in the eight-spored ascus. This is important because without access to the entire meiotic output it is not possible to unambiguously distinguish between the different types of recombination event. Pomraning and co-workers identified 168,579 single nucleotide polymorphisms that distinguish the Oakridge (OR) and Mauriceville (MV) genomes and which have been used to determine the provenance of chromosomal regions amongst progeny from a cross between these strains. With the advent of next-generation sequencing, these two features made obtaining a genome-wide picture of recombination events possible. We have now sequenced four complete octads from a cross between OR and MV. The two octads (4 and 7) for which we have done a full analysis come from a cross in which both parents lack the *msh-2* gene. Since MSH2 repairs post-recombination mismatches its absence serves to preserve the original recombination footprint. We identified 151 and 109 recombination events in octads 4 and 7 respectively with some events being quite complex. There were 20 crossovers in octad 4 and 15 in octad 7, a number similar to the two crossovers per chromosome thought to be necessary for proper chromosome disjunction and typical of creatures as diverse as fungi and mammals. Segregation of markers in an aberrant 4:4 fashion is indicative of symmetric heteroduplex which results from Holliday junction migration in the recombination intermediate. In both octads, aberrant 4:4 segregation was associated with crossing over suggesting that Holliday junction migration is a feature of recombination intermediates resolved as a crossover.

The *Neurospora crassa* COT1 kinase – a regulator of polar growth: Interactions with type 2A phosphatase and the RNA-binding protein GUL1

Oded Yarden, Liran Aharoni-Kats, Hila Shomin and Inbal Herold. Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 7610000, Israel

COT1 is the founding member of the NDR kinase family, whose members are involved in regulation of polar growth in many eukaryotic cells. Inactivation of *cot-1* or altering COT1 phosphorylation states affects hyphal elongation and branching in *N. crassa*. MPS One Binder (MOB) proteins 2A/B are coactivators of COT1 and their interaction with COT1 is affected by the phosphorylation state of the kinase. We have determined the presence of a physical interaction between the type 2A phosphoprotein phosphatase (PP2A) catalytic and RGB1 regulatory subunits with COT1 suggesting that PP2A is a counter-acting phosphatase in the COT1 pathway. In addition, RGB1::GFP localizes to hyphal tips and along the plasma membrane, in a manner similar to COT1. Consequences of COT1 inactivation include cell wall abnormalities, concomitant with altered expression of genes encoding cell wall remodeling enzymes. At least part of these downstream effects are mediated by the COT1 substrate GUL1 [a homologue of the yeast SSD1p (Suppressor of SIT4 Deletion)]. Inactivation of *gul-1* partially suppresses the *cot-1* phenotype along with curbing the increased (20-250%) expression of chitin synthase (*chs*) 1-7, glucan synthase 1 (*gls-1*) and the chitinase *gh18-5*. We conclude that the severity of the *cot-1* phenotype is dependent on COT1 phosphorylation state and interactions with other COT1 complex components and that changes in cell wall remodeling machinery which are at least in part regulated by GUL1, contribute to the phenotypic alterations observed.

The BEM46 protein in *N. crassa*: Eisosomal localization and its link to tryptophan and auxin biosynthesis

Krisztina Kollath-Leiß, Puspendu Sardar & Frank Kempken

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The BEM46 homologous proteins are evolutionary conserved in eukaryotes (1). Although their function remains elusive, some studies indicated a role in processes associated with cell polarity. We previously reported that BEM46 in *N. crassa* is localized to the perinuclear ER and it is part of the fungal eisosome (2, 3), as it co-localizes with PilA, which is an integral part of the fungal eisosome. In addition we showed that the tryptophan transporter MTR is localized in the eisosome.

Over expression of *bem46*, RNAi mediated knock-down and knock-out strains show impaired germination of ascospores with reduced germination rate and non-directed germination tubes (2). The BEM46 protein interacts with the anthranilate-synthase (*trp-1*) and may influence the auxin biosynthesis of the fungus (3), as the gene expression of several genes encoding auxin biosynthesis enzymes and the tryptophan transporter gene *mtr* differs strongly in *bem46* over-expressing and down-regulated lines. Currently our investigations are focused on the complete description of the auxin biosynthesis gene network of *N. crassa* using bioinformatical tools and by investigating double and triple knock-out strains for their ability to synthesize auxin. In addition new components of the fungal eisosome have been identified with a special focus on amino acid transporter and permeases.

(1) KUMAR A, KOLLATH-LEIB K, KEMPKEN F (2013) Characterization of bud emergence 46 (BEM46) protein: sequence, structural, phylogenetic and subcellular localization analyses. *Biochem Biophys Res Comm* 438:526-532

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

(3) KOLLAT-LEIB K, BÖNNIGER C, SARDAR P, KEMPKEN F (2013) BEM46 shows eisosomal localization and association with tryptophan-derived auxin pathway in *Neurospora crassa*. *Euk Cell* 13:1051-1063

The Kinetochore Interaction Network (KIN) of Neurospora

Stephen Friedman, Shauna Otto, Pallavi A. Phatale, Kristina M. Smith, Michael Freitag

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331-7305

Chromosome segregation requires coordinated activity of a large assembly of proteins, the “Kinetochore Interaction Network” (KIN). More than 50 different proteins comprise the KIN or are associated with fungal centromeric DNA. CENP-A, sometimes called “centromere-specific H3” (CenH3), is incorporated into nucleosomes within or near centromeres. The “constitutive centromere-associated network” (CCAN) assembles on CenH3 chromatin, based on interactions with CENP-C. The outer kinetochore comprises the Knl1-Mis12-Ndc80 (“KMN”) protein complexes that connect the CCAN to microtubule spindles and in the process generate load-bearing assemblies for chromatid segregation. Several conserved binding motifs or domains within KMN complexes have been described recently, and these features of ascomycete KIN proteins are shared with metazoan proteins. We identified several ascomycete-specific domains and will report on our ongoing studies linking centromeric chromatin to the KMN via CCAN subcomplexes, with emphasis on the CENP-O complex.

Organelle Biogenesis in *Neurospora*: Characterization of a Novel Prevacuolar Compartment

Barry J. Bowman,^a Marija Draskovic,^a Robert R. Schnittker,^b Tarik El-Mellouki,^b Michael D. Plamann,^b Eddy Sánchez-León,^c Meritxell Riquelme,^c and Emma Jean Bowman^a Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, California^a; School of Biological Sciences, University of Missouri-Kansas City, Kansas City, Missouri^b; Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada (CICESE), Ensenada, Baja California, Mexico^c

Organelle biogenesis and intracellular trafficking pathways, intensively studied in yeast, have received much less attention in filamentous fungi. Analysis of fungal genomes indicates that filamentous fungi have many proteins found in animal cells but not in yeasts. In *Neurospora crassa* and related fungi the family of Rab GTPases (which confer identity to vesicles in trafficking pathways) include RAB-2, RAB-4 and RAB-A, proteins not found in *S. cerevisiae*. Using confocal microscopy we have investigated the cellular location of Rab-GTPases, SNARE proteins, and other effectors involved in organelle biogenesis. Rab-2, Rab-4, and Rab-7 (a known marker of vacuoles), were observed in a novel prevacuolar compartment (PVC). This PVC organelle had integral membrane proteins found in vacuoles (V-ATPase, calcium/H⁺ transporter, polyphosphate polymerase) but did not have soluble vacuolar proteins (carboxypeptidase Y, alkaline phosphatase). PVCs are unusually large, many as big or bigger than nuclei. They can change shape quickly and appear to produce tubular protrusions. Globular clusters of dynein and dynactin were observed in the interior space of many PVCs. The boundary layer of the PVCs appears to have a complex structure and preliminary observation with a new high-resolution microscope suggests that the PVC could be a sphere of tubules. The size, structure, dynamic behavior and protein composition of the PVCs are significantly different from the well-studied prevacuolar compartments of yeasts.

The Spitzenkörper of *Neurospora crassa*: a sublime valve for vesicle flow control

Merixell Riquelme¹, Natasha Savage² and Robert W. Roberson³

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³School of Life Sciences, Cellular and Molecular Faculty, Arizona State University, Tempe, Arizona 85287-1601.

Over the last 10 years a collection of proteins participating in the polarized tip growth of *Neurospora crassa* have been identified. Among those proteins, the cell wall biosynthetic enzymes have been found concentrated at the hyphal apex, arranged in a highly organized manner within the Spitzenkörper (Spk). Chitin synthases are contained in microvesicles (chitosomes), which concentrate at the core of the Spk, while β -1,3-glucan synthases are contained in macrovesicles, which occupy the outer layer of the Spk. FRAP analysis on each of these vesicle subgroups has revealed recovery rates of $t^{1/2} = 21$ sec for the microvesicular core and of $t^{1/2} = 32$ sec for the macrovesicular outer layer, indicating the existence of high vesicular flow rates into and out of the Spk, which itself maintains a homeostatic state. Transmission electron micrographs of 70 nm hyphal sections have allowed us to count the number of microvesicles and macrovesicles present in hyphal tip slices. These counts, along with hyphal tip geometry, have been used to estimate vesicle numbers for whole hyphal tips. Assuming vesicle density homeostasis, and that all vesicles in the tip are secretory, the FRAP recovery rates described above can be used in conjunction with vesicle number calculations to estimate vesicle insertion rates. Considering vesicles to be spheres of an average diameter, the total surface area inserted into the tip plasma membrane per min is then calculated. A flow rate ranging from 5,000 to 11,000 vesicles per min (including both macro and microvesicles) would account for an increase of surface area ranging from 76 to 108 $\mu\text{m}^2/\text{min}$, values that are within the range of previously measured growth values (66 to 288 $\mu\text{m}^2/\text{min}$).

Signal exchange and integration during vegetative cell fusion in *Neurospora crassa*

André Fleißner

Technische Universität Braunschweig, Germany

Fusion of conidial germlings or hyphal branches is prevalent in the life cycle of filamentous ascomycete fungi. In order to fuse, hyphae need to establish physical contact by tropically growing towards each other. In *Neurospora crassa*, this cellular interaction is mediated by an unusual signaling mechanism, in which the two fusion partners take turns in signal sending and receiving. This unique mode of communication requires the cells to coordinate their behavior over a spatial distance before they physically touch.

In recent years, numerous molecular factors mediating this process have been identified, including the MAK-1 and MAK-2 MAP kinase modules and the SO protein. In interacting hyphae, the MAK-2 signaling complex and the SO protein are recruited to the plasma membrane of the growing hyphal tips in an alternating manner. By manipulating the spatial and temporal dynamics of these recruitments, we found that the activity of MAK-2 strongly correlates with its subcellular localization. The presence of phosphorylated MAK-2 at the plasma membrane prevents recruitment of SO. This negative dependency might represent a key feature in coordinating the cellular signaling. In addition, the characterization of a novel SO-interaction partner revealed that fusion competent cells alternate between two physiological stages even in the absence of a fusion partner. The switching behavior is therefore cell autonomous, but becomes coordinated once two cells have sensed each other.

Preliminary experiments indicate that *N. crassa* also undergoes tropic interactions with other fungal species, for example *Botrytis cinerea*. In these interspecies pairings, MAK-2 recruitment to the plasma membrane is still oscillating in the *N. crassa* cell. This surprising finding suggests that the described mode of cellular communication is conserved in ascomycete species and that fungi master foreign molecular languages.

Is hyphal morphogenesis impacted by endocytosis?

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In filamentous fungi the machinery for endocytosis is found mainly in a collar formed by small actin patches with several associated proteins. In this work we examined the proteins related to the recognition stage of endocytosis such as clathrin, AP-180, SLA-1 and the recruitment stage LAS-17/WSP-1 (Wiskott - Aldrich Syndrome Protein) and MYO-1 (myosin class I) by fluorescent-protein tagging and deletion mutants analysis to observe the effect of endocytosis in hyphal morphogenesis. The mutations of the *chc* (clathrin heavy chain), *clc* (clathrin light chain) and AP-180 were lethal for *N. crassa*. SLA-1, LAS-17/WSP-1 and MYO-1 mutants showed marked but different reductions in growth rate, irregular branching, reduced aerial mycelia and poor or no conidiation. All these proteins were located in the endocytic collar and in scattered patches along the hypha. Faint clathrin fluorescence was detected in the endocytic collar, and in the cytoplasm apparently associated to Golgi equivalents. By visualizing actin with Lifeact-GFP, we found that the absence of *myo-1* or *sla1* alters the actin cytoskeleton, leading to periods of polarized and isotropic growth. Our findings indicate that clathrin and AP-180 are essential for cell viability but proteins associated to the endocytosis recruitment stage are not, although they are important for growth and hyphal morphogenesis and for the stability of the actin cytoskeleton.

Identifying carbohydrate sensing pathways in *Neurospora crassa*

Lori B. Huberman, Samuel T. Coradetti, and N. Louise Glass
Energy Biosciences Institute, University of California Berkeley

Identifying and utilizing the nutrients available in the most efficient manner is a challenge common to all organisms. In humans inaccurate or failed nutrient sensing can result in a variety of diseases including diabetes and obesity, and cancer progression has been shown to rely on increased glucose uptake and changes in nutrient sensing. Many of the nutrient sensing pathways are conserved from yeast to humans, and studies on nutrient sensing in unicellular eukaryotes have been instrumental in elucidating nutrient sensing pathways in humans. The model filamentous fungus, *Neurospora crassa*, is capable of utilizing a variety of carbohydrates: from simple sugars to the complex sugar chains found in plant cell walls. In order to efficiently exploit the available resources, *N. crassa* must be capable of sensing and responding to the presence of these different carbohydrates. Several transcription factors have been identified that activate the transcription of plant cell wall-degrading enzymes including CLR1, which is responsible for activating the transcription of cellulases in the presence of cellulose. However, expression of CLR1 in the absence of an inducer does not lead to significant transcriptional activation of its target genes, indicating that activation or derepression of CLR1 is necessary. We performed a screen to identify mutants that are unable to properly regulate the transcription of cellulases, and identified genes which appear to be involved in the activation of cellulase transcription. As we continue to characterize these mutants, we expect to shed light on the cellulose sensing pathway in *N. crassa* and, more generally, provide insight into nutrient sensing in other fungi and multicellular eukaryotes.

Two light receptors, the fast-evolving phytochrome PHY-2 and the oxygen-sensitive opsin NOP-1, modulate sexual development by perception of light in *Neurospora*

Zheng Wang^{1,2}, Ning Li¹, Jigang Li³, Frances Trail⁴, Jay C. Dunlap⁵, Jeffrey P. Townsend^{1,2,6*}

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² Department of Biostatistics, Yale School of Public Health, New Haven, CT 06520, USA.

³ State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100193, China. ⁴ Department of Plant Biology, Department of Plant Pathology, Michigan State University, East Lansing, MI 48824, USA. ⁵ Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH 03755, USA. ⁶ Program in Microbiology, Yale University, New Haven, CT 06510, USA. *Speaking author: Jeffrey P. Townsend (Jeffrey.Townsend@Yale.edu)

Rapid responses to changes in incident light are critical to the guidance of behavior and development in most species. Phytochrome light receptors in particular play key roles in bacterial physiology and plant development, but their functions and regulation are less well understood in fungi. The same is true of opsins, well-known sensory molecules homologous to bacterial green-light sensory rhodopsins. One of these four opsin-like proteins has been molecularly characterized as a proton pump, and no known functions at the organismal level have been recognized for either phytochromes or opsins—organism-level mutant or knockout phenotypes have not been identified in fungi. Nevertheless, genome wide expression measurements provide key information that can guide experiments that reveal how genes respond to environmental signals and clarify their role in development. We performed functional genomic and phenotypic analyses of the phytochrome *phy-2* in *Neurospora crassa*, which is adapted to a post-fire environment in which it experiences dramatically variable light conditions. Expression of *phy-2* was low in early sexual development, and in the case of *phy-2*, increased in late sexual development. Under light stimulation, strains with the phytochromes deleted exhibited increased expression of sexual development related genes. Moreover, under red light, the knockout strain of *phy-2* commenced sexual development early. In the evolution of phytochromes within ascomycetes, at least two duplications have occurred, and the faster-evolving *phy-2* has frequently been lost. Additionally, the three key cysteine sites that are critical for bacterial and plant phytochrome function are not conserved within fungal *phy-2* homologs. Through the action of phytochromes, transitions between asexual and sexual reproduction are modulated by light level and light quality, presumably as an adaptation for fast asexual growth and initiation of sexual reproduction of *N. crassa* in exposed post-fire ecosystems. We also examined functional divergence of key amino acids on the phylogeny of fungal opsins. In *N. crassa* and related species, the opsin genes are up-regulated during sexual development. Early development of protoperithecia was observed for an *N. crassa nop-1* knockout mutant cultured in unsealed plates under constant blue and white light, potentially due to light-dependent loss of sensitivity to oxygen level in the mutant. When compared to wild type strains, the knockout mutant of *nop-1* in *N. crassa* abundantly expressed genes involved in oxidative stress response, NAD/NADP binding, proton trans-membrane movement and catalase activity, and the homeostasis of protons. These lines of evidence, along with recently revealed phenotypes of gene knockouts in oxidative pathways along with the decreased sensitivity to oxidative stress of the *nop-1* knockout mutant, support the role of NOP-1 in the negative regulation of the initiation of sexual reproduction via ROS pathways in *N. crassa*.

Melanin Biosynthesis in the Perithecium.

Jie Ao, Sumit Bandyopadhyay, Stephen J. Free, SUNY University at Buffalo.

Dihydroxynaphthalene (DHN) melanin is a black colored polymer found in the cell walls of a number of fungi. It helps strengthen the cell wall, and provides protects the fungus from dessication, UV light, antibiotics, and from cell wall degrading enzymes produced by other microbes. In some fungal pathogens the cell wall melanin is an important virulence factor. In *Neurospora crassa* two different cell types become highly melanized, the peridium cells and the ascospores. Our research is focused on answering three key questions about DHN melanin. First, we want to identify the enzymes involved in creating DHN melanin in *N. crassa*. Second, we want to learn how the fungus regulates the expression of these genes to create melanized cell walls in a tissue type-specific manner. Third, we want to determine how the dihydroxynaphthalene, which is synthesized by cytosolic enzymes, becomes polymerized into extracellular melanin.

Global analysis of predicted G protein coupled receptor genes

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G protein-coupled receptors (GPCRs) regulate growth, development, and environmental sensing in filamentous fungi. The largest predicted GPCR class identified in Pezizomycotina ascomycete filamentous fungi, including *Neurospora crassa*, is the Pth11-related, with members similar to a protein required for disease in the plant pathogen *Magnaporthe oryzae*. However, the Pth11-related class has not been functionally studied in any fungal species. We analyzed phenotypes in available mutants for 36 GPCR genes, including 20 Pth11-related, in *N. crassa*. We also investigated patterns of gene expression for all 43 predicted GPCR genes in available datasets. A total of 17 mutants (47%) possessed at least one growth or developmental phenotype. We identified 18 mutants (56%) with chemical sensitivity or nutritional phenotypes (11 uniquely), bringing the total number of mutants with at least one defect to 28 (78%), including 15 mutants (75%) in the Pth11-related class. Gene expression trends for GPCR genes correlated with the phenotypes observed for many mutants and also suggested overlapping functions for several groups of co-transcribed genes. Several members of the Pth11-related class possessed phenotypes and/or were differentially expressed on cellulose, suggesting a possible role for this gene family in plant cell wall sensing or utilization.

A code within the code: codon usage affects translational dynamics to regulate protein folding

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Codon usage bias is a universal feature of eukaryotic and prokaryotic genomes and has been proposed to regulate translation efficiency, accuracy and protein folding based on the assumption that codon usage affects translation dynamics. We have previously demonstrated that non-optimal codons regulates circadian clock protein function and structure in *Neurospora*. In addition, we observed genome-wide correlations between codon usage and protein structure motifs. Here we show that codon usage bias plays an important role in regulating translational dynamics in vitro and in vivo. We further demonstrate that codon-regulated translational rhythm affects protein function by modulating co-translational protein folding. Together, these results resolve a long-standing fundamental question and demonstrate the importance of codon usage for co-translational protein folding.

Regulation of cross pathway control by CPC-2 via the bZIP transcription factor CPC-1 in *Neurospora crassa*

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Cross pathway control is a global response mechanism by which eukaryotic cells can respond to changes in amino acid starvation. Depletion of one amino acid can lead to upregulation of various enzymes vital to other amino acid biosynthetic pathways. In *Neurospora crassa*, de-repression of the bZIP transcription factor *cpc-1* (cross pathway control-1) is essential for transcriptional induction of amino acid biosynthetic genes when cells are artificially starved for histidine through supplementation with 3-aminotriazole. The WD40 protein/ RACK1 homolog encoding gene – *cpc-2* has also been found to be an important component of the cross pathway network in *N. crassa*. We are currently interested in understanding how CPC-2 regulates cross pathway control and how it might regulate the transcription factor CPC-1 – which is the central hub for controlling how the cell responds to amino acid starvation.

Investigating the mechanism of cellulase translocation through the secretory pathway of *Neurospora crassa*

Jun, Darae, Liu, J. and Glass, NL. (UC Berkeley)

As scavengers of plant biomass in the environment, *Neurospora crassa* has evolved to be exceptionally good at secreting large amounts of enzymes. However, the mechanism that regulates this increase in protein secretion under lignocellulosic conditions is still obscure. To elucidate these unknown mechanisms, we are in the process of doing a forward genetic screen to identify and characterize defective trafficking mutants that cannot translocate cellulases into the ER or that retain cellulases in the ER on their way to the extracellular environment. To conduct the screen, we used a mutant library created by random mutagenesis on a strain with a GFP tagged endoglucanase (EG-2). Using this mutant library, we screened for mutants with mis-localized EG-2 by microscopy and identified a particularly interesting mutant. We plan to further characterize this mutant using bulk segregant analysis to map the casual mutation; we also plan to assess the accuracy of cellulase mRNA targeting, cellulase translocation, and ER to the Golgi trafficking. Identifying unknown components that play a role in secretion and trafficking of cellulases will provide a better understanding of the regulation of the secretory pathway and improve the efficiency of biofuel production.

Measuring individual size in Neurospora

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It is challenging to delimit filamentous fungal individuals, because a fungal mycelium can contain millions of genetically diverse but totipotent nuclei, each capable of founding new mycelia. Moreover a single mycelium can potentially stretch over kilometers and it is unlikely that its distant parts share resources or have the same fitness. Using *Neurospora crassa* heterokarya we show that a filamentous fungal mycelia can be decomposed into Reproductive Units (RUs); subpopulations of nuclei that propagate together as spores, and function as reproductive individuals. RUs are dynamic units of fungal individuality, that change their size in response to environmental conditions and the geometry of the growing mycelium. They provide a concept of fungal individuality that is directly connected to reproductive potential, and therefore to theories of how fungal individuals adapt and evolve over time. Time permitting, I will describe how population genetics models for the different nuclei that may coexist within the Neurospora mycelium can explain the growth and diversity of Reproductive Units.

Localization and stability of the regulator VE-1 during conidiation in *Neurospora crassa*

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The *N. crassa ve-1* gene is a homolog of *veA* in *Aspergillus nidulans*. In *A. nidulans* mutations in *veA* results in constitutive conidiation that is independent of light, and the VeA protein forms a complex with blue and red photoreceptors. The *N. crassa ve-1* mutant has defects in aerial hyphal growth and increased conidiation. We have characterized the light-dependent accumulation of carotenoids in strains with a deletion in *ve-1* and in the wild type for a comparison. A ten-fold reduction in sensitivity was observed in the *ve-1* mutant, an indication for a role of VE-1 in light sensing in *N. crassa*. VE-1 is a protein with a nuclear localization signal and a velvet factor domain that is highly conserved in fungi. We observed a minor increase in the accumulation of *ve-1* mRNA after light exposure in vegetative mycelia (30 min), which did not lead to a major change in VE-1 accumulation. The mutation in *ve-1* results in decreased light-dependent accumulation of mRNA for several genes, including the carotenogenesis genes (*al-1*, *al-2*, *al-3*, *cao-2*), *wc-1*, *vvd*, and *frq*. We characterized the cellular localization of VE-1 under different light conditions and we have observed that VE-1 is preferentially located in the nucleus under all conditions, but VE-1 was also detected in the cytoplasm. We detected VE-1 in vegetative mycelia in the dark but light promoted the accumulation of VE-1 in vegetative mycelia, aerial hyphae and conidia through the activity of the WC complex. We didn't observe any major changes in the accumulation of *ve-1* mRNA during conidial development but exposure to light modify the stability of VE-1. The light-dependent degradation of VE-1 requires CSN-5 and FWD-1 and the activity of the WCC. We propose that the absence of VE-1 in aerial hyphae is a key step in the regulation of conidial development.

Shelterin is required for telomeric integrity in *Neurospora crassa*

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A telomere-specific protein complex, shelterin, caps and protects chromosome ends against inappropriate DNA damage, telomeric fusion and telomere length in eukaryotes. However, in any filamentous fungi, the corresponding shelterin has not been identified. Here we show *Neurospora* shelterin which is composed of five core proteins: the two components, POT-1 and RAP1, are conserved from yeasts to mammals whereas the others named TRF-1, TPP-1 and TIN-2 are only conserved in the closely-related species but are functional orthologues of mammalian TRF1/2, TPP1, TIN2, respectively. By ChIP-seq analyses, we confirmed that the double-strand telomeric repeats-binding domain protein TRF-1 is specifically localized to chromosome ends. Fluorescence microscopic analyses revealed that the GFP-tagged TRF-1 forms 2~4 foci which are mostly associated with nuclear envelope and heterochromatic foci but not a single centromeric spot and the telomeric foci formation is dependent of the telomerase but not H3K9 methylation that directs heterochromatic foci in *Neurospora*. We confirmed that TRF-1-GFP is colocalized with all the other RFP-tagged components of *Neurospora* shelterin. We found that mutants lacking the single-strand telomeric repeats-binding domain protein POT-1 shows circular chromosomes, highly sensitive to genotoxic chemicals and significant accumulation of gamma-H2A, which is a marker of DNA damage, at telomeres. In addition, *pot-1* mutants display increased RPA foci which are known to be required for the recruitment of the DNA damage response ATR to sites of DNA single-strand breaks. Furthermore, a temperature-sensitive *mus-9* (ATR) mutants lacking POT-1 show growth defects at the permissive temperature, supporting the requirement of ATR for DNA repair in *pot-1* mutants. We demonstrate that *Neurospora* shelterin indeed protects telomeres against improper DNA damage and telomeric fusion.

***Neurospora mus-30* encodes an LSH/DDM1-type chromatin remodeling enzyme required for genome maintenance**

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LSH/DDM1 enzymes are required for DNA methylation in higher eukaryotes and have poorly defined roles in genome maintenance in yeast, plants, and animals. The filamentous fungus *Neurospora crassa* is a tractable system that encodes a single LSH/DDM1 homolog (NCU06306). We report that the *Neurospora* LSH/DDM1 enzyme is encoded by *mutagen sensitive-30* (*mus-30*), a locus identified in a genetic screen over 25 years ago. We show that MUS-30-deficient cells have normal DNA methylation, but are hypersensitive to DNA damaging agents. MUS-30 is a nuclear protein, consistent with its predicted role as a chromatin remodeling enzyme, and levels of MUS-30 are increased following DNA damage. MUS-30 co-purifies with *Neurospora* WDR76, a homolog of yeast Changed Mutation Rate-1 and mammalian WD40 repeat domain 76. Deletion of *wdr76* rescued DNA damage-hypersensitivity of *Dmus-30* strains, demonstrating that the MUS-30-WDR76 interaction is functionally important. DNA damage-sensitivity of *Dmus-30* is partially suppressed by deletion of methyl adenine glycosylase-1, a component of the base excision repair machinery (BER); however, the rate of BER is not affected in *Dmus-30* strains. We found that MUS-30-deficient cells are not defective for DSB repair, and we observed a negative genetic interaction between *Dmus-30* and *Dmei-3*, the *Neurospora* RAD51 homolog required for homologous recombination. Together, our findings suggest that MUS-30, an LSH/DDM1 homolog, is required to prevent DNA damage arising from toxic base excision repair intermediates. Overall, our study provides important new information about the functions of the LSH/DDM1 family of enzymes.

Elements of constitutive heterochromatin determine the distribution of facultative heterochromatin

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We are exploring the interactions between facultative and constitutive heterochromatin in *Neurospora crassa*. Methylated lysine 27 on histone H3 (H3K27me) marks facultative heterochromatin, consisting of transcriptionally repressed, gene-rich chromatin. Constitutive heterochromatin marks gene-poor, AT-rich DNA and is formed by a largely unidirectional pathway consisting of three steps: 1) the DCDC methylation complex catalyzes the trimethylation of H3K9 (H3K9me3) 2) H3K9me3 is bound by HP1 and 3) DNA methylation catalyzed by dim-2. We tested how disruption of each step might affect H3K27me. H3K27me became massively redistributed by disrupting either of the first two steps in this pathway, elimination of any member of DCDC or by loss of HP1. The features of this redistribution are twofold: regions of facultative heterochromatin lost H3K27me3, while regions that are normally marked by H3K9me3 gained H3K27me2. Since loss of HP1 leaves H3K9me3 largely intact, the normally non-overlapping H3K27me2 and H3K9me3 marks became superimposed, as revealed by mass spectrometry from an *hpo* strain. Elimination of the final step in the pathway, catalyzing DNA methylation, had no obvious effect on the distribution of H3K27me. Elimination of H3K27me machinery did not impact constitutive heterochromatin. Additional work on the control of H3K27me will also be discussed.

Investigating unpaired DNA detection during meiosis in *Neurospora crassa* with ectopic fragments of the Round Spore gene.

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Neurospora crassa uses a process called Meiotic Silencing by Unpaired DNA (MSUD) to detect unpaired DNA between homologous chromosomes during meiosis. For example, in crosses between two strains, one of which carries a fragment of the Round spore gene (referred to as a ref marker) at an ectopic location on chromosome VII, MSUD efficiently detects the ref marker as unpaired. The proteins and mechanisms involved in detecting the ref marker as unpaired are mostly unknown. In previous work, we performed crosses between strains carrying ref markers at different locations on chromosome VII. We found that the efficiency of unpaired DNA detection increased with increasing distance between the markers. Here, we present a number of new findings. First, ref markers are efficiently detected as unpaired at several different euchromatic locations across chromosome VII. Second, large unpaired DNA loops within the vicinity of an unpaired ref marker decrease the efficiency of MSUD. Third, the specific arrangement of homology (i.e. microhomology) appears to play an important role in determining whether two genes are considered paired or unpaired. These results provide new knowledge on the unpaired DNA detection process in *N. crassa*. Future experiments will be performed to examine each of these findings in more detail as part of an effort to fully elucidate the mechanism of unpaired DNA detection during MSUD.

The effects of meiotic drive on genome architecture in *Spore killer* strains of *Neurospora*

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Selfish genes, which act in their own interest and can spread without increasing the fitness of the individual as a whole, are thought to be a major driver of the evolution of genome structure and composition. Meiotic drive is the phenomenon where a selfish genetic element causes itself to end up in more than the expected 50% of the meiotic products. In the seventies, strains displaying meiotic drive were identified in natural populations of *Neurospora intermedia* and *N. sitophila*. When these strains were crossed with “sensitive” strains, half of the spores in each ascus were killed, and the surviving spores would all show the killing phenotype in further crosses. For this reason these strains were named *Spore killers*.

In order to study the genomic architecture of the region surrounding the *Spore killer* genetic elements we used PacBio long-read sequencing (to >50x coverage) together with short-read Illumina data to create high quality, full chromosome genome assemblies of fifteen strains of *Neurospora* which are representative of all known *Spore killer* types as well as of sensitive and resistant strains.

These data have revealed that *Spore killer* strains of *N. intermedia* carry complex patterns of tandem inversions, interspersed with large clusters of RIP:ed transposable elements. These structural differences cover a ~3 Mbp region surrounding the *Spore killer* genetic element where recombination with sensitive strains is absent and may be instrumental in maintaining linkage between the genes causing the killer phenotype. The two different *Spore killer* types found in *N. intermedia* both show similar patterns of structural variation compared to sensitive strains, but they also differ from each other. This suggests that they have evolved separately and accumulated structural variation independently, even though they are found within the same species and exhibit very similar phenotypes.

We make the case that the need for linkage between the necessary genes is the primary driver of the evolution of *Spore killer* genome architecture, but that relaxed selection due to sheltering by the meiotic drive and degeneration due to reduced recombination may also play important roles.

The *Neurospora Spore killers* have here given us an excellent opportunity to study how the proliferation of selfish genes can play a significant role in shaping eukaryote genomes.

Neurospora crosses with hybrid translocation strains uncover a novel meiotic drive that targets homokaryotic progeny derived from alternate segregation.

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Four insertional or quasiterminal translocations (T) were recently introgressed from *Neurospora crassa* into *N. tetrasperma*. Crosses of some of the resulting T^{Nt} strains with *N. tetrasperma* N strains (N = normal sequence) produced many more Dp than T and N progeny. T and N progeny are generated by alternate segregation (ALT), whereas Dp and Df arise from adjacent-1 segregation (ADJ). Although the same genes are shared by both $[T + N]$ and $[Dp + Df]$ ascus types, their distribution in the *mat A* and *mat a* nuclei differs, and this results in differential survival of ALT- versus ADJ-derived homokaryotic progeny ascospores in a novel and unprecedented type of meiotic drive. We suggest that incompatibility between *N. crassa*- and *N. tetrasperma*-derived genes causes an insufficiency for a product required for ascospore maturation. In $[Dp + Df]$ asci, only four ascospores (Dp or $[Dp + Df]$ types) share this limited resource because the Df homokaryons are inviable, whereas four to eight ascospores can compete for it in $[T + N]$ asci, increasing the likelihood that none properly matures. Thus, Dp homokaryons are over-produced relative to T and N types, whereas $[T + N]$ and $[Dp + Df]$ heterokaryons are produced in equal numbers.

A Novel DNA transposon is recognized and silenced by Meiotic Silencing by small RNAs

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Genome defense likely evolved to curtail the spread of transposable elements and invading viruses. A combination of effective defense mechanisms has been shown to limit colonization of the *Neurospora crassa* genome by transposable elements. A novel DNA transposon we named *Sly1-1* was discovered in “wild-type” strain FGSC 2489 (OR74A). Meiotic silencing by unpaired DNA, also simply called meiotic silencing, prevents the expression of regions of the genome that are unpaired during karyogamy. This mechanism is posttranscriptional and is proposed to involve the production of small RNA, so-called masiRNAs, by proteins homologous to those involved in RNA interference-silencing pathways in animals, fungi, and plants. Here, we demonstrate production of small RNAs when *Sly1-1* was unpaired in a cross between two wild-type strains. These small RNAs are dependent on SAD-1, an RNA-dependent RNA polymerase necessary for meiotic silencing. We present the first case of endogenously produced masiRNA from a novel *N. crassa* DNA transposable element.

Untold Diversity in North American *Neurospora* Species Suggests Intrinsic Speciation

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Barriers to recombination can maintain integrity between diverging populations and prevent maladaptive hybrid genotypes. In this way, barriers to recombination can facilitate ecological and allopatric speciation. When the barrier to recombination is absolute, or when it is selected for strongly, a genetic barrier to recombination may be an intrinsic mechanism of sympatric speciation. Our recent sampling efforts across North America have revealed numerous sympatric populations of *Neurospora* species in areas previously thought to be dominated by *Neurospora discreta*. These samples include pairs of sister species that are divergent for the ability to self-cross. Selfing has been previously shown to have evolved at least nine separate times in *Neurospora*. We hypothesize that if selfing represents an absolute barrier to recombination, or if there is strong selection for selfing, that transitions to selfing lifestyles could be driving diversification within *Neurospora*, leading to the observed abundance of sympatric species. Previous studies have shown that selfing *Neurospora* lineages accumulate point mutations at a higher rate, indicative of a reduction in recombination. We show further that the rate of genomic rearrangements is accelerated in selfing lineages, indicating a relaxation of selection on genome architecture. Because genomic rearrangements are barriers to recombination themselves, we hypothesize a positive feedback cycle between selfing and rearrangements that drives sympatric speciation in a ratchet-like manner.

***Neurospora discreta* as a model to assess adaptation of soil fungi to warming**

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Short-term experiments have indicated that warmer temperatures can alter fungal biomass production and CO₂ respiration, with potential consequences for soil carbon (C) storage. However, we know little about the capacity of fungi to adapt to warming in ways that may alter C dynamics. To understand the effects of the fungal community in an increasingly warmer environment and the repercussions on ecosystems' processes, we used *Neurospora discreta* as a model organism to answer questions in relation to global warming and fungal metabolism. We exposed *N. discreta* to warm selective temperatures (16 and 28 °C) for 1500 mitotic generations, and then examined changes in mycelial growth rate, biomass, spore production, and CO₂ respiration (Mass specific respiration, MSR). We hypothesized that strains will adapt to its selective temperature. More specifically, we expected that adapted strains would grow faster, and produce more spores per unit biomass (i.e., relative spore production). In contrast, they should generate less CO₂ per unit biomass due to higher efficiency in carbon use metabolism.

In support to our hypothesis, *N. discreta* adapted to warm temperatures, based on patterns of relative spore production. Adapted strains produced more spores per unit biomass than parental strains in the selective temperature. Nonetheless, this increase was accompanied by an increase in MSR and a reduction in mycelial growth rate and biomass, compared to parental strains.

Our results suggest that adaptation of *N. discreta* to warm temperatures may have elicited a tradeoff between biomass production and relative spore production, possibly because relative spore production required higher MSR rates. Our results do not support the idea that adaptation to warm temperatures will lead to a more efficient carbon use metabolism. Our data might help improve climate change model simulations and provide more concise predictions of decomposition processes and carbon feedbacks to the atmosphere.

New resources, research, and progress at the Fungal Genetics Stock Center.

Kevin McCluskey

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Having successfully relocated in November 2014 with only minimal disruption of normal distribution, the FGSC has continued its impact in diverse research communities. While the removal of US National Science Foundation support has brought change to all US living microbe collections, the FGSC has set a positive example by moving to a new institution better aligned with the support, research, and outreach missions of the FGSC. Similarly, the FGSC has emerged as a leading collection in both the US Culture Collection Network and also the World Federation for Culture Collections.

Many changes are impacting the FGSC, however, including the diminished role in organizing meetings, publishing the *Fungal Genetics Reports*, serving as central resource for gene naming, and supporting diverse research communities through ex officio roles. Having secured a long-term home for the biological materials, FGSC digital archival resources will be increasingly migrated to digital repositories to assure their future availability. This will also enable the provision of new resources, such as digital deposit sheets from the early years, robust material management, and website revisions to better showcase newer and highly impactful materials. Among these are gene deletion resources for clinically relevant yeasts, strains for gene manipulation, *Neurospora* from sugar beet bagasse, and new plasmids for protein tagging and visualization. The FGSC has served for over 50 years and remains central to the conduct of research with filamentous fungi.

Neurospora meets Synthetic Biology: optogenetic tools to manipulate gene expression for scientific and artistic purposes

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Light is a strong environmental cue that informs organisms about their whereabouts, and can reprogram gene expression allowing a better adaptation to the surrounding environment. The fungus *Neurospora crassa* has been one of the main models for the study of photobiology, providing great insights on how microorganisms perceive and respond to light. This ascomycete responds specifically to blue-light (but not to other wavelengths) through a transcriptional heterocomplex named White Collar Complex (WCC). One of its components, WC-1, possesses a LOV (Light Oxygen Voltage) domain capable of detecting blue light, which promotes a conformational change that leads to a light-dependent dimerization that results in strong transcriptional activation. Thus, the expression of WCC-target genes is rapidly and precisely controlled in a dose-dependent manner when light is present. In order to design and improve optogenetic switches that can be utilized in other organisms as orthogonal controllers, we have been exploring the dynamics of light responses in this organism. Through the development of simple synthetic switches we have successfully implemented a blue-light responding transcriptional system in *Saccharomyces cerevisiae*. Therefore, now in yeast (which naturally does not respond to light) we can efficiently and orthogonally induce gene expression. Most importantly, in order to better identify the kinetics of light-responses in *Neurospora*, we have explored the sensitivity and spatial resolution of this system. In doing so, we have been able to genetically program images in this organism. Thus, we can project a photograph on a surface covered with *Neurospora* carrying a real-time reporter under the control of a light responsive promoter and obtain back a response that mimics the pattern of the original image. Thus, we have established a live canvas in which images can be genetically interpreted and reconstituted with real-time dynamics. Funded by MN-FISB120043, FONDECYT 1131030.

Circadian Clock Regulation of mRNA Translation: eEF2 and the Ribosome Code

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Our long-term goal is to understand the fundamental mechanisms by which the circadian clock regulates rhythmic gene expression and thus cell function and metabolism. To date, most of the focus on understanding clock control of gene expression has been at the level of transcription. However, in many systems, there are examples of specific proteins that show a circadian rhythm in levels, while levels of the associated mRNA are relatively constant throughout the day. This suggests that the clock regulates aspects of translation, but which proteins cycle in abundance in cells, and the mechanisms of clock control of translation, were not known. We discovered that the clock in *Neurospora* controls the phosphorylation and activity of the highly conserved translation elongation factor 2 (eEF2). Using ribosome profiling in WT and mutant cells disrupted for rhythms in eEF2 phosphorylation, we found that rhythmic eEF2 activity leads to rhythmic translation of specific mRNAs. Additional evidence for clock control of translation comes from our recent findings that, counter to the view that all the ribosomes in a cell function identically, there are clock-controlled changes in the protein composition of the ribosome, which may lead to rhythmic translation of specific mRNA classes.

Local adaptation by losing circadian control of asexual development in *Neurospora discreta*.

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The 24-hour circadian clock has been attributed as a fitness trait in multiple organisms. However, the mechanism of how circadian clock variation influences organismal survival is still not well understood. We found that habitat-specific-clock-variation is involved in local adaptation in *N. discreta*, a species that is adapted to two different habitats, under or above tree bark. African *N. discreta* strains, whose habitat is above the tree bark, have higher fitness under a light/dark cycling condition relative to a constant ambient light condition. North American strains, whose habitat is under the tree bark, gained overall fitness in comparison to that of African strains, regardless of light conditions, but lost their clock regulation of asexual development. Our results demonstrate a mechanism by which local adaptation involving circadian clock regulation influences fitness.

Circadian control of global proteomic output in *Neurospora crassa*.

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Eukaryotic circadian clocks involve transcriptional/translational feedback loops that drive 24hr rhythms in transcription. It is hypothesized that these transcriptional rhythms underlie oscillations of protein abundance, thereby mediating circadian rhythms of behavior, physiology, and metabolism. Research in *Neurospora crassa* pioneered the isolation of clock-controlled genes (ccgs) through the use of subtractive hybridization, microarrays and RNA-seq, as well as a host of other techniques. While these transcriptomic methods have been the traditional way to identify clock-regulated elements, new data show that concordance between mRNA and protein levels, including the relationship between circadianly oscillating mRNAs and proteins, has consistently been low. In order to better understand the correlation between circadian output at the level of expression, as compared to the level of protein, we used Tandem Mass Tag Mass Spectrometry to identify global protein levels over a period of 48 hours with a resolution of 2 hours. We then subjected these rhythmic proteins to categorization using Gene Ontology (GO), demonstrating enrichment in specific ontological classifications. Finally, we matched this data to previously published analyses of rhythmic levels of mRNA determined via RNA-seq. This analysis showed that while there are many rhythmic proteins correlated with oscillating mRNAs, the association of expression levels with proteomic levels was less than expected. This suggests that while circadian expression is important to clock-regulated element rhythmicity, there are other factors that determine rhythmicity at the protein level. Furthermore, it suggests that in order to have a complete picture of what the clock regulates, one must investigate all levels of cellular output.

Facultative Heterochromatin at the clock gene frequency

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The circadian clock allows organisms to anticipate daily changes in environmental conditions and controls developmental and physiological processes. In *Neurospora*, the core mechanism underlying circadian clock-regulated transcription is a negative feedback loop where the White Collar-1 (WC-1) and WC-2 drive rhythmic expression of the *frequency (frq)* gene. Recent developments indicate that part of circadian clock-regulated gene expression involves facultative heterochromatin at the central clock gene; A process that is conserved in species ranging from *Neurospora* to *mammals*. Facultative heterochromatin formation at *frq* is distinct from heterochromatin at repeated regions throughout the genome in that it requires the *frq* natural antisense transcript (NAT) *qrf*, and components of the COMPASS complex, including the histone H3 lysine 4 methyltransferase SET1, SWD1, and SWD3 but not SWD2. Despite these differences, there still a requirement for components of the DCDC complex. Consistent with previous findings, facultative heterochromatin at *frq* is needed to establish the appropriate phase of circadian regulated gene expression. Efforts are currently underway to further define the molecular mechanisms of facultative heterochromatin at *frq* and attempts are being made to understand the enigmatic role of COMPASS.

Structure/function analysis of WC-1 reveals mechanisms of White Collar Complex differential activation of light-regulated versus clock-regulated genes

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The core of the negative feedback loop comprising the *Neurospora* circadian clock includes the heterodimeric transcription factors WC-1 (WC-1) and White Collar-2 (WC-2) that form the White Collar Complex (WCC). WCC in turn drives the rhythmic daily expression of the frequency (*frq*) gene whose product, FRQ, in conjunction with FREQUENCY-Interacting RNA Helicase (FRH), forms the FRQ-FRH complex (FFC), and inhibits its own expression by blocking WCC activity. Additionally, the WCC is also the principal *Neurospora* photoreceptor, responsible for the acute light-induction of hundreds of genes including *frq* whose induction resets and entrains the phase of the clock. However, not all acutely light-induced genes are also clock regulated, and conversely, not all clock-regulated direct targets of WCC are light induced; the structural determinants governing the shift from WCC's dark circadian role to its light activation role have been poorly described. Structure/function analysis of WC-1 and WC-2 has revealed transactivation domains essential for *frq* circadian expression and a domain required for assisting the WC-1 zinc-finger to bind DNA in the dark and for mediating interactions with the FFC. A proteomics-based search for WCC coactivators identifies the SWI/SNF (SWItch/Sucrose NonFermentable) complex. SWI/SNF binds to the clock-box in the *frq* promoter and is required both for circadian remodeling of nucleosomes at *frq* and for rhythmic *frq* expression in the dark.

Phase Response Analysis of the Circadian Clock in *Neurospora crassa*

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The circadian clock plays a vital role in maintaining the daily activities of ~24 hours in many organisms including *Neurospora crassa*. This endogenous oscillator processes different input signals such as light and nutrient to align its internal phase to external time cues to optimize physiological function of organisms. In this research, we used mathematical models to explore molecular mechanisms that regulate light-dependent phase shifts of circadian rhythms in *Neurospora crassa*.

***period-1* encodes an ATP-dependent RNA helicase that influences nutritional compensation of the *Neurospora* circadian clock**

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The long period phenotype and its associated gene, *period-1* (*prd-1*), were initially observed and described as part of a UV mutagenesis screen for period length mutants. We have now used genomic sequencing to identify *prd-1* as well as to identify the mutation defining it. We confirmed this by rescuing the mutant circadian phenotype via transformation. PRD-1 is an RNA helicase whose orthologs, DDX5 [DEAD (Asp-Glu-Ala-Asp) Box Helicase 5] and DDX17 in humans and DBP2 (Dead Box Protein 2) in yeast, are implicated in various processes, including transcriptional regulation, elongation, and termination, ribosome biogenesis, and mRNA decay. Although *prd-1* mutants display a long period (□25 h) circadian developmental cycle, they display a WT period when the core circadian oscillator is tracked using a *frq*-luciferase transcriptional fusion under conditions of limiting nutritional carbon; the core oscillator in the *prd-1* mutant strain runs with a long period under glucose-sufficient conditions. PRD-1 is transiently induced by glucose availability. Immunofluorescence microscopy shows localization of PRD-1 in the nuclei that is sharply diminished after complete depletion of glucose. Interestingly, addition of glucose back to the depleted media yields full recovery of nucleus localized fluorescence. PRD-1 appears to act in the nucleus because mutants display normal circadian period lengths in low glucose when PRD-1 is not in the nucleus and display long periods only under high glucose conditions where PRD-1 is in the nucleus. Because circadian period length is carbon concentration-dependent, *prd-1* may be formally viewed as a clock mutant with defective nutritional compensation of circadian period length.

Molecular characterization of HET domain mediated programmed cell death.

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Programmed cell death (PCD) plays a critical role in homeostasis and immunity in Eukaryotes. In Fungi, PCD has been described in the phenomenon of Heterokaryon Incompatibility (HI) – a cell death reaction occurring between somatic fusions of genetically distinct conspecific strains. Although there is a relatively good description of the genetic control of PCD in HI, the molecular mechanisms and signal transducing pathways remain poorly characterized.

However, some recent reports draw parallels between proteins involved in PCD in plants and metazoan and determinants of HI PCD. Of note are the reported similarities between the fungal specific HET domain – a cell death effector – and plant and metazoan TIR (Toll/Interleukin-1 receptor) domains. The TIR domain is also a cell death effector domain, mediating homotypic protein-protein interactions involved in immunity processes. The similarity between the HET domain and the TIR domain, suggests that the HET domain may function as a protein scaffold for assembly of signal transduction components required for fungal PCD. It also suggests that there are potentially conserved molecular mechanisms in the execution and downstream signaling of PCD in plants, metazoans and fungi. We decided thus to better characterize the PCD reaction mediated by the HET domain in *Neurospora crassa*.

First, we identified 25 significantly conserved motifs (e-value < 2.3e-002) outside of the HET domain using the online MEME suite tool. The patterns of these motifs defined three major protein families. Of particular interest is the high occurrence of CxxC—CxxxHxxC (Zn-finger like motif) in the N-terminus of HET domain proteins in Family A. Several domains involved in cell death reactions in metazoans belong to Zn-finger families. Exploring the functional importance of the conserved regions will help address long standing questions regarding the signal transduction pathways of PCD in fungi.

Epistatic relationship between nuclear localized suppressors of meiotic silencing and perinuclear localized suppressors of meiotic silencing: A hypothesis

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Meiotic silencing by unpaired DNA (MSUD), an RNAi based mechanism, causes silencing of unpaired genes and their paired homologues during meiosis. Semi-dominant mutations *Sad-1* and *Sad-2* suppress MSUD. These mutations disrupt the normal pairing of wild type alleles *sad-1*⁺ and *sad-2*⁺, respectively, and induces self-silencing, resulting in silencing of MSUD. However, it has been a dilemma since the discovery of MSUD that who the suppressor of the suppressor is. Recently, additional suppressors (*sad-3*, *sad-4*, *sad-5*, *sad-6*, *qip*) are discovered to understand the process of MSUD in depth. Both *sad-5* and *sad-6* localize in the nucleus whereas all other suppressors localize at the perinuclear region. I hypothesize that suppressors that are localized in the nucleus are the suppressors of *Sad-1* (and *Sad-2*, *sad-3*, *sad-4*, and *qip*). This could be easily tested by epistatic relationship between them. Except *sad-5* and *sad-6*, all other suppressors suppress the round spore phenotype that is due the *Round spore* (*R*) locus at the IR chromosome. Crosses heterozygous for *sad-5* don't suppress the *R* phenotype whereas homozygous crosses suppress the *R* phenotype. Homozygous and heterozygous crosses involving *sad-6* produce more than 60 % round spores, suggesting that *sad-6* cannot suppress MSUD completely. Insensitivity of the *R* locus against *sad-5* and *sad-6* suggests that, only, suppressors at the perinuclear region can suppress the *R* phenotype. Therefore, my hypothesis is that there might be two different levels of meiotic silencing: nuclear and cytoplasmic or perinuclear. Components of the nuclear meiotic silencing (*sad-5* and *sad-6*) might sense unpaired genes (e.g. *Sad-1*) or might have role in production of aberrant mRNA, whereas components of the cytoplasmic or perinuclear meiotic silencing might be involved only in recognition of aberrant mRNA for degradation. That's why; I hypothesize that the components of nuclear meiotic silencing might be involved in self-silencing of the components of perinuclear meiotic silencing. Thus, the components of nuclear meiotic silencing might be the suppressors of suppressors. If this is true, *sad-5* and *sad-6* might be epistatic to the suppressors from the perinuclear complex (e.g. *Sad-1*). Therefore, *sad-5* and *sad-6* might suppress the self-silencing of *Sad-1* (or *Sad-2*), resulting active meiotic silencing. Insensitivity of the *R* phenotype against *sad-5* and *sad-6* provides a tool to test this hypothesis easily in a cross *sad-5* x *R*; *Sad-1* (or *Sad-2*, *sad-3*, *sad-4*, and *qip* at the place of *Sad-1*). Also, role of *sad-6* could be tested in a cross *sad-6* x *R*; *Sad-1*; *sad-6*. These crosses might produce round spore even in the presence of unpaired *Sad-1* due to lack of detection of unpaired *Sad-1* that produces active SAD-1 protein. Crosses of *Sad-4* x *R*; *Sad-1* genotype that involve *Sad-4* (or preinuclear localized suppressors) in place of *sad-5* might produce spindle shaped ascospores due to absence of meiotic silencing. Even if, dominant MSUD suppressors in Spore killers (*Sk*) and in the Carrefour Mme. Gras (CMG) don't suppress the *R* phenotype, they are not characterized molecularly. Crosses of CMG and *Sk* (*Sk* x *R*; *Sad-1* and CMG x *R*; *Sad-1*) could give information about their role in the nucleus or in the perinuclear part of MSUD. If CMG and *Sk* suppress silencing of unpaired *sad-1*⁺ (i.e., producing round spore), they might be the part of the nuclear meiotic silencing complex. If CMG and *Sk* don't suppress silencing of unpaired *sad-1*⁺ (i.e., producing spindle spore), they might be the part of the perinuclear meiotic silencing complex.

Ceramides and Neurospora

Daneisha Bowles

Neurospora crassa is a type of red bread mold from the phylum Ascomycota, and serves as a common model organism in genetics. Over the course of my research I tested eleven different genes to find the genes or gene responsible for clock synchronization in single cells. To detect whether the genes could be the one accountable for synchronization I performed crosses with knockouts of eleven different genes with a strain that were equipped with mCherry recorder gene for single cell measurements and the *bd* mutation for clock experiments. (I then tested isolates from the 11 crosses for the knockout (having hygromycin resistance (test 1)), the *bd* mutation (test 2), and the *recorder* gene (test 3). The first test included the use of hygromycin to test for hygromycin resistance. The second test was to observe if the genes were able to band; therefore, the different genes were grown on race tubes and marked every 24 hours. The last test that was performed was the luminescence test, which is why it was very important to have the genes equipped with mCherry (a fluorescent marker that shows single cell measurements on the clock.) The isolates that were positive were then examined for viability with and without ceramide, an important signaling molecule. We also looked at the effect of ceramide on three different mutants of *Neurospora crassa* (*lag-1*, *bd*, wt, and *lac-1*). Ceramides are a family of waxy lipid molecules. A ceramide is composed of sphingosine and a fatty acid, but differs from sphingosine in having an amide link to a fatty acid, rather than a free amino group. (Plesofsky, 2008) Ceramide is supposed to have a direct effect on cell viability.