NEUROSPORA 2008

March 27-30
Asilomar Conference Center
Pacific Grove, CA

Invitation

Information

Plenary Session Abstracts
Poster Abstracts

Scientific Program

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Metzenberg Memorial

Activities at Asilomar

Neurospora Awards

A brief history of the Neurospora meeting

Neurospora 2008 logo by Alan Radford

November 5, 2007

To: *Neurospora* Community
From: *Neurospora* Policy Committee
Subject: *Neurospora* 2008

We are happy to present information on the upcoming *Neurospora* meeting, ***Neurospora* 2008**, which will be held at Asilomar Conference Grounds, Pacific Grove, California, starting this coming March 27 (Thursday) and ending after lunch on Sunday, March 30. David Catcheside and Yi Liu are arranging the scientific program this year, and Nora Plesofsky and Oded Yarden are handling other organizational matters. You probably recall that the *Neurospora* community decided several years ago to reestablish meetings devoted exclusively to *Neurospora*. The main purpose was to reclaim the spirit of the fabulous meetings that many of us enjoyed prior to expanding the meeting to become the Fungal Genetics Meeting. ***Neurospora* 2008** will be the sixth reincarnated *Neurospora* meeting and all indications are that it will be a great success.

Thursday night will include a special Metzenberg Memorial followed by a reception/mixer. Research talks will be presented Friday morning and afternoon, Saturday morning and afternoon, and Sunday morning. Poster presentations will be made Friday and Saturday evenings, with the posters available for viewing throughout the meeting from Friday through Sunday morning. There will be some free time in the afternoons (1:00-2:30), but those wishing to take excursions may wish to do so Thursday or Sunday afternoon.

All participants are encouraged to present their work on posters. Approximately 80% of the research talks will be by invited speakers. Additional speakers will be selected from those submitting poster abstracts. Those selected will be notified in early February and invited to present a short talk in one of the plenary sessions.

A preliminary listing of plenary sessions is presented below:

I. Gene Silencing, Sex, Repair, and Recombination
II. Clocks and Light
III. Cell Growth, Morphogenesis, and Populations
IV. Gene Regulation and Cell Signaling
V. Genomics and Methodology

The final program will be posted on the Fungal Genetics Stock Center website in two weeks. We expect ***Neurospora* 2008** to be an exceptionally fine meeting and we hope you can come!

NEUROSPORA 2008

The *Neurospora* 2008 Conference will be held at the Asilomar Conference Grounds, Pacific Grove, California (near Monterey, California) from the afternoon of March 27 to noon, March 30, 2008 (Thursday-Sunday). The meeting will consist of five plenary sessions and two poster sessions.

SCHEDULE

DAY	MORNING	AFTERNOON	EVENING
Thursday March 27		Arrival Registration	Dinner Metzenberg Memorial Mixer
Friday March 28	Breakfast Plenary Session I	Lunch Plenary Session II	Dinner Poster Session I
Saturday March 29	Breakfast Plenary Session III	Lunch Plenary Session IV	Banquet Poster Session II
Sunday March 30	Breakfast Plenary Session V	Lunch Departure	

NEUROSPORA POLICY COMMITTEE

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NEUROSPORA 2008 TREASURER

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PLENARY AND POSTER SESSIONS

David Catcheside and Yi Liu will co-chair the Scientific Program Committee of the *Neurospora* 2008 Conference. Approximately 80% of the research talks will be by invited speakers. Poster abstracts should be submitted online. A number of short talks will be selected from the submitted poster abstracts. Participants selected for possible short talks will be contacted in early February and invited to speak in one of the plenary sessions. David and Yi will make the final decisions on speakers for the plenary sessions. For further information, contact a Policy Committee member.

REGISTRATION AND HOUSING
(see enclosed form)

Deadline: January 15, 2008

FOR ABSTRACT SUBMISSIONS ACCESS THE FUNGAL GENETICS STOCK CENTER WEB SITE

www.fgsc.net

Deadline: January 15, 2008

AD HOC SESSIONS

Time is available in the early afternoons for ad hoc meetings. Those wishing to convene such informal sessions should contact one of the scientific organizers.

DAVID PERKINS AWARD

A fund has been established in honor of our colleague, Dr. David Perkins, to support students attending the Neurospora and Fungal Genetics Conferences. Nominations for the Perkins Award are requested for graduate students and postdoctoral scientists that utilize Neurospora as an experimental system. Nominations should include a 200 word description of the individual's contributions from the candidate's research advisor, as well as reprints or preprints of recent work using Neurospora. Nomination letter and reprints should be submitted by January 15, 2006 to:

Dr. Nora Plesofsky
Chair, *Neurospora* Policy Committee
Department of Plant Biology
University of Minnesota
St. Paul, MN 55108

ASILOMAR CONFERENCE CENTER

Asilomar Conference Center is located on 105 acres of forest, dune and beach at the tip of California's historic Monterey Peninsula. Overlooking the Pacific, living and meeting accommodations are set among Monterey pines and cypresses. As a "Natural Area," Asilomar retains many of the original flora and fauna of the peninsula. The tide pools of Asilomar State Beach are just a pleasant stroll across the dunes. The famous "17 Mile Drive," beginning half a mile from Asilomar, winds through the Del Monte Forest to Carmel-by-the-Sea. In Monterey, Cannery Row and Fisherman's Wharf provide excellent shopping, sightseeing and dining. Nearby Carmel is famed for its shopping, art and craft galleries. Group tours of the Peninsula, the Del Monte Forest and historic Monterey may be arranged through the Asilomar Conference Center. The weather is brisk (40 - 60°F) with a possibility of rain. Bring warm clothing and a raincoat.

Transportation

Monterey is the closest commercial airport to Asilomar. Taxis cost approximately $25 to Asilomar (about 20 minutes). The closest major airports are, in order of convenience, San Jose, San Francisco, and Oakland.

Accommodations

All rooms have private baths. There are very few singles available. Requests for single rooms will be honored in the order received. Rates vary with accommodation. All rates include meals, beginning with Thursday dinner and concluding with Sunday lunch. No refunds are available for missed meals. Rates for children are as follows: Infants under 3 years of age may stay in any room with no charge. Children from 3-17 years old are charged the special rate of $200 for three nights, provided there is triple or quadruple occupancy, or double occupancy with one adult in certain rooms. Otherwise, they pay regular rates. There are no camping facilities at Asilomar, and sleeping bags are not permitted in the rooms. No pets are allowed on the grounds. We strongly encourage all participants to stay at Asilomar. For individuals staying off the grounds, meals are available at a special rate. There is a $60 facilities fee for those staying off site.

Check-in

Check-in begins 3:00 PM Thursday at the Administration Building. Check-out is noon Sunday. For those wishing to arrive early, some rooms may be available at Asilomar and can be reserved by contacting Asilomar at the number listed below no more than 30 days prior to the meeting.

Telephone and Mail

There are no phones in rooms. The address and phone number for messages is:

c/o *Neurospora* 2008
Asilomar Conference Center
P.O. Box 537
Pacific Grove, California 93950
Phone: (831) 372-8016
Fax: (831) 372-7227

FOR INFORMATION REGARDING ACCOMMODATIONS, CONTACT

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NEUROSPORA 2008 PLENARY SESSION ABSTRACTS

Session I: Gene Silencing, Sex, Repair and Recombination

Beadle and Tatum Award Lecture

Genetic Dissection of Meiotic Silencing by Unpaired DNA

Patrick Shiu. University of Missouri- Columbia

A gene present in an abnormal number of copies is usually a red flag for mischief. One way to deal with these potential intruders is by destroying their transcripts, a phenomenon known as RNA interference (RNAi). Meiotic Silencing by Unpaired DNA (MSUD) is an RNAi-based mechanism that surveys the genome of *Neurospora crassa*. In this system, genes that are not paired during meiotic prophase I, as well as all homologous copies, are silenced during the sexual phase. MSUD is likely to involve the production of double-stranded RNA, which is mediated by the *sad-1*-encoded RNA-directed RNA polymerase. Additional genetic loci that are important for the silencing pathway, the interactions among different components of the MSUD machinery, as well as the tools developed for their studies (e.g. Bimolecular Fluorescence Complementation, targeted gene placement), will be discussed.

Sexual development in *Neurospora crassa*: Elusive recessive mutations

Mary Anne Nelson, Christine Chee, Joseph Kunkel, Diego Martinez, and Charles Sanchez. Department of Biology, University of New Mexico

Knockouts (KOs) of each of the ~10,000 genes of the filamentous fungus *Neurospora crassa* are being constructed by the NIH-supported Program Project, Functional Analysis of a Model Filamentous Fungus, Jay Dunlap, PI. One of the goals of this project is to characterize phenotypes for each of the genes in *N. crassa*. The NIH P01 award supports limited vegetative and sexual phenotypic analyses. In a pilot project, we have extended those analyses to include self or homozygous crosses of the KO strains, in order to identify strains with recessive sexual defects. We are screening the KO strains for dominant and recessive sexual development defects, as well as male-specific defects. In preliminary work, about 5% of the KO strains made no protoperithecia (female sexual structures), and 3% made no fruiting bodies (perithecia). One KO strain formed perithecia but no sexual progeny (ascospores). The challenges for extending this analysis to include all *Neurospora* genes will be discussed.

Getting connected: Formation and function of the hyphal network

Louise Glass. University of California, Berkeley

Hyphal fusion occurs at different stages in the vegetative and sexual life cycle of filamentous fungi, such as *Neurospora crassa*. Similar to cell fusion in other organisms, the process of hyphal fusion requires cell recognition, adhesion and membrane merger. Cell fusion during vegetative growth in *N. crassa* is a self-signalling process that occurs between both genetically identical and genetically dissimilar germlings/colonies. Analysis of fusion mutants in *N. crassa* has revealed that genes previously identified as having non-fusion related functions in other systems, have novel hyphal fusion functions in *N. crassa*. We have focused on a MAP kinase, MAK-2, and SO, a protein of unknown molecular function, which are required for hyphal/germling fusion in *N. crassa*. Subcellular localization of GFP fusion constructs showed that both proteins localize to the tips of germling fusion cells during chemotropic interactions. We constructed a *mak-2* allele whose kinase activity can be specifically inhibited by addition of inhibitor. Disruption of MAK-2 kinase activity by drug addition disturbs the signalling process between MAK-2 and SO. Localization and inhibitor studies also suggest that kinase activity of MAK-2 is required for late fusion events, while SO is not. Understanding the molecular basis of cell fusion in filamentous fungi provides a paradigm for cell communication and fusion in eukaryotic organisms. Furthermore, the physiological and/or developmental role of hyphal fusion is not understood in these organisms; identifying these mechanisms will provide insight into environmental adaptation.

Recent advances understanding the control of DNA methylation

Eric U. Selker. Institute of Molecular Biology, University of Oregon, Eugene, OR 97403. selker@uoregon.edu

We are interested in better defining the mechanisms that control the distribution of DNA methylation in eukaryotic genomes. Twenty-five years ago, while in Bob Metzenberg's laboratory, I discovered DNA methylation in *Neurospora crassa* and decided to exploit this organism to explore its function and control. Other model lower eukaryotes, including yeasts, Drosophila and *C. elegans* showed no methylation. Our early work led to the discovery of RIP (the first described genome surveillance mechanism in eukaryotes), identified sequences that trigger DNA methylation and demonstrated that DNA methylation can interfere with transcriptional elongation. Analyses of the genomic distribution of DNA methylation revealed that it is almost exclusively in relics of RIP, which are mostly in the centromeric regions but are also found near telomeres and at scores of dispersed sites. In addition the tandemly repeated rDNA is lightly methylated. Throughout our studies we have used traditional genetic methods to identify components of the DNA methylation machine and this approach has become increasingly fruitful, especially in conjunction with biochemical and proteomic methods. The availability of the genome sequence and the development of methods to carry out reverse genetics has also accelerated our progress. Previously we reported the identification of several key players in DNA methylation, including a DNA methyltransferase (DIM-2), a histone H3 lysine-9 methyltransferase (DIM-5), a homolog of heterochromatin protein-1 (HP-1) and histone deacetylases (HDACs). Recently we developed an efficient method to identify additional methylation mutants and have placed 14 new mutants into five new complementation groups. In addition, we have applied a new microarray-based method to detect restriction site polymorphisms and used this to map the mutations in some of the new *dim* (*d*efective *i*n DNA *m*ethylation) mutants. I will describe several newly identified *dim* genes that we have found to be essential for DNA methylation and will present a model summarizing our current understanding. I will also describe two novel genes that are involved in the controlling the spread of DNA methylation in Neurospora.

Centromeres of filamentous fungi

M. Freitag, Lanelle R. Connolly and Kristina M. Smith. Dept. of Biochemistry and Biophysics, Center for Genome Research and Biocomputing, Oregon State University, Corvallis, USA

Centromeres are complex structures composed of DNA, proteins and RNA. We still do not fully understand how centromeres assemble, how they are maintained and how they are inherited. Early studies revealed specific centromeric DNA motifs and associated DNA-binding proteins, but this simple model does not hold true outside of budding yeasts. Centromeres are now functionally defined by the presence of a centromere-specific histone H3 variant, CenH3. In addition, a combination of histone modifications differentiates "centrochromatin" from "heterochromatin" and "euchromatin", but a cause and effect relationship between histone modifications and centromere identity has not been established. One key question regards the relative importance of DNA composition *vs.* epigenetic modifications in marking the regional centromeres of higher eukaryotes. Similarly, the molecular mechanisms that govern centromere assembly and maintenance remain largely obscure, even though numerous proteins have been implicated in these processes. We are using *Neurospora crassa* and *Fusarium graminearum* to address these questions. Centromeric regions in Neurospora are of similar complexity as those found in most animals, bridging the gap between simple (e.g., yeasts) and complex eukaryotes, while lacking the ~180-bp tandem repeats typically associated with plant and mammalian centromeres. The *F. graminearum* genome appears virtually repeat-free, which makes it ideally suited to investigate the relative contributions of DNA composition and epigenetic modifications to centromere identity. We will show evidence for the localization of two centromeric proteins (CenH3 and Cenp-C) in Neurospora and Fusarium, detected by cytological (GFP fusions) and molecular means (ChIP-Sequencing).

Region specific regulation of recombination - How the discovery of MSUD unmasked a troublesome phantom

F.J. Bowring, P.J. Yeadon and D.E.A. Catcheside. School of Biological Sciences, Flinders University, PO Box 2100, Adelaide, South Australia, Australia

rec-1$^+$, *rec-2*$^+$ and *rec-3*$^+$ are dominant *trans*-acting genes that suppress meiotic recombination in specific regions of the *Neurospora crassa* genome. As an example, up to 1% of progeny from a *rec-2* by *rec-2* cross experience recombination in *his-3* but this falls to about 0.005% when one or both parents carries *rec-2*$^+$. While our attempt to clone *rec-2*$^+$ has encountered some difficulty, without Patrick Shiu and Bob Metzenberg's discovery of meiotic silencing by unpaired DNA (MSUD), it would have been impossible.

RFLP mapping after a long walk along chromosome V indicated that *rec-2*$^+$ resides in a 10kb stretch of DNA. A comforting corollary was that this region is absent from *rec-2* strains which have in its place a 3kb stretch of unique DNA. Decidedly non-comforting however, were the observations that putting *rec-2*$^+$ DNA into *rec-2* strains failed to yield a *rec-2*$^+$ phenotype and that deletion of *rec-2*$^+$ DNA failed to yield a *rec-2* phenotype. On top of this, bioinformatic analysis of the 10kb region failed to identify any plausible *rec-2*$^+$ candidates. Having reached this dead-end, the discovery of MSUD suggested another possibility: *rec-2*$^+$ does not exist! We wondered if *rec-2*$^+$ is a phantom that simply appears as a dominant suppressor of recombination because it deprives *rec-2* of an opportunity to pair in *rec-2*$^+$ by *rec-2* heterozygotes?

This appears to be so. Disabling MSUD in *rec-2*$^+$ by *rec-2* heterozygotes substantially increases recombination at *his-3* and a *rec-2* deletion behaves, like *rec-2*$^+$, as a dominant suppressor of recombination. Indeed, MSUD is responsible for the dominance of all three *rec* genes. In the absence of MSUD, recombination frequencies at *his-1* in *rec-1*$^+$/*rec-1* heterozygotes and at *am* in *rec-3*$^+$/*rec-3* heterozygotes are indistinguishable from those of *rec-1* and *rec-3* homozygotes respectively. Thus, rather than *rec*$^+$ genes producing suppressors of recombination we have shown that the products of *rec-1*, *rec-2* and *rec-3* act to promote recombination in specific regions of the genome, a discovery that would not have been possible without the prior discovery of MSUD.

Meiotic silencing in Neurospora

Rodolfo Aramayo. Texas A&M University

In Neurospora, if a segment of DNA is not present on the opposite homologous chromosome in meiosis, the resulting "unpaired" DNA segment is targeted for silencing. This situation occurs when a DNA element gets inserted at a particular chromosomal position (e.g., a situation akin to the "invasion" of a genome by transposable DNA elements). It can also occur when a normal region gets deleted. In both situations, the resulting loop of "unpaired" DNA activates a genome-wide "alert" system that results in the silencing not only of the genes present in the "unpaired" DNA segment, but also of those same genes if present elsewhere in the genome, even if they are in the paired condition. This phenomenon is called, meiotic silencing and was originally described in *Neurospora crassa*, but has since been observed in nematodes and mammals. In all these organisms, "unpaired or unsynapsed" regions (or chromosomes) are targeted for gene silencing. We think that meiotic silencing is a two-step process. First meiotic trans-sensing compares the chromosomes from each parent and identifies significant differences as unpaired DNA. Second, if unpaired DNA is identified, a process called meiotic silencing silences expression of genes within the unpaired region and regions sharing sequence identity. We are using a combination of genetics, molecular biology and biochemistry aimed at identifying all the molecular players of the process and at understanding how they work together. In this work we describe the genetic, molecular, cytogenetic and biochemical characterization of key components of the system: Sms-2, Sms-3, Sms-4, Sms-5, Sms-6, Sms-7, Sms-8, Sms-9 and Sms-10. In addition, we describe and discuss how mutants in key genes required for recombination and chromosome pairing are not required for gene-specific meiotic silencing.

Genetic interaction between mutagen sensitive mutants

Hirokazu Inoue. Lab of Genetics, Dept of Regulation Biology, Saitama University, Japan

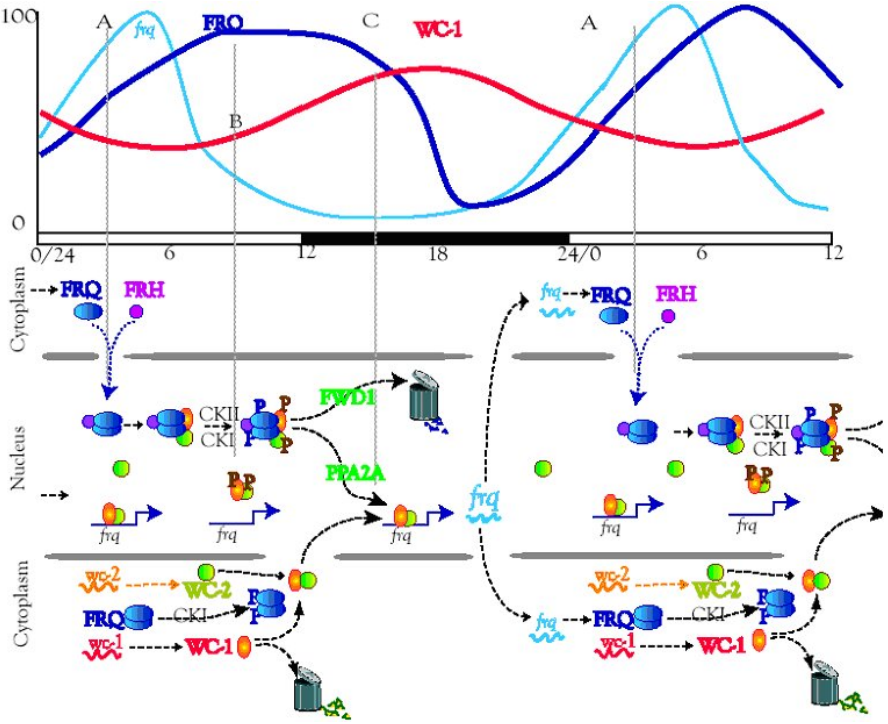
Mutagen-sensitive mutants have been classified into several groups, based on sensitivity to various mutagens, sensitivity of double mutants, spontaneous and induced mutability, vegetative growth and fertility in homozygous crosses. Recently information of DNA sequencing was added to this characteristic. Major groups are excision repair, recombination repair, post-replication repair, damage checkpoint and mismatch repair. Some mutants did not belong to any groups or belong to plural groups in epistasis interaction. Also some combinations resulted in synthetic lethal. We first described synthetic lethal relationship between *uvs-3* and *uvs-6* 20 years ago. What does "synthetic lethal"

mean? In this talk I introduce characters of the *mus-16* mutant that is highly sensitive to MMS, but not to IR and UV, and is synthetic lethal in combination with some DNA repair-deficient mutants. As *mus-16* was not epistatic to other DNA repair mutations, the repair group belonging to was not determined. We cloned this *mus-16* gene from Linkage Group V genomic library. The inferred MUS-16 protein did not show any prominent motifs, but it was homologue of Rtt109 of *Saccharomyces cerevisiae*. Function of MUS-16 was discussed.

Metzenberg Award Lecture

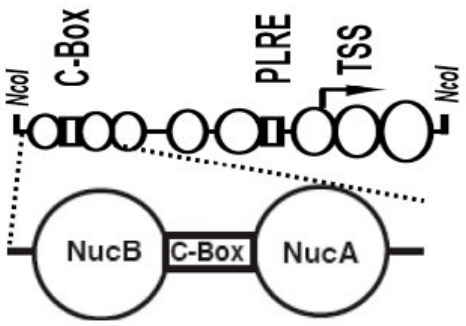
Changing Times.

Jay C. Dunlap. Department of Genetics, Dartmouth Medical School, Hanover, NH 03755



The formal part: Transcription/ translation feedback loops involving *frq* are central to circadian rhythms in *Neurospora*. The transcription factors WC-1 and WC-2 activate *frq* expression in a circadian and light- dependent manner by binding to the Clock Box in the *frq* promoter. In cell extracts, WC-1 has always been found complexed with WC-2, so we expected ChIP experiments to show WC-1 and WC-2 entering and leaving the Clock Box as a unit at phases associated with increasing and decreasing *frq* expression. ChIP, however, indicates that the WC proteins do not act solely as an obligate complex: *in vivo* binding of WC-2 to the *frq* promoter occurs in a rhythmic fashion coincident with the peak in *frq* transcription, whereas WC-1 is bound continuously (Belden et al., 2007). Nuclease accessibility experiments show chromatin rearrangements at *frq*; a nucleosome occupying the region near the Clock Box is moved/disassembled in a rhythmic fashion. Deletion of all 19 genes encoding ATP-dependent chromatin-remodeling enzymes revealed only 2 genes, *clockswitch* (*csw-1* a homolog of the yeast *Fun30*, mouse *Etl1* and human *SMARCAD* genes) and *chd-2* (a homolog of the mammalian *mi-2* and yeast *Chd1/2* genes) required for normal clock function; CSW-1 localizes rhythmically to *frq*, suggesting it is required at specific times for the sharp transition from

the transcriptionally active to the repressed state. These data provide a first picture of events happening at the *frq* promoter that are important for its essential, correctly timed daily expression.



That's the formal part, which I expect I'll get to. Beyond this watch for invocation of Isaac Newton, Bob Metzenberg, David Botstein, the 1st Congregational Church in Thetford, our kitchen, my lab design, and whatever else gets written in and for which there is time.

Session II: Clocks and Light

Circadian Output Pathways in Neurospora.

Deborah Bell-Pedersen. Center for Research on Biological Clocks and Department of Biology, Texas A&M University dpedersen@mail.bio.tamu.edu

Organisms from bacteria to humans use a circadian clock to control daily biochemical, physiological, and behavioral rhythms. We are using the model organism *Neurospora crassa* to study the circadian clock and its output pathways. Using microarrays we found that ~20% of Neurospora genes are under control of the circadian clock system at the level of transcript accumulation, and that the bulk of the clock-controlled mRNAs have peak accumulation in the late night to early morning. These data suggest the existence of global mechanisms of rhythmic control of gene expression. Consistent with this idea, we found that the Neurospora OS pathway, a phosphorelay signal transduction pathway that responds to changes in osmotic stress, functions as an output pathway from the Neurospora clock system that regulates daily rhythms in expression of downstream genes. Hijacking conserved signaling pathways by the circadian clock provides a new paradigm for global rhythmic control of target genes of the pathway.

Functional Characterization of the White Collar Complex

Tobias Schafmeier, Astrid Schäfer and Michael Brunner. University of Heidelberg Biochemistry Center, INF328, 69120 Heidelberg, Germany

The *Neurospora* circadian clock protein Frequency (FRQ) participates in interconnected negative and positive feedback loops.

In the negative loop FRQ it inhibits its own transcriptional activator, the White Collar Complex (WCC), and in the positive loop it supports expression of WCC. These apparently contradictory functions are coordinated in a spatial

and temporal fashion. Newly synthesized FRQ recruits CK1 and supports phosphorylation of WCC in the nucleus. This leads to inactivation of the transcription factor. Maturation of FRQ causes its progressive accumulation in the cytosol. When high amounts of cytosolic FRQ have accumulated WCC becomes hyperphosphorylation and redistributes from the nucleus into the cytosol. Our data suggest that WC proteins rapidly shuttle between cytosol and nucleus. FRQ affects the subcellular localization of the WCC by controlling its phosphorylation status.

Genome-wide analysis of light induced gene expression and the role of CK2 in temperature compensation.

Jennifer Loros, Chen-Hui Chen, Arun Mehra, Mi Shi, Jay C. Dunlap. Departments of Biochemistry and of Genetics, Dartmouth Medical School, Hanover, NH 03755 USA

Biological rhythms provide organisms with the ability to anticipate environmental changes arising from the Earth's rotation. The genetically determined ability to gauge time of day in concert with the perception of environmental light represents one of the most widespread and closely coupled forms of cellular, tissue and organismal regulation across a broad range of taxonomic groups. Neurospora has played a pioneering role in the description of the molecular basis of both photobiology and circadian rhythmicity. Neurospora has numerous chromophore-binding photoreceptive molecules capable of detecting changes in light fluence and wavelength via the harvesting of photons and thereby allowing immediate signaling of important environmental changes. Genome-wide analysis of changes in transcript abundance has revealed a hierarchical cascade of light regulated responses. Under vegetative conditions most light-inducable genes were found to be dependant on the White-collar 1 and 2 proteins. Additionally, correspondence between timing of expression and gene function was also observed. This work has allowed identification of a cis-acting motif and dedicated transcription factor involved in the control of a specific subset of late light responses. A defining characteristic of all clocks is the ability to maintain constant periodicity over a range of environmental changes including temperature and metabolic rate. Mutations in two Neurospora clock mutants, *prd-3* and *chr*, that result in both period and temperature compensation defects identify subunits of the CK2 enzyme. The *prd-3* mutation is in CK2-alpha while *chr* is defective in CK2-beta. An inducible wild-type CK2-beta subunit that allows graded expression results in temperature compensation phenotypes ranging from anti-compensation to a near wild-type compensation that corresponds to the level of expression of the wild-type protein. The clock protein FRQ is identified as a direct substrate of CK2. FRQ stability is shown to increase with increased temperature in CK2-beta hypomorphs but not with another kinase, CK1, involved in clock function. This suggests that different types of FRQ phosphorylation can lead to changes in either period length alone or period length and compensation.

Neurospora mutants altered in their regulation of transcription by light

Laura Navarro-Sampedro, Maria Olmedo, Carmen Ruger-Herreros and Luis M. Corrochano. Departamento de Genetica, Universidad de Sevilla, Spain

Light activation of transcription in Neurospora is transient due to photoadaptation. The gene *con-10* of Neurospora is expressed during conidiation and after illumination of vegetative mycelia. Photoactivation of *con-10* is transient and disappears after two hours of light. We have used a *N. crassa* strain bearing a translational fusion of *con-10* to a selectable bacterial gene conferring hygromycin resistance (hph). This con-10::hph fusion is preceded by the *con-10* regulatory region. Growth of this strain was shown to be sensitive to hygromycin, upon continuous culture in the light. Five mutants were isolated that were resistant to hygromycin when cultured under constant light. Three of these mutant strains displayed elevated, sustained, accumulation of con-10::hph mRNA, during continued light exposure. This behavior suggests that they bear mutations that reduce or eliminate the presumed light-dependent repression mechanism that normally blocks con-10 transcription upon prolonged illumination.

The gene *rco-1*, encoding a putative gene repressor, and the gene *rco-3*, encoding a putative glucose sensor, are required for the repression of *con-10* in vegetative mycelia. In addition, we have observed that *rco-1* and *rco-3* mutants have an enhanced and sustained photoactivation of *con-10* and *con-6*, a phenotype they share with *vivid* mutants. We propose that VVD, RCO-1, and RCO-3 participate directly or indirectly in the mechanism of adaptation to light.

Does circadian clock matter for *Neurospora crassa* in nature?

Kwangwon Lee. Cornell University, Ithaca, NY.

Neurospora crassa has been a superb model organism for revealing the molecular nature of circadian clock regulation in filamentous fungi. However, there is, as yet, limited knowledge regarding the ecological significance of *N. crassa* clock regulation. To understand the quantitative nature and the ecological significance of the clock phenotype in nature, we focused on two research areas; natural variation and QTL (Quantitative Trait Loci) analysis. We found that there is significant natural variation in clock phenotypes and in the known molecular components, WC-1, WC-2, FRQ, and VVD. We focused our study on WC-1, a blue-light receptor and a core circadian clock component. By statistical tests and genetic analysis, we have shown that molecular variation at the amino-terminal activation domain, NpolyQ, in WC-1, contributes to the circadian period variation. Since WC-1 plays a key role in light perception and circadian clock period, we suggest that the molecular variation in WC-1 fine tunes the clock function for ecological adaptation. However, the real question is "does clock regulation matter for fungi in nature?" We addressed this question using two different experimental approaches: 1) comparing fitness of two different strains, wild type and a *wc-1* knockout strain, under ecologically-relevant conditions, and 2) measuring fitness of strains with extreme circadian clock period stains. Our data suggest that clock impacts the fitness of an organism. To have a comprehensive understanding of genetic determinants of the circadian clock phenotypes, we preformed QTL analyses on three different populations. We identified 30 QTL for clock phenotype that were not linked to previously known clock genes. Characterizing these QTL will reveal novel insights on quantitative variation of circadian regulation.

White Collar Complex (WCC): acetylation and evolution

B.Grimaldi, A.Brenna, P.Filetici and P. Ballario. Dip Genetica and Mol .biol, UNI and IBPM, CNR, Rome. Italy

Blue light–induced transcription is mainly regulated by the White Collar complex (WCC) in *Neurospora* and in the other filamentous fungi so far investigated. The *white collar-1* (*wc-1*) and *wc-2* genes encode for zinc-finger – PAS trascription activators which constitute the functional core of this complex. When activated by a light stimulus, the WCC drives the transient-transcriptional activation of several photo-regulated genes involved in different physiological processes.

Recently, we have shown that the activation of light-regulated genes by WCC is coupled to light-dependent changes in histone acetylation at their promoters (Grimaldi et al., 2006), indicating that transcription-permissive chromatin states are dynamically established in a light-specific manner.

Indeed, upon a light pulse the promoters of photo-induced genes (i.e. *al-3* and *vivid*) appear acetylated in the residue K14 of histone H3. As the light-driven transcriptional activation, H3 K14 acetylation is transient, depends on WC-1 and follows the RNA kinetic. The enzyme responsible for this chromatin remodelling is NGF-1 (*Neurospora Gcn-Five-1*), the homologous of *S.cerevisiae* Gcn5p, a member of the GNAT family of lysine acetyl transferases coactivators. Notably, the structure of WC-1 proteins resembles that of the vertebrate Nuclear Receptor factors (NRs) which interact with transcriptional co-activators (i.e. GCN5, PCAF, p300) upon activation by a stimulus (steroid hormone for NRs, light for WCC). Consistent with this analogy, we demonstrated a physical *in vitro* interaction between WC-1 and yeast Gcn5p. The interaction requires the presence in the carboxy terminal of WC-1 of the consensus sequences corresponding to the already identified region of binding between NRs and co-activators.

In addition, we will present the comparison between Nc*wc-1* versus its hortologous found in an"underground living" fungus, showing adaptative mutations of the function of WCC protein domains in other species.

The effects of prd mutations on the FRQ-less oscillator (FLO)

Patricia Lakin-Thomas

Poster 56

Session III: Cell Growth, Morphogenesis and Populations

Neurospora as a model for evolutionary biology

Jason E. Stajich, Thomas J. Sharpton, Christopher Ellison, Christopher Villalta, David Jacobson, N. Louise Glass, Donald O. Natvig, John W. Taylor.

Research using phylogenetics to characterize *Neurospora* species, including finding new species that now are being described and extending the ranges of some species in the Northern hemisphere, combined with the many available genetic and developmental tools, make the outbreeding species exceptional candidates for studies of natural evolutionary processes. In addition, research published just last year has initiated the era of experimental, evolutionary studies in *Neurospora*. Most recently, genome sequencing of *N. discreta* and *N. tetrasperma* has opened the door to comparative genomics as a tool to study evolution. We have applied the power of comparative genomics to problems of assembly and annotation that remained for one of the first and best-studied fungal genomes, *N. crassa*. With improved assemblies and annotation for the three *Neurospora* genomes, and with genomes for close outgroup species *Podospora anserina* and *Chaetomium globosum*, we now are able to investigate gene content, gene family expansion and contraction, synteny and rearrangement, and the effects of selection.

Where is calcium sequestered within cells, and how does it get there

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Calcium is an important signaling molecule in all cells. In fungi calcium has been proposed to play a key role in polar growth, although we know little about the mechanisms by which calcium is sequestered and released. Analysis of the *Neurospora crassa* genome led to the identification of at least 5 genes that encode putative calcium transporters (*nca-1*, *nca-2*, *nca-3*, *pmr*, and *cax*). We have characterized the phenotypes of strains with mutations in these genes. To investigate the cellular locations of the transporters we made use of both GFP and dsRED tags. Marker enzymes for the vacuolar network, Golgi, ER and nuclei have also been tagged with GFP and dsRED. Analysis of the fluorescent-tagged proteins in living cells shows that the organelles are part of a dynamic system of small vesicles and tubules. The data also suggest that the nuclear envelope may be a significant part of the ER in *N. crassa*. Different calcium transporters appear to be located in the ER, the vacuolar network and the Golgi.

An Analysis of the Microtubule-Binding Domain of Neurospora Dynein

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Cytoplasmic dynein is a large, microtubule-associated motor complex that facilitates minus-end-directed transport of various cargoes. Using the model organism *Neurospora crassa*, we utilized a genetic screen to isolate mutants defective in dynein functions. Here we report on two mutations, one within the microtubule-binding domain (MTBD) of the dynein heavy chain and another in the first coiled-coil of the MTBD. In a suppressor screen we found that dynein MTBD mutations are suppressed only by intragenic mutations. Interestingly, the two MTBD mutations discussed here suppress each other's defects in hyphal growth and nuclear distribution. We purified recombinant MTBD fragments from these strains and determined that the single amino acid mutations alter microtubule-binding frequency in opposite manners. When both mutations were present the frequency of microtubule binding was restored to near wild-type levels. To study the underlying basis of these mutations we tagged the dynein intermediate chain and purified the native motor. Using an in vitro bead motility assay we determined that *N. crassa* dynein moves with an average velocity of ~2.0 um/s. In the presence of either single amino acid mutation we observed a decrease in the average velocity and distance of dynein translocation. When we examined strains that contain both mutations the velocity was partially restored. Together these data indicate that opposing defects in different domains of dynein heavy chain can compensate for one another.

Regulation of polar tip extension by Rho-type GTPases and COT1 kinase signaling

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Regulation of Rho GTPase signaling is critical for cell shape determination and polarity. LRG1, a novel member of the GTPase activating proteins (GAPs) was found to be essential for apical tip extension and to restrict excessive branch formation in subapical regions of the hypha and is involved in determining the size of the hyphal compartments. LRG1 localizes to hyphal tips and sites of septation via its three LIM domains. The accumulation of LRG1 as an apical cap is dependent on active growth and influenced by opposing microtubule-dependent motor proteins dynein and kinesin-1. Genetic evidence and *in vitro* GTPase assays identify LRG1 as a RHO1 specific GAP affecting several output pathways of RHO1, based on hyposensitivity to the glucan inhibitor caspofungin, synthetic lethality with a hyperactive b1,3-glucan synthase mutant, altered PKC/MAK1 pathway activities, hypersensitivity to Latrunculin A and the suppression of *lrg-1* by the over expression of the dominant-negative acting N-terminus of the formin BNI1. The morphological defects of *lrg-1* are highly reminiscent to the Ndr pathway mutants *cot-1* and *pod-6*, and genetic evidence suggests that LRG1 functions in parallel with COT1 in coordinating apical tip growth.

Mitochondrial Biogenesis

Frank E. Nargang, E. Laura Sherman, Nancy E. Go, and Jeremy G. Wideman. Department of Biological Sciences, University of Alberta, Edmonton, Alberta.

Mitochondria are composed of roughly 1000 different proteins. The vast majority of these are encoded by nuclear genes, synthesized as mitochondrial precursor proteins on cytoplasmic ribosomes, and imported into the organelle. Virtually all mitochondrial precursor proteins carry targeting information that is recognized by the translocase of the outer mitochondrial membrane (TOM complex), which initiates import of the precursors into mitochondria. The core TOM complex contains five different proteins named after their apparent molecular weights: Tom40, Tom22, Tom7, Tom6, and Tom5. Tom40 is the pore forming component of the complex. We have investigated the role of the three small molecular weight Tom proteins by genetic and biochemical analysis. All three proteins appear to play a role in the stability of the complex, but to different extents. Mitochondria lacking Tom6 and Tom7 are affected in their ability to import mitochondrial precursor proteins. Unexpectedly, the absence of any of the small Tom proteins results in an increased rate of assembly of Tom40 into the TOM complex.

Conidial sex tubes and phytochrome signalling

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A new cell type, the conidial sex tube (CST), that can act as a male fertilizing agent, has been discovered in *Neurospora crassa*. It is morphologically and physiologically distinct from germ tubes and conidial anastomosis tubes (CATs) that also arise from conidia, and under separate genetic control. CSTs are characterized by being long, thin, straight and unbranched, and they do not avoid or grow towards each other. The surface texture and chemistry of CSTs is different to that of germ tubes and CATs. CSTs require the presence of the peptide sex pheromone of opposite mating type to be induced. Nuclear division in CSTs is arrested. Besides being stimulated by sex pheromone, CST induction is also regulated by red light and this is phytochrome-mediated. *Neurospora crassa* possesses two phytochromes: PHY-1 and PHY-2, and they play contrasting and mating type dependent roles in the photoregulation of CST induction

Genetic and functional characterization of three K+ transport systems in Neurospora

Alberto Rivetta

Poster 3

Identification and partial characterization of Neurospora anastomosis mutants

Stephen Free

Poster 7

Session IV: Gene Regulation and Cell Signaling

Investigation of functional domains of RRG-1, a response regulator that regulates the OS-2 MAPK cascade

Carol Jones

Poster 48

Post-transcriptional control of gene expression in *Neurospora*

Matt Sachs. Texas A&M University

Fungal mRNAs containing upstream open reading frames (uORFs) can be subject to post-transcriptional regulation. First, translation of the uORF can modulate translation of the main reading frame specified in the mRNA. Second, the uORF termination codon can be recognized as a "premature" termination codon by the machinery responsible for nonsense-mediated mRNA decay (NMD). Fungal mRNAs specifying the small subunit of arginine-specific carbamoyl phosphate synthetase contain a uORF specifying the evolutionarily conserved arginine attenuator peptide (AAP). The synthesis and amino acid sequence of the AAP are critical for *cis*-acting Arg-specific negative regulation of translation. In cell-free translation systems, AAP-mediated regulation depends on the AAP's ability to stall ribosomes at the uORF termination codon in response to Arg. Certain single missense mutations eliminate ribosome stalling *in vitro* and Arg-specific regulation *in vivo* and *in vitro*. The *S. cerevisiae CPA1* and *N. crassa* AAPs are specified by uORFs whose start codons are in relatively poor translation initiation contexts, resulting in much leaky-scanning past the uORF. Furthermore, when Arg levels are low, there is no stalling of ribosomes on the uORF even when it is translated. However, when Arg is high, ribosomes that have synthesized the AAP stall, and this stalling blocks other scanning ribosomes from initiating translation at the downstream start codon. In addition to translational regulation, *CPA1* expression is naturally regulated at the level of mRNA stability via a mechanism involving nonsense-mediated mRNA decay (NMD). Arg-regulated stalling at the *CPA1* uORF stop codon triggers NMD and the data suggest a simple model that controlling the density of ribosomes at the uORF termination codon modulates NMD. Analyses of *N. crassa arg-2* in isogenic NMD^+ and nmd^- strains indicate that its levels are also controlled by NMD. We are developing methods to analyze *N. crassa* mRNA half-life to determine directly how the stability of *arg-2* and other mRNAs is controlled, and to determine which *trans*-acting factors contribute to this control. These studies provide a basis for a general understanding of how ribosome occupancy of a uORF termination codon controls both translation and mRNA stability in eukaryotes.

Regulation of the RNAi pathway in Neurospora

Heng-Chi Lee

Poster 43

The *ylo-1* gene encodes an aldehyde dehydrogenase responsible for the last oxidative reaction in the Neurospora carotenoid pathway

Alejandro Fernández Estrada

Poster 1

Trans-species activity of the *Neurospora crassa* ribonucleotide reductase incompatibility domain

Robert P. Smith, Fuad Tanha, Kenji Wellman, Leila Hadari and Myron L. Smith. Department of Biology, Carleton University, Ottawa, Ontario, Canada

In *Neurospora crassa*, nonself recognition occurs during vegetative growth. Cell fusion may result in the formation of heterokaryons, cells that contain more than one genetically distinct nucleus. Viability of heterokaryotic cells is governed by at least 11 het (heterokaryon) genes. The *het-6* locus contains two genes one of which, *un-24*, has a dual function as it also encodes the large subunit of a class I ribonucleotide reductase (RNR). *un-24* has two alleles, Oakridge (OR) and Panama (PA), which differ in their carboxy termini. Cells that contain both OR and PA, either as heterokaryons or partial diploids, are non-viable or grow slowly with an aberrant morphology. Extended periods of incompatible colony growth result in a phenomenon called 'escape,' a sudden shift to wild-type growth rate and morphology. Recently, this incompatibility reaction was demonstrated to occur when fusion constructs expressing 63 amino acids of the $un\text{-}24^{PA}$ C-terminus (hygunPA) were transformed into $un\text{-}24^{OR}$ strains.

To further our understanding of this system, we transferred *un-24* constructs into *Saccharomyces cerevisiae*. Several lines of evidence suggest that hygunPA is toxic to yeast. Expression of hygunPA at low levels results in a characteristic incompatible phenotype that includes sensitivity to physical and chemical stressors, an extended lag phase and interruption of the cell cycle, and an aberrant, granulated morphology. Western analysis with RNR antibodies shows that when hygunPA is expressed at low-levels there is a decrease in oxidized RNR and an increase in reduced RNR. During this presentation, we present evidence that the *un-24* incompatibility system functions in yeast in a similar fashion to *N. crassa*. In addition, we will demonstrate how *S. cerevisiae* can be used to further characterize fungal incompatibility systems.

Plasmid-induced senescence in Neurospora crassa triggers mitochondrial-nuclear communication

Jack Kennell

Poster 11

Characterization of the Neurospora VS ribozyme in vivo

Andrew Keeping

Poster 27

New features over the expression of Neurospora crassa genes in response to extracellular phosphate levels and pH

Fabio Squina

Poster 38

Session V: Genomics and Methodology

Serine-Threonine Kinases and *Neurospora* Biology

Gloria Turner and Katherine Borkovich. University of California, Los Angeles and University of California, Riverside

Serine-threonine protein kinases are well-known for their involvement in critical signal transduction pathways regulating growth, development and environmental responses in eukaryotes. In order to address the roles of these proteins in *Neurospora* biology, we attempted to create knockout mutants in all serine-threonine kinase-encoding genes. A total of 89 serine-threonine protein kinases were annotated in the *Neurospora crassa* genome sequence. Heterokaryotic transformants were obtained for all 89 genes, but we were only able to isolate viable ascospore progeny for 60 kinases. Phenotypic analysis of the viable mutants revealed ~40% exhibited defects in the sexual cycle. Of these, 15 mutants did not produce protoperithecia, while another 10 mutants had defects in perithecial development or ascospore shooting/production. Approximately 19% of the mutants had asexual developmental defects, with the most severe phenotype, the complete absence of conidia, found only in the female-sterile kinase mutants. A total of 27% of the mutants exhibited abnormal growth rates, measured as basal hyphal extension over 72 hours. Of interest, all but one of the female-sterile mutants are included in this mutant phenotype category. In conclusion, serine-threonine kinases were shown to play an important role in growth and asexual and sexual development in *N. crassa*, and a small group of genes were essential for all three processes.

Neurospora Functional Genomics Projects: Annotation

Heather M. Hood[1] and Matthew S. Sachs[2]. [1]Oregon Health & Science University, Beaverton OR 97006; [2]Texas A&M University, College Station, TX 77843

Refinement of the *Neurospora crassa* genome annotation is proceeding on multiple fronts. Published scientific literature from 2001 through the present was reviewed; data were extracted and added to the *N. crassa* database. To date, nearly 1000 articles were reviewed and over 500 were associated with specific genes in the Broad Institute database

(http://www.broad.mit.edu/annotation/genome/neurospora/Home.html). Information about cloned genes contained within the *Neurospora* Compendium (Perkins, Radford, and Sachs, 2000) was curated; data from the large-scale genome annotation paper (Borkovich et al., 2004) were also added to corresponding loci. All known and predicted peptides were functionally analyzed using the Gene Ontology (GO) Consortium's BLAST server. Functional information acquired from these GO BLAST results were subjected to stringent criteria before inclusion in the *N. crassa* database. Over 1000 genes have curated annotations ascertained from these literature and functional analyses. Finally, each of the 251 contigs were individually inspected a minimum of three times, resulting in over 25% of the predicted genes being manually annotated. When possible, intron/exon boundaries were confirmed or corrected, and untranslated regions were extended using existing and newly sequenced cDNA libraries that are a part of this functional genomics project. The incorporation of data from multiple literature sources, functional analyses, and manual curation of gene models has greatly expanded and refined the information available to the research community from the *N. crassa* database.

Dissecting colony development of _Neurospora crassa_ using mRNA profiling and comparative genomics approaches

Takao Kasuga and N. Louise Glass. Department of Plant and Microbial Biology, University of California, Berkeley 94720-3102

Colony development, including hyphal extension, branching, anastomosis, and asexual sporulation, is a fundamental part of the lifecycle of filamentous fungi, yet genetic mechanisms underlying these phenomena are poorly understood. We conducted transcriptional profiling during colony development of a filamentous model fungus, *Neurospora crassa* using 70-mer oligonucleotide microarrays. First, six sections of defined age groups were excised from a 27 hour old colony and subsequently Bayesian inference of relative mRNA expression levels was made. A functional category database was then used to identify biological processes participating during the developmental stages. Genes involved in polar growth and cellular signaling were enriched for expression at the periphery of the colony. The middle part of the colony was engaged in protein synthesis and energy production. In the older part of the colony, genes for proteins and peptide degradation, and unclassified proteins were active. A cross-examination of the *N. crassa* dataset with a

published dataset of *Aspergillus niger* revealed shared patterns in the spatiotemporal regulation of gene orthologs during colony development. At present less than 50% of *N. crassa* genes have functional annotations, which impose the chief limitation on data analysis. We developed an alternative method by incorporating an evolutionary approach. First, a phylogenetic distribution of each of the *N. crassa* genes was used to classify genes into mutually exclusive lineage specific (LS) groups: (1) Eukaryotes/Prokaryotes (Euk/Prok)-core, (2) Dikarya-core, (3) Ascomycota-core, (4) Euascomycetes (Euasco)-specific, and (5) *N. crassa*-orphans. We then cross-examined mRNA profiles with the LS groups. An enrichment of Euasco-specific genes was detected in the colony periphery. On the other hand, Euk/Prok-core and Dikarya-core genes were enriched in the middle part of the colony. In the order part of the colony, where the region was in the early stage of conidiation, an enrichment of *N. crassa*-orphans was observed.

Construction and Characterization of New cDNA Libraries from Neuorospora Mauriceville.

Meray Basturkmen[1], Junhuan Xu[2], Mary Anne Nelson[2], Chinnappa D. Kodira[3], Bruce Birren[3], Matthew S. Sachs[1]. [1]Texas A&M University, Department of Biology, College Station, TX, [2]University of New Mexico, Department of Biology, Albuquerque, NM, [3]The Broad Institute, Cambridge, MA

Neurospora crassa cDNA libraries were constructed from the Mauriceville Mat A strain (FGSC# 2225). Libraries were prepared from cells grown under 13 different conditions including growth in Vogel's and Westergaard's media, heat shock, oxidative stress, osmotic stress, glucose deprivation, nitrogen deprivation and sexual induction. Cells were extracted for total RNA and poly(A) RNA was purified by oligo-dT cellulose chromatography. First strand cDNA was synthesized at high temperature to minimize problems arising from RNA secondary structure. The Gubler and Hoffman RNAse H method was used for second strand synthesis. Double-strand cDNA was directionally cloned into the UNIZAP XR vector using EcoRI and XhoI sites. Lambda libraries were amplified and excised to obtain single-strand phage containing pBlueScript for sequencing. Each individual library of amplified lambda phage was also deposited at the Fungal Genetics Stock Center. Test sequencing provided evidence for more than 700 new genes with a high rate of full-length cDNA sequences. Deeper sequencing and the analysis of the libraries are continuing.

Cross-kingdom patterns of alternative splicing and splice recognition

Abigail Manson McGuire*, Matthew D. Pearson*, Daniel E. Neafsey, and James E. Galagan (*these authors contributed equally)

We have performed a comprehensive survey of alternative splicing across 42 eukaryotes – including 14 fungi - to gain insight into the recognition of spliceosomal introns. The observed variations in transcript splicing reveal potential differences in how eukaryotes recognize intronic splice sites. Intron recognition by intron definition (ID) implies a greater number of shorter retained introns (RIs) whereas intron recognition by exon definition (ED) implies a greater number of shorter cassette exons (CEs).

All eukaryotes we examined exhibit RIs, which appear more frequently than previously thought. CEs are also present in all kingdoms and most of the organisms that we studied. We observe that the ratio of CEs to RIs varies substantially among kingdoms, while the ratio of competing 3' acceptor and competing 5' donor sites remains nearly constant. In addition, we find the ratio of CEs to RIs in each organism correlates with the length of its introns.

In all 14 of the fungi that we examined, as well as in most of the 9 protists, RIs far outnumber CEs. This trend was observed for both ascomycetes and basidiomycetes and was independent of the number of introns present in the organism. This differs from the trend seen in the 13 multicellular animals in our analysis, where CEs occur more frequently. The 6 plants we analyzed have substantial amounts of both CEs and RIs. Our results suggest that most extant eukaryotes are capable of recognizing splice sites via both ID and ED, although ED predominates in multicellular animals and ID is more common in fungi and most protists.

***Neurospora* Lights Up!**

Van Gooch†, Arun Mehra§, Luis Larrondo§, Julie Fox†, Jennifer Loros§ and Jay Dunlap§. †Division of Science and Mathematics, University of Minnesota—Morris, Morris, MN 56267, and § Department of Genetics, Dartmouth Medical School, Hanover, New Hampshire 037552.

We have synthesized a firefly *luciferase* gene to be compatible with the codon usage of *Neurospora crassa*. The third codon position of the native firefly *(Photinus pyralis) luciferase* gene is rich in adenine and thymine while usage tables show that *Neurospora* tends to have a preference for cytosine and guanine in that position. The optimized gene was designed to recognize these *Neurospora* preferences while the eventual amino acid sequence of the luciferase would be identical to the native firefly luciferase. A total of 426 individual nucleotide changes were made out of the 1653 base pair sequence of the native firefly *luciferase*. Eighty-six oligonucleotides of 40 bp in length with 20 mer overlaps were purchased and assembled into a gene using assembly PCR. The gene was designed so that promoters of choice could be easily inserted. Initially we created strains using a segment of the *frq* promoter (known to be integral in the circadian mechanism) and the *eas*/*ccg-2* promoter (known for its vigorous ability to induce translation as well as being controlled by the circadian clock). *Neurospora* transformed with *eas*/*ccg-2* optimized *luciferase* construct in the presence of luciferin yields a glow strong enough to be seen by the dark-adapted eye. The bioluminescence from the *frq* optimized *luciferase* construct demonstrates all of the classic properties of *Neurospora* circadian rhythms. This new system allows for detailed and high volume monitoring of the molecular level circadian oscillator in *Neurospora*. Detailed phase response curves for light and temperature have been generated. We believe the codon optimized *luciferase* gene should be a valuable tool to all researchers of *Neurospora* and fungal genetics and that the concept of codon optimization is an important consideration for those looking for high protein expression.

Expression of the red fluorescent protein mCherry During the circadian rhythm of *Neurospora crassa*

Ernestina Castro-Longoria

Poster 8

Development of Neurospora host strains with reduced codon bias for elevated protein expression

Christina Chung

Poster 60

NEUROSPORA 2008 POSTER ABSTRACTS

BIOCHEMISTRY AND METABOLISM

1 The *ylo-1* gene encodes an aldehyde dehydrogenase responsible for the last oxidative reaction in the *Neurospora* carotenoid pathway.

Alejandro F. Estrada[1], Loubna Youssar[1], Daniel Scherzinger[2], Salim Al- Babili[2], and Javier Avalos[1] [1]Departament of Genetics, Faculty of Biology, University of Seville, E-41012 Seville, Spain. [2]Faculty of Biology, Albert-Ludwigs University of Freiburg, Schaenzlestr. 1, D-79104 Freiburg, Germany.

The surface cultures of *Neurospora* are orange because of the accumulation of the acidic apocarotenoid neuroporaxanthin and variable amounts of precursor carotenes. The enzymes identified so far in the *Neurospora* carotenoid pathway, Al 1, Al 2, Al 3 and Cao 2, lead to the synthesis of beta apo 4´ carotenal, the aldehyde version of neurosporaxanthin. The yellow mutant, *ylo 1*, lacks this xanthophyll and accumulates a mixture of carotene intermediates, but the biochemical basis of this phenotype has been elusive. Based on former genetic data and unpublished observations, we identified the gene *ylo-1* in the *Neurospora* genome and demonstrated that it is responsible for the yellow phenotype by complementation. Accordingly, two independent yellow strains contained mutated *ylo-1*alleles. The predicted YLO-1 protein belongs to the aldehyde dehydrogenase family, and contains a presumptive transmembrane domain in its carboxy-end. In contrast to the precedent structural genes of the carotenoid pathway, light does not induce the synthesis of *ylo-1* mRNA. To investigate its enzymatic activity, the *ylo-1* gene was expressed in *E. coli*and the YLO-1 protein was purified. In vitro incubation of YLO-1 with beta apo 4´ carotenal produced neurosporaxanthin through the oxidation of the terminal aldehyde into a carboxyl group. YLO-1 was also active on shorter aldehyde carotenoids, including acyclic ones, such as 8'-apo-lycopenal, indicating the irrelevance of the cycled end of the molecule for substrate recognition. The identification of YLO-1 closes the set of enzymes required to produce neurosporanthin in *Neurospora*.

2 Functional characterization of phosphorylation sites in White Collar Complex.

Gencer Sancar, Erik Malzahn and Michael Brunner. University of Heidelberg Biochemistry Center, Im Neuenheimer Feld328, Heidelberg, Germany.

Frequency (FRQ) and the White Collar complex (WCC), consisting of the GATA-type transcription factors White Collar-1 (WC- 1) and WC-2, are essential element of interconnected negative and positive feedback loops of the circadian clock of *Neurospora crassa*. Posttranslational modification of clock proteins is crucial for the function of the circadian clock. In particular, the activity of the WCC is regulated by FRQ-dependent and FRQ-independent phosphorylation. Using a tandem affinity purification tag (TAP-tag) we purified the WCC to homogeneity and identified in vivo phosphorylation sites in WC-1 and in WC-2 by mass spectrometry. Site directed mutagenesis of newly identified phosphorylation sites in WC-1 alters period and phase of the conidiation rhythm of *Neurospora*.

3 Genetic and Functional Characterization of Three K+ Transport Systems in Neurospora

Alberto Rivetta, Kenneth Allen, & Clifford Slayman; Yale School of Medicine, New Haven CT

Molecular characterization of the primary active transporter for K in Neurospora requires identification of the established electrophoretic H-K symport process(1) with one product from three possible ORF's: NCU06449 (*TRK1*), NCU02456 (*TRK2*), and NCU00790 (*HAK1*). At our request, the 'knockout team' within the Neurospora Genome Project(2) produced homokaryon knockout strains for all three genes, and we subsequently crossed these strains to obtain all combinations of knock-outs. Combinations were checked by PCR, and growth tests were carried out on Vogel-sorbose-agar medium, plus KCl at concentrations between 0.03 mM and 100 mM. The following unequivocal results were obtained: i) deletion of all three genes (*trk1 trk2 hak1*) completely abolishes growth below 1 mM KCl; ii) *TRK1* alone (*TRK1 trk2 hak1*) sustains robust growth at KCl concentrations as low as 0.1 mM, essentially identical with the wild-type control (*TRK1 TRK2 HAK1*); iii) *HAK1* alone (*trk1 trk2 HAK1*) allows weak growth at 0.1 mM KCl; iv) *TRK2*has no influence on growth. Thus, TRK1 clearly mediates high-affinity transport, but HAK1 could be either a low-affinity system or a high-affinity system of low velocity. The overall results imply that TRK1 supplies the major current and voltage signals observed in K-starved wild-type cells(1), with perhaps a minor contribution from HAK1. Direct electrophysiological experiments are now under way. We are also exploring the use of Neurospora as

an expression system for other fungal and plant K-transporters, by means of a strain (*trk1 trk2 hak1 mus51*) optimized for homologous recombination at the NcTRK1 locus. References: (1) Rodriguez-Navarro, et al., 1986. J. Gen. Physiol. 87: 649. (2) Colot, et al., 2006. Proc. Natl. Acad. Sci. 103:10353. [We are especially indebted for the advice and assistance of Ms. Hildur Colot.]

4 Development of new biochemistry laboratory exercises using knockout mutants in *Neurospora crassa*
Kelly Keenan, Matt Scarnati, Xiao Shao, and Amber Cardani, Richard Stockton College, Pomona, NJ

The goal of this project is to develop new laboratory exercises for an undergraduate biochemistry laboratory using knockout mutants created in*Neurospora crassa*. The goal in one lab is to identify various extracts by measuring arginase specific activity in heat treated as well as in the knockout mutant for the gene that encodes arginase. The specific activity results cannot distinguish these extracts and immunoprecipitiation was used and results showed that a commercially antibody detected a protein with the expected molecular weight of arginase in the heat treated but not the knockout mutant. The second project involves the oxidative phosphorylation and alternate oxidase pathway and uses a knockout mutant in an enzyme of the oxidative phosphorylation pathway. Enzyme assays have been developed that allow measurement of sum of oxidative phosphorylation and alternate oxidase pathway as well as each component individually. Immunoprecipitation experiments with a commercially available antibody have been used to show a protein that appears in wild type but not the knockout mutant and has the expected molecular weight. These experiments are being repeated using the Western blot method.

CELL BIOLOGY

5 A Screen for mutations displaying rate-dependent branching in *Neurospora crassa.*
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In a previous study of branch frequency in Neurospora crassa focused on the wild-type, no relationship between growth rate and the frequency of hyphal branching was observed. In subsequent experiments, it became clear that while this independence is valid for the wild-type and most mutant strains, it fails to hold for a subset of morphological mutants. This study distinguishes a subset of Neurospora morphological mutants for their morphological response to altered growth rate. The observed effect provides an additional method for characterizing morphological mutants. It also provides support for models of branching in which control of branching is tightly linked to mechanisms of tip growth. It further seems likely that the mutants showing a morphological response to growth rate are more likely to be involved in the homeostatic system previously proposed to govern branch initiation.

6 Calcium sequestration and transport within cells: using green and red fusion proteins to identify organelles in *N. crassa*.

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Calcium is an important signaling molecule in all cells. In fungi calcium has been proposed to play a key role in polar growth, although we know little about the mechanisms by which calcium is sequestered and released. Analysis of the *Neurospora crassa* genome led to the identification of at least 5 genes that encode putative calcium transporters (*nca-1, nca-2, nca-3, pmr,* and *cax*). We have characterized the phenotypes of strains with mutations in these genes. To investigate the cellular locations of the transporters we made use of both GFP and dsRED tags. Marker enzymes for the vacuolar network, Golgi, ER and nuclei have also been tagged with GFP and dsRED. Analysis of the fluorescent-tagged proteins in living cells shows that the organelles are part of a dynamic system of small vesicles and tubules. The data also suggest that the nuclear envelope may be a significant part of the ER in *N. crassa*. Different calcium transporters appear to be located in the ER, the vacuolar network and the Golgi.

7 Identification and partial characterization of Neurospora anastomosis mutants.
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We have been able to identify seventeen genes that are required for the process of cell fusion (anastomosis) in the filamentous fungus Neurospora crassa. Some of these genes were identified by a map and sequence strategy while others were identified from among the mutants found in the KO gene library available from the Neurospora genome project. Many of these genes encoded transcription factors and elements of a MAP kinase signal transduction pathway. There are also cell wall proteins, transmembrane proteins, and cytosolic proteins that have been identified as being required for cell fusion.

8 Expression of the Red Fluorescent Protein mCherry During the Circadian Rhythm of *Neurospora crassa*
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The circadian rhythm in *Neurospora crassa* is expressed by the production of conidiation bands alternated by interbands with low or null conidiation. The rhythm is regulated by a transcription/translation clock that revolves around a feedback loop involving the central *frequency* (*frq*) gene. In *N. crassa*, a number of clock-controlled genes (*ccgs*) have been identified. In this work, we describe the behavior of a band (*bd*) strain of *N. crassa* containing the *ccg- 2*promoter driving the production of the red fluorescent protein (RFP) mCherry (Abs/Em 587/610 nm). Colonies were grown in special chambers and scanned from the edge inward by confocal microscopy. In fully grown colonies scanned at low magnification (10x), we noticed a strong accumulation of mCherry in the conidiation bands, but not in the interbands. To trace the expression or accumulation of mCherry we scanned growing colonies at higher magnification (100x) every hour for an entire circadian cycle. During development of the interband, mCherry was detected in vegetative hyphae at the growing edge of the colony. The level of expression increased gradually and reached maximal intensity during the first 3h. The intensity remained high for the following 7h and then decreased rapidly during the next 2 h. During development of the conidiation band, the expression of mCherry in vegetative peripheral hyphae was very low during the entire phase (11h), but a strong fluorescence developed in aerial hyphae and conidia. It remains to be seen whether the observed differences in mCherry accumulation in the colony represent circadian control of gene expression or are due to differential transport of the RFP from vegetative hyphae to aerial hyphae and conidia, or both. If differential transport is excluded, do differences in mCherry expression suggest the existence of spatially different morphogenetic control of the promoter? Clearly, the Pccg-2 - mCherryNC construct is a useful tool for studying gene dynamics in aerial hyphae development and conidiogenesis of fungi and may provide a new tool for labeling proteins involved in the morphogenesis and apical growth of *N. crassa*.

9 Characterization of the Neurospora cell wall

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We have identified the major cell wall proteins from different stages of the Neurospora life cycle, and demonstrated that the cell wall is a cell type-specific structure. We have focused our analysis of the cell wall on the vegetative hyphal cell wall and havecharacterized KO mutants affected in most of the identified proteins within the vegetative hyphal cell wall. We identified two cell wall proteins that appear to function as upstream elements in the MAP kinase pathway controlling cell development in Neurospora. We also found that Neurospora has gene families encoding cell wall enzymes that function in cross-linking cell wall polymers (glucans and chitin) and demonstrate that such enzymes are expressed in the cell wall. The analysis of KO mutants shows that these cell wall polymer cross-linking enzymes are necessary for the formation of a normal cell wall.

10 Withdrawn

11 Plasmid-induced senescence in *Neurospora crassa* triggers mitochondrial-nuclear communication
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Wild-type strains of *Neurospora* grow indefinitely and senescence (growth cessation) is generally restricted to strains that harbor certain mitochondrial plasmids. These plasmids can integrate into mitochondrial DNA (mtDNA) or over-replicate, which inhibits the synthesis of vital mitochondrial proteins. Here, we study senescence in the MS4416 strain that harbors a variant form of the Mauriceville mitochondrial retroplasmid (pMS). Senescence in MS4416 strains is associated with plasmid over-replication and pre-senescent cultures express increased levels of nuclear-encoded mitochondrial genes involved in respiratory function and mtDNA synthesis. Induction of these genes suggests that plasmid over-replication triggers a mitochondrial-nuclear communication pathway that leads to the synthesis of genes involved in mitochondrial function and biogenesis. Significantly, spontaneous isolates of MS4416 that harbor high levels of pMS plasmid, yet escape senescence, fail to induce these genes. Genetic studies of these long- lived mutants suggest that longevity is controlled by a single nuclear mutation that interferes with the expression of nuclear- encoded mitochondrial genes that are induced when mitochondrial function is compromised. Confocal microscopy analyses reveal that the abundance of mitochondria is elevated in senescent cultures, although the relative levels of active mitochondria remain the same. Interestingly, the pMS/mtDNA ratio increases in senescent cultures, while the mtDNA/nuclear DNA ratio is not elevated. Our findings support the hypothesis that senescence is associated with a mitochondrial-nuclear communication pathway that leads to the accumulation of dysfunctional mitochondria.

12 SpaNc localizes at the cellular apex of germlings and mature hyphae of *Neurospora crassa*

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In polarized fungal cells multiple protein complexes assemble at sites of apical growth to ensure that cellular components get incorporated in to the plasma membrane and provide precursors and enzymes required for building new cell wall. One of these protein complexes is the polarisome, which in *Saccharomyces cerevisiae* contains Spa2p, Pea2p, Bud6p, Aip3p and Bni1p. Analysis of the genome sequence of *N. crassa*allowed us to identify the homologues of Spa2p, Bud6p and Bni1p. To investigate the spatial and temporal distribution of the polarisome in *N. crassa*we labeled SpaNc (NCU03115.1) with the green fluorescent protein (GFP) and analyzed growing cells by laser scanning confocal microscopy. The distribution of SpaNc-GFP was monitored from germination phase until mature hyphae were formed. SpaNc-GFP was localized at the cellular apex during germ tube elongation phase and in mature hyphae. SpaNc-GFP was observed in the apical region of all cells as a crescent shape with higher intensity at the center. Also it was found that SpaNc is not required for septum formation, but it may be involved in the development of lateral branches. The vital dye FM4-64 was used to determine the colocalization of SpaNc with the Spitzenkörper (Spk). As the germ tube elongated, SpaNc-GFP accumulated gradually at the apex of germlings of more than 24 µm in length. Subsequently the Spk was detected, when the germlings reached about 40 µm in length. From this point on, the localization of the Spk and Spa2-GFP was identical. *SpaNc*mutant (FGSC #11140) cells displayed distorted hyphae with uneven constrictions, apices with a smaller Spk (1.5 + 0.3 µm; n=30) compared to wild type strain (2.3 + 0.2 µm; n=30), slower growth rate and higher number of branches. The evidence presented here strongly suggests that SpaNc is required for the stability, behavior and morphology of the Spk and maintenance of regular hyphal growth in *N. crassa*.

13 Confocal and Total Internal Reflection Fluorescence Microscopy (TIRFM) imaging of Fimbrin in *Neurospora crassa.*

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The actin cytoskeleton in filamentous fungi is fundamental for the establishment and maintenance of polarized growth via vesicle delivery, endocytosis, and septal formation. The various functions of actin are determined by its association to its numerous binding proteins (ABPs). Fimbrin is an ABP of the calponin homology domain family. It has two actin binding domains, and causes the formation of microfilament bundles. To determine the localization and dynamics of

actin-associated fimbrin we made two fusions, one using the full length protein (Fim) and another using the second binding domain (ABD2), to the N-terminus of GFP in *Neurospora crassa*. Confocal imaging of Fim-GFP and ABD2-GFP revealed that Fimbrin is distributed throughout the hypha forming cortical patches, which were mainly concentrated in the subapical region forming a broad ring just below the Spitzenkörper. The number of patches diminished in the basal region. Some of the subapical patches become lagged and accumulate in sites where branching will occur. Both proteins localize at the septa, forming a transient pair of rings flanking the contractile ring. TIRFM shows that the patches are highly motile and mainly localized in the membrane.

14 Localization of gamma-tubulin in *Neurospora crassa*.

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Gamma tubulin is the major microtubule (Mt) nucleator. Together with the gamma ring proteins they constitute the Microtubule- Organizing Center (MTOC) at the Mt minus end. In most fungal cells this protein complex is localized at cytoplasmic MTOCs (i.e., spindle pole bodies (SPB)). However, by immunolabeling gamma tubulin was found in the Spitzenkörper (Spk) of *Allomyces macrogynus*, although it has not been found in the Spk of higher fungi. In order to determine the localization of gamma-tubulin in *Neurospora crassa*, the protein was tagged with the green fluorescent protein (GFP). A transformant of *N. crassa*was constructed fusing the gamma-tub gene to the N-terminus of sgfp gene. By laser scanning confocal microscopy, we observed that gamma-tubulin was localized at SPBs. All interphase nuclei had one SPB, although in mitotic nuclei, located mostly in basal regions, two bright fluorescent spots were observed indicating a pair of SPBs. Free gamma-tubulin was also observed in the cytoplasm. Contrary to our expectation, we found that gamma-tubulin is not localized in the Spk of*N. crassa*, as has been described for the Spk of *A. macrogynus*where the Spk is believed to function as an MTOC.This evidence suggests that, at molecular level, the hyphal apices of these fungi are organized differently.

15 *In vivo* localization of *Neurospora crassa* GS-1, a protein involved in beta-1,3-glucan synthesis. Jorge Verdín, Salomón Bartnicki and Meritxell Riquelme. Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada (CICESE). Km 107 Carr. Tijuana-Ensenada, Ensenada 22860, B.C., México.

GS-1 is a protein necessary for beta-1,3-glucan synthase activity and normal hyphal development of *N. crassa*. GS-1 shares several sequence motifs with Knr4/Smi1 from *S. cerevisiae*, a cell wall synthesis regulator that coordinates the action of proteins involved in cell wall maintenance and the establishment of cell polarity. To date, the role of GS-1 in beta-1,3- glucan synthesis is unknown. In order to determine the localization and dynamics of GS-1, the *N. crassa* gene gs-1 (NCU04189.3) was endogenously fused to the green fluorescent protein gene sgfp. Total internal reflection fluorescence (TIRF) and laser scanning confocal microscopy (LSCM) were used to analyze the transformant strain. GS-1::sGFP arranges in an apical ring which corresponds to the outer layer of the Spitzenkörper (Spk). Occasionally, the "Spitzenring" moves retrogradly and disintigrates while a new Spitzenring is assembled in the apex. In subapical regions, discrete fluorescent corpuscles move in anterograde fashion along paths that reach the Spitzenring, where GS-1::sGFP is recruited. Together with the previously reported localization of chitin synthases in the core of the Spk, these results suggest an architectural and functional stratification of the cell wall synthesis machinery within the Spk that should be considered in the models that describe hyphal growth.

16 PAC-1A DYNAMICS IN *Neurospora crassa*

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The *pac-1A* gene of *Neurospora crassa* has a high identity with the *A. nidulans nudF* gene and the human gene *lis1*. These genes seem to interact with the dynein/dynactin complex at microtubules (Mts) plus end and are required for nuclear distribution and cell migration. In order to analyze the dynamics of PAC-1A in *N. crassa*, we recorded by confocal microscopy time-lapse movies of growing hyphae labeled with *pac-1A::gfp*. We also analyzed the knockout strain of *pac-1A*. PAC-1A was distributed with different density through the hyphal regions. Single fluorescent particles could be seen in the subapical region; as these particles moved, they lined up forming short filament-like structures. At the hyphal tip, there was a higher accumulation of PAC-1A, although it seemed to disappear when they reached apex. The fluorescent structures moved exclusively in an anterograde fashion following the microtubular cytoskeleton pathway. Fluorescence was not associated with nuclei. FRAP experiments and the fast speed of PAC-1A, indicate that the fluorescent particles move independently of the cytoplasmic flow. In cells treated with 2.5 µg $ml^{-1}$of benomyl, there were disorganized small fluorescent spots of PAC-1A in the subapical region that did not reach the apex. The deletion of the *pac-1A* gene causes a severe effect in the cell growth and organelle distribution. The organized displacement of PAC-1A from individual particles at the base of the hypha to the filament-like pattern at the tip, suggests that PAC-1A participates in unique fashion in the dynamics of polarized growth. In *ro- 3* mutant containing PAC-1A::GFP, the fluorescent filaments-like structures were not formed, an indication that the behavior of PAC-1A depends on dynactin.

17 Characterization of the nucleotide excision repair genes *mus-43* and *mus-44* in *Neurospora crassa*. Masahito Sato, Takaharu Niki, Takeru Toko, Kouhei Suzuki, Makoto Fujimura, Akihiko Ichiishi. Dept of Life Sciences, Toyo University, Gunma, Japan.

Nucleotide excision repair (NER) is the main mechanism responsible for removing a wide range of bulky DNA lesions, including UV-induced cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs). We identified and characterized the *RAD14* and *RAD10* homolog genes in *N. crassa*, which we named *mus-43* and *mus-44*, respectively. Disruption of *mus-43* and *mus-44*conferred sensitivity to UV and 4NQO, but not to MMS, MNNG, camptothecin, hydroxyurea, or bleomycin. Genetic analysis indicated that *mus-43* and *mus-44* are epistatic to *mus-38* which is a homolog of the *S. cerevisiae RAD1*, but not to *mus-18*which belongs to a second excision repair pathway. The roles of these genes in the excision of UV damage were characterized by direct assay of CPDs and 6-4PPs. In wild-type cells, binding sites for the antibodies to both CPDs and 6-4PPs decreased rapidly with incubation time after UV irradiation. The *mus-18* mutant was slow in removing both types of damage, with approximately 70% of the CPDs and 35% of the 6-4PPs remaining after 3 h. In contrast, the rate of repair of UV-damage in *mus-43* and *mus-44* mutants were only slightly lower than that in wild-type. Furthermore, there was no decrease in antibody binding sites in the *mus-18 mus- 43* and *mus-18 mus-44*double mutants over the 3 h of the assay, indicating a failure to excise both types of UV damage in these strains. These results suggest that *N. crassa* has two redundant repair mechanisms -NER and *mus-18* excision repair pathways- for UV-induced CPDs and 6-4PPs, and that the *mus-18* excision repair pathway is predominant in the removal of these lesions.

18 Biochemical basis of ribonucleotide reductase – associated nonself recognition in *Neurospora crassa*. Robert P. Smith, Denis Lafontaine, Kenji Wellman, Leila Haidari, and Myron L. Smith Carleton University, Ottawa, Ontario, Canada, rsmith4@connect.carleton.ca

During the assimilative growth phase of filamentous fungi, cell fusion between conspecifics may result in the formation of heterokaryons, cells that contain genetically distinct nuclei. In *Neurospora crassa*, proliferation of heterokaryotic cells is governed by at least 13 genes. The *het-6* locus of *N. crassa* contains two genes that have heterokaryon incompatibility function. One of these genes, *un-24*, has a dual function as it also encodes the large subunit of a class I ribonucleotide reductase (RNR), the enzyme responsible for the conversion of nucleotides into

deoxynucleotides. *un-24* has two alleles, Oakridge (OR) and Panama (PA), which differ in their carboxy termini. Cells that contain both OR and PA alleles, either as heterokaryons or partial diploids, are non-viable or grow slowly with an aberrant morphology. Western blot analyses of *un-24*incompatible colonies suggests that toxic OR-PA dimers mediate incompatibility. Interestingly, incompatibility is also observed when 63 aa of the PA C-terminus or 595 aa of the OR form are expressed in strains carrying *un- 24*^{OR} or *un-24*^{PA}, respectively. Substitutions of conserved redox-reactive cysteines near the C-terminus that are involved in RNR catalysis results in loss of incompatibility function. We present a detailed model and supporting data of how OR-PA dimerization results in incompatibility-associated cell death.

19 Centromere assembly and maintenance in *Neurospora crassa*.

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Centromeres serve as platforms for the assembly of kinetochores, the attachment points for spindle microtubules that transport chromosomes into daughter nuclei during nuclear division. It remains unclear how centromeres are assembled and maintained in most eukaryotes. The histone H3 variant CenH3 is present in specialized nucleosomes that are associated with centromeric DNA, which is typically AT- and repeat-rich. The centromere-specific protein CenpC connects centromeric nucleosomes to inner kinetochore proteins. Centromeric nucleosomes also carry a unique set of posttranslational histone modifications. One of our goals is to assess the contribution of DNA sequence content versus histone variants and modifications to the placement of functional centromeres. To this end we have generated fusions of CenH3 and CenpC with fluorescent proteins and affinity tags to study their localization and function, and to isolate DNA associated with both proteins by ChIP. Both CenH3 and CenPC GFP fusions localize to a single "chromocenter" in each nucleus. We also address which forces may drive the evolution of centromeric DNA and proteins, a fundamental question in cell biology. Because CenH3 and CenpC are thought to co-evolve with rapidly changing centromeric DNA, we compared the CenH3 and CenpC genes from divergent *Neurospora* and *Gelasinospora*species, and used interspecific complementation of a null CenH3 allele to determine requirements for CenH3 function. The Neurospora CenH3 amino-terminal region appears under positive selection, while the carboxy-terminus remains under strong purifying selection. Our findings support the idea that meiotic drive may be involved in centromere evolution in filamentous fungi.

20 Analysis of Protein Interactions in the *Neurospora crassa* Circadian Clock

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The *Neurospora* circadian clock is a biochemical oscillator comprising interconnected positive and negative feedback loops. Many of the core clock components have been identified including*frequency (frq), white collar-1* and *2 (wc-1, wc-2), frequency-interacting RNA helicase(frh)*. When these, and the other proteins known to interact with them, are considered together we can describe an emerging portrait of a dynamic multi-protein complex required to accurately maintain temporal control. With advances in technology the ability to identify proteins in complex peptide mixtures is becoming a routine procedure. However, when considered at the level of a single purified protein, these methods generate large protein-protein interaction datasets that require laborious biological validation. Here we describe a subtractive proteomics strategy to identify new protein components of the *Neurospora crassa*circadian clock. Our hypothesis is that by separately purifying multiple known proteins with their interactors, and comparing the resulting lists, that new functional components will appear enriched on more than one list. Additionally, we developed a fast and reliable method to create C-terminal tandem tagged proteins. We have applied this method to three well characterized clock proteins. These data confirm the direct physical interaction between FRQ, WCC, and FRH as well as potentially revealing novel protein-protein interactions within the clock.

21 The Tip+ MAL-3 in *Neurospora crassa*.

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Microtuble (Mt) plus end proteins (TIPs +) regulate the polymerization and depolymerization of the Mts during dynamic instability. End binding proteins (e.g., Eb1) are a highly conserved family of Mt plus end stabilizer proteins. MAL-3 represents the member of this family in *S. pombe*, which sequence was used to identify the encoding gene in *Neurospora crassa*. MAL3 was molecularly tagged by the fusion of the *mal3* gene with the *sgfp*gene (Mal3::sGFP). Using different fluorescent microscopy techniques (wide field epifluorescence microscopy, laser scanning confocal microscopy and total internal reflection fluorescence microscopy) we analyzed the distribution and dynamics of Mal3::sGFP in *N. crassa*. We observed abundant fluorescent comet-like structures in all hyphal regions. The comets had a length of 1.6 + 0.4 µm (mean + standard deviation), they moved in an anterograde manner, and had an average motility rate of 1.9 µm s^{-1}, almost 5-folds faster than the cell growth (0.2 µm s^{-1}). In the subapical region, the Mal3 comets displacement was bidirectional and we also observed a few instances of comets moving in retrograde direction from the apex. The latter, suggests that MAL3 supports the polymerization of Mts that are nucleated in the Spk. Membrane organelles stained with FM4-64 did not colocalized with Mal3::sGFP. Cells treated with 5 µg ml of Benomyl did not show fluorescent comets, only a punctuated distribution of the fluorescence in the subapical region and some accumulations around the nuclei. The similarity between Mal3 displacement rate and the Mts polymerization rate and the localization of this protein along the Mts indicate that Mal3 participate in the regulation of rescue during dynamic instability.

22 Development and characterization of a FREQUENCY-LUCIFERASE translational fusion to light up the core of the *Neurospora crassa* circadian oscillator.

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In the past years our lab has developed a codon-optimized luciferase gene for *Neurospora crassa*. This reporter system has proved itself as a valuable tool to reveal known and unknown properties of the Neurospora clock (Gooch et al., 2008). In order to gain a deeper understanding of the molecular details governing circadian oscillations we sought to generate a FREQUENCY- LUCIFERASE translational fusion at the endogenous *frq* locus. This fusion protein reports in real time the abundance and kinetics of this central core component of the Neuropora circadian oscillator. Initial characterization of this reporter strain shows robust molecular rhythms in FRQ-LUC levels, with a period *circa* 24 hrs and strong light response dynamics. Using this strain we have developed a high-throughput RIP-based screening protocol to obtain new *frq* alleles. With this system, non- sense mutations can be effortlessly discarded and full length *frq* mutants can be easily selected for further characterization.

23 Live Imaging of Chitin Synthase 1 (Chs-1) Traffic in Growing Cells of *Neurospora crassa*

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The biosynthesis of chitin, a minor but very important cell wall structural component, is catalyzed by a diverse group of enzymes named chitin synthases (CHS). In earlier studies we visualized the cellular localization of two CHS, CHS-3 (Class I) and CHS-6 (Class VI), tagged with the green fluorescent protein (GFP) in mature cells of *Neurospora crassa*. Both fusion proteins were found in specific cell structures: at the core of the Spitzenkörper (Spk), developing septa, endomembranous compartments, and small particles. To study the localization and dynamics of a class III CHS, exclusive of filamentous fungi, we fused the *gfp* gene to *chs1*, and expressed it under the control of a strong promoter (*ccg1*) or the native promoter. *N. crassa* strains (FGSC 9717; *mus-51 delta::bar+*; *his-3*; FGSC 9718, *mus-51 delta::bar+*) that are deficient in non-homologous end joining were used as host strains for transformation. Live imaging visualization was done by Laser Scanning Confocal Microscopy. There were no significant differences between strains expressing the fusion gene controlled by either promoter or between these strains and the strains previously studied expressing CHS-3-GFP or CHS-6-GFP under the control of *ccg1*. Observations with Widefield Fluorescence Microscopy revealed a cloud of putative vesicles reaching the Spk. Hyphae exposed to the actin inhibitor Cytochalasin A (100µg/ml) lost fluorescence in the Spk while fluorescent patches accumulated adjacent to the plasma membrane; cells treated with the microtubule inhibitor Benomyl (100µg/ml) retained the fluorescence of the Spk. These studies suggest that transport of CHS-1 to the Spk is dependent on the actin cytoskeleton.

24 Effects of Light on the Circadian Rhythms of *Neurospora crassa*.
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Recently an optimized firefly luciferase gene has been successfully introduced into *Neurospora crassa*. The frequency (frq) gene is known to be a core genetic component to the circadian rhythmicity in Neurospora and when the frq promoter is placed in front of the reporter luciferase gene, its transcriptional regulation can be easily monitored by bioluminescence. Classically, Neurospora circadian rhythms have been measured using conidial banding within race tubes in constant dark at 25C. Since conidiation is strongly stimulated by even low levels of light, then measuring the effects of light on the circadian rhythms of Neurospora has been hindered. Using this new frq-luciferase reporter system, one can now more easily monitor molecular level changes of the circadian clock even when exposed to light. Under constant light conditions, we have shown that the rhythmic activity is not expressed (as suggested by other experimenters and a phenomenon that is commonly featured in circadian systems). Entrainment using 12 hours light and 12 hours of dark show very large amplitudes and angular waveforms in comparison to free running rhythms. In addition, entrainment is demonstrated to persist even at extremely low light levels (0.27 nanomoles/sec/m^2). In other experiments, we have generated a detailed phase response curve where we see that a 15 minute LED white light pulse causes strong phase shifting (type 0). Finally, we have examined in more detail the phenomenon of "light induction" whereby light stimulates the frq promoter. We have shown that the rate of light induction plateaus quickly as a function of light intensity and that the ultimate level of light induction more strongly depends upon the light duration and the phase of the cycle at which the light exposure occurs.

25 The Role of Coronin in Polarized Growth in *Neurospora crassa*
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Coronin is an actin binding protein that plays a major role in regulating actin patch formation. The mutation of the *coronin*gene affects locomotion, growth and actin cytoskeleton organization. Actin is important for both polarized hyphal growth and Spitzenkörper (Spk) function. To determine the role of coronin in polarized growth and Spk function and organization, we have described the cellular features of the *coronin* null mutant in *Neurospora crassa*and compare it with the wild type (wt) strain. Growth rate of coronin mutant was around 40% slower than the rate for wt strain. The Spk size and shape of the coronin mutant was smaller with an ovoid shape. Looking at the effects of coronin mutation in other organelles, such as mitochondria, we observed that they had a spherical shape instead of the wt

elongated mitochondria. The direction of the hyphal apical growth was affected showing a bumping and zig-zag morphology. Branching rate was 5-folds higher than the wt. Coronin mutant produced half of the conidia than the wt (1.5×10^5 conidia ml^{-1}). The biomass production per day was 40 % less than the wt. The mutation of the coronin gene in *N. crassa*is not essential but strongly reduces the growth rate, affects the direction of polarized growth, increased the frequency of branching and the defects in septal construction.

COMPARATIVE AND FUNCTIONAL GENOMICS

26 Amplification of glycosyl hydrolase genes in *Neurospora crassa*

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A previous paper (Radford, A. (2006) FGN. 53: 12-14) described multiple menbers of a number of glycosyl hydrolase families, and further families are now to be found in the CAZy database (Coutinho & Henrissat (1999)). Based on these, the e-Compendium now contains representatives of thirty-seven different families of glycosyl hydrolases, with up to thirteen members of the largest class, family 61, and twenty-one of the families containing more than one representative. A "search for genes" among other filamentous fungal species databases at Broad for "glycosyl hydrolase" gives comparable numbers of hits for Fusarium, Botrytis and Magnaporthe, but rather fewer for Ustilago, Rhizopus, Candida and Puccinia. Glycosyl hydrolases have many functions, including carbon nutrition (cellulases, amylases and chitinases), wall polymer synthesis in hyphal growth, branching and conidiation, protein glycosylation in the ER and Golgi, etc., so the requirement for multiple functionally divergent glycosyl hydrolases is very ancient. Sequence alignments of families show homologies, but in general sequences are sufficiently diverse to be immune from RIP. Details of the glycosyl hydrolase families will be given, and investigations will be presented to show if RIP has had any detectable role in their evolution.

27 Characterization of the Neurospora VS ribozyme in vivo.

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The Varkud satellite (VS) plasmid is found in many Neurospora strains, though no corresponding phenotype has yet been identified. The plasmid encodes a ribozyme that is abundantly transcribed, and of significant interest as a model for RNA catalysis. We have pursued a proteomics approach using difference gel electrophoresis (DIGE) in order to identify differences in the mitochondrial proteome in the presence and absence of the Varkud and VS plasmids. These differences may provide insight into the effect of the VS plasmid and its encoded ribozyme in vivo.

28 Revealing Gene Function Using Phenotypic Analysis of *Neurospora crassa* Knock-out Mutants

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Analysis of the genome sequence of *N. crassa* reveals many proteins of unknown function. To understand the role of these novel proteins in the biology of filamentous fungi and further annotate*N. crassa* genome, knockout mutants were made using a systematic deletion of each predicted gene in the *N. crassa*genome. We performed basic phenotypic analysis to assess the loss of each gene on growth, morphology, linear growth rates, and asexual and sexual development in knockout mutants. We selected 20 out of 70 knockout mutants that exhibited phenotypes. The most dramatic phenotype was seen in NCU06205, a knockout mutant missing a tup-1 like protein. This protein is responsible for global transcriptional repression in the budding yeast *Saccharomyces cerevisiae*, mediating this

process through chromatin remodeling. In *N. crassa*, loss of this regulatory protein slows its growth dramatically and impedes completion of its sexual cycle. Examination of information on the other missing genes revealed representation of several important classes of proteins such as transcription factors, ras proteins, chromatin remodeling factors, and DNA repair proteins. N. crassa is representative of the filamentous fungi that are responsible for agricultural and human diseases. Understanding the biology of this organism will aid researchers who are interested in preventing or controlling fungal pathogenesis and disease.

29 The on-going *Neurospora* e-Compendium

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The e-Compendium at http://www.bioinf.leeds.ac.uk/~gen6ar/ newgenelist/genes/gene_list.htm maintains the data in successive printed *Neurospora crassa*gene compilations dating back through 2001 and 1982 to Barratt et al. (1954), but presented in structured and readily searchable format. Many of the data included, including those on genes currently defined only by phenotype, are not available in the sequence database and associated annotations at the Broad Institute. Moreover, the e-Compendium continues to be developed, as further genes are added as they are described in publications, and correlations are made between sequences, functions and homologs. As of January 2008 the e- Compendium contains data on 2474 sequenced and 441 unsequenced nuclear genes (including 399 tRNA genes, 79 5s rRNA genes and many of the rRNA repeat sequences in the nucleolus organiser region) and 49 mitochondrial genes, together with comprehensive genetic and physical maps of all seven linkage groups. It continues to serve as a readily available and comprehensive reference of gene symbols and names already in use, and therefore as an essential guide for workers naming new genes, in order to avoid duplicate use and resulting ambiguities.

30 Dissecting colony development of *Neurospora crassa* using mRNA profiling and comparative genomics approaches

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Colony development, including hyphal extension, branching, anastomosis, and asexual sporulation, is a fundamental part of the lifecycle of filamentous fungi, yet genetic mechanisms underlying these phenomena are poorly understood. We conducted transcriptional profiling during colony development of *N. crassa*using 70-mer oligomer microarrays. First, six sections representing different ages and developmental stages were excised from a 27 hour old colony and relative mRNA expression levels were inferred using Bayesian analysis. It was found that genes involved in polar growth and cellular signaling were enriched for expression at the periphery of the colony (0-1 hr old). The middle section of the colony (6-9 hr old) was engaged in biosynthesis of amino acids and nucleotides, protein synthesis and energy production. In the older part of colony (18~21 hr old), genes for proteins and peptide degradation and unclassified proteins were actively expressed. At present, less than 50% of *N. crassa*genes have functional annotation, which imposes the chief limitation on data analysis. We have developed an alternative method by incorporating an evolutionary approach. First, a phylogenetic distribution of each of the *N. crassa* genes was used to classify genes into mutually exclusive lineage specific (LS) groups using SIMAP database developed by MIPS: (1) Eukaryotes/Prokaryotes-core, (2) Dikarya-core, (3) Ascomycota-core, (4) Euascomycetes-specific, and (5) *N. crassa*-orphans. We then cross-examined mRNA colony profiles with LS groups. An enrichment for expression of Euascomycetes- specific genes was detected in the periphery of a colony. On the other hand, expression of Eukaryotes/Prokaryotes-core and Dikarya-core genes were enriched in middle section of a colony. In the older part of the colony where the region is engaged in conidiation, an enrichment for expression of *N. crassa*-orphans genes was observed.

31 Carbon sensing involves all three G protein alpha subunits and multiple putative G protein coupled receptors in *N. crassa*

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Our previous studies demonstrate that the G protein coupled receptor (GPCR) GPR-4 and the three Galpha proteins, GNA-1, GNA- 2 and GNA-3, are all important to mass accumulation on poor carbon sources in *N. crassa*. We also showed that GPR-4 is required for the transient increase in cAMP levels that are observed in wild type glycerol-grown cultures after the addition of glucose. In this study, we demonstrate that the cAMP transient is lost in mutants lacking any of the three Galpha genes, supporting the need for GNA-1, GNA-2 and GNA-3 in carbon sensing. Microarrays were successfully performed for wild type, delta*gna- 1* and delta*gpr-4* strains. The results indicate that genes are differentially regulated in delta*gna-1* and delta*gpr- 4*mutants under four different carbon conditions compared to the wild type. Many genes are up- or down-regulated with the same or similar patterns in both delta*gna-1* and delta*gpr-4* mutants, further supporting functioning of GPR-4 and GNA-1 in the same pathway for carbon sensing. We also analyzed the putative GPCRs GPR-5 and GPR-6 that are homologous to the putative nutrient sensor Stm1 from Schizosaccharomyces pombe. Highest expression of *gpr-5* and *gpr-6* is observed in cultures with glycerol as the carbon source. Phenotypic analysis showed that delta*gpr-5* and delta*gpr-6* mutants form less mass than wild type when cultured on glycerol plates, and strains lacking *gpr-5* and/or *gpr-6* in addition to *gpr-4* have dry masses similar to delta*gpr-5* or delta*gpr-4* single mutants. These findings suggest that GPR-4 and GPR-5 are redundant, while GPR-6 plays a minor role in mass formation when grown on glycerol medium. Taken together, the results support the notion that all three Galpha proteins as well as the putative GPCRs GPR-4, GPR-5 and GPR-6, are involved in carbon sensing in *N. crassa*.

32 High throughput gene knockouts in *Neurospora*

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More than 4,100 gene disruptions have been made in predicted open reading frames in the annotated *Neurospora crassa*genome using a high-throughput procedure as part of an on-going NIH-funded Program Project (P01)*. Knock-out (KO) cassettes were created using yeast recombinational cloning techniques and electroporation of *N. crassa* conidia can be performed in a 96-well format. In order to expedite molecular confirmation of KO mutant strains, we have developed a program (http://borkovichlims.ucr.edu/southern/) that allows automated identification of the appropriate restriction enzyme to use during Southern analysis. Completed *N. crassa* KO mutant strains are submitted to the Fungal Genetics Stock Center after targeted gene replacements are confirmed by Southern analysis and the list of submitted strains is available at the Neurospora genome project website

(http://www.dartmouth.edu/~neurosporagenome/knockouts_completed.html). Use of these tools and our current progress in creating KO mutants will be presented.
*Colot and Park et al., 2006. A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. PNAS 103:10352-10357

33 Assembling the *Neurospora crassa* genome: Construction of a genomic library in a linear plasmid.
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The complete genome sequence of *Neurospora crassa* has been difficult to assemble, in part because AT-rich DNA may have been lost during the preparation of traditional shotgun, cosmid, and BAC libraries. Much of the missing Neurospora sequence is expected to map to centromeric regions, as there are significant gaps between contigs that are associated with most, if not all, of the seven centromeres. Previous analyses suggested, that - as in many other eukaryotes - Neurospora centromeric regions are AT- rich and contain much repeated DNA. Unlike in mammals or plants, these regions do not harbor satellite repeats, but rather an assembly of retrotransposon relics that have undergone RIP. Various novel direct sequencing approaches promised useful to finish the Neurospora genome.

Nevertheless, preliminary results suggest that new sequencing methodology alone will likely not suffice to generate finished sequence of this relatively small eukaryotic genome, simply because the assembly of AT-rich repeats proves to be a major obstacle. To understand centromere formation and maintenance, we wish to complete the assembly of the predicted centromeric regions by alternative means. To this end, we generated a Neurospora genomic library in a linear cloning vector, pJAZZ-OC. This plasmid allows the cloning and maintenance of long AT-rich fragments. Direct and inverted repeats are tolerated. Here we describe the construction and characterization of a pilot library. Preliminary results suggest that we succeeded in cloning many AT-rich inserts that should help to complete the assembly of centromeric sequences of *Neurospora crassa*.

34 Development of an overexpression screen for new genes involved in the circadian clock. Hildur V. Colot, Luis F. Larrondo, Jennifer J. Loros and Jay C. Dunlap, Genetics Dept., Dartmouth Medical School, Hanover NH 03755;

With the proliferation of whole genome sequences, the use of multiple approaches for studying the functions of unkown genes has become imperative. We have been engaged in a high-throughput gene deletion project with *Neurospora*for a few years and the strains thus created have proved useful to many researchers. As a companion to that project, we have undertaken an overexpression screen using many of the techniques, tools and reagents developed for the knockout project. Overexpression studies allow functional analysis of essential genes and can yield unique insights when compared with underexpression or deletion studies: In genetic terms, deletions provide loss-of-function whereas, ideally, overexpression can mimic gain-of-function alleles. With the goal of finding new genes involved in circadian clock function and/or output, we have placed numerous genes under the control of the inducible *qa-2* promoter by using high-throughput gene replacement strains and techniques to replace the native promoters with the *qa-2*promoter. Both low expression and high/constitutive expression of a given gene can be analyzed in this system. Preliminary data indicate that there is a great deal of variability in the extent to which true overexpression of a gene can be achieved by the inducible promoter; expression levels of a variety of genes with respect to that achievable by *qa-2* will be presented.

35 Soluble multiprotein complexes in Neurospora mitochondria

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We have treated highly-purified mitochondria with low concentrations of mild detergents to extract soluble multiprotein complexes. Complexes were separated by blue native gel electrophoresis; individual subunits were dissociated in a second dimension SDS gel and identified by MALDI-TOF peptide fingerprinting mass spectrometry. Hsp60, hsp70, F1 ATPase, pyruvate dehydrogenase, isocitrate dehydrogenase and several other Kreb's cycle enzymes each were found in discrete complexes.

36 *Neurospora* comparative genomics using *Neurospora discreta* and *N. tetrasperma* genomes
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The sequencing of the *Neurospora crassa*genome enabled a new era of molecular and evolutionary analysis in filamentous fungi. The availability of whole genome sequences from *Neurospora discreta* and *N. tetrasperma* from the Joint Genome Institute elevates *Neurospora*as a platform for evolutionary genomics. Using comparative genomics and phylogenomics we have identified gene conservation and changes among *N. crassa*, *N. discreta* and *N. tetrasperma*. Specifically, we identify species-specific genes, significant expansions and contractions in gene family

size, and genes under positive selection. Using comparative methods with three Neurospora genomes and the outgroup species *Podospora anserina* and *Chaetomium globosum* we have constructed a refined set of *Neurospora*-specific and shared genes found among these filamentous fungi. In addition to genic content, we have explored the extent of genome structure conservation by cataloging syntenic sequence conservation between the taxa and determining if repeat density correlates with breaks in synteny. Finally, given its importance in *N. crassa*, we surveyed for signatures of RIP and gene family size in the *N. discreta* and *N. tetraspermag*enomes to ascertain the age and influence of this mechanism and the evolutionary dynamics of how RIP has impacted genome evolution.

37 The evolution of the mating-type chromosome in *Neurospora tetrasperma*: syntenic comparisons to *Neurospora crassa* and *Neurospora discreta*

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The reproductive strategy of *Neurospora tetrasperma*involves the packaging of two haploid nuclei of opposite mating type into a single ascospore. This pseudohomothallic condition results in self-fertility. Blocked recombination proximal to the mating type locus during meiosis I is necessary for the correct packaging of alternative mating type alleles into a single heterokaryotic ascospore and previous genetic studies have shown that recombination is suppressed along the majority of the mating type chromosome (75% / 7Mbp). This recombination blockage has been hypothesized to be the result of a series of chromosomal rearrangements. The Joint Genome Institute has recently sequenced the *N. tetrasperma* genome, including both mating types (*mat a* [FGSC #2509] and *mat A* [FGSC #2508]), and the *N. discreta* genome (*mat A* [FGSC #8579]), thus allowing the opportunity to test this hypothesis. Using *N. crassa*linkage groups as a reference, we constructed syntenic alignments between the mating type chromosomes as well as the autosomes. After identifying regions of rearrangement we then compare the degree of rearrangement between 1) *N. tetrasperma mat a* and *mat A*chromosomes and 2) autosomes and mating type chromosomes between taxa. Finally, we place the specific locations of rearrangements in the context of the *N. tetrasperma* genetic map. Elucidating the degree and sites of rearrangement along the *N. tetrasperma*mating type chromosome will lead to a better understanding of the origin of the psuedohomothallic condition as well as the evolution of sex chromosomes in plants and animals.

GENE REGULATION

38 New features over the expression of *Neurospora crassa* genes in response to extracellular phosphate levels and pH.

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Microorganisms have evolved complex signal transduction networks that enable them to adapt effectively in response to environmental changes in nutrients and extracellular pH. The phosphorus (Pi) acquisition system in *Neurospora crassa* includes four regulatory genes: *nuc-2, preg, pgov* and *nuc-1*. In response to signals of Pi starvation, NUC-2 inhibits the functioning of the complex PREG-PGOV allowing the translocation of transcription regulator NUC-1 into the nucleus and expression of Pi-repressible phosphatases. In an attempt to further understand the molecular responses to exogenous Pi sensing, six subtractive cDNA libraries were constructed to identify genes differentially expressed in the *nuc*-1^{RIP} and *nuc*-2A mutant strains of *N. crassa*grown in low Pi, at acidic and alkaline pH. We identified genes involved in diverse cellular processes such as cellular transport, cellular metabolism, protein biosynthesis, regulation of transcription, development and signal transduction, revealing novel molecular aspects of the functioning of *nuc*-1 and *nuc*-2 genes. We also found that transcription of two heat shock protein genes is affected by growth pH while

another one is overexpressed when *nuc*-1 gene is non functional. Financial support: FAPESP, CNPq, FAEPA and CAPES

39 Definition of the DNA-Binding Specificity of the CYS3 Transcription Factor of *Neurospora crassa* by Binding-site Selection.

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The CYS3 transcription factor is a basic region-leucine zipper (bZIP) DNA-binding protein that is essential for the expression of a coordinately regulated group of genes involved in the acquisition and utilization of sulfur in *Neurospora crassa*. An approach of using binding site selection from random-sequence oligonucleotides was used to further define CYS3 binding specificity. The derived consensus binding site of AT(G/t)(g/a)C(g/a)C(C/a)AT defines a symmetrical sequence that resembles that of other bZIP proteins such as CREB and C/EBP . By comparison, CYS3 shows a greater range of binding to a central core of varied Pur-Pyr-Pur- Pyr sequences than CREB as determined by gel shift assays. The derived CYS3 consensus binding sequence was further validated by demonstrating *in vivo*sulfur regulation using a heterologous promoter construct. The CYS3 binding site data will be useful for the genome-wide study of sulfur-regulated genes in *Neurospora crassa*, which has served as a model fungal sulfur control system. The overall pattern of regulation in the *Neurospora crassa* sulfur metabolic network based on our gene expression

40 A molecular mechanism for light-dependent conidiation: the fluffy gene, a major regulator of conidiation in Neurospora, is activated by light

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Light stimulates the development of conidia in Neurospora crassa. The products of the wc genes, wc-1 and wc-2, are required for all the Neurospora responses to light, including the activation by light of conidiation. WC-1 is a blue-light photoreceptor and transcription factor that interacts with WC-2 to form a complex (WCC) that binds to the promoters of light-regulated genes. The fluffy gene is a major regulator of conidiation that encodes a 88-kDa polypeptide (FL) containing a Zn2Cys6 binuclear zinc cluster domain that is necessary and sufficient to induce conidiophore development in Neurospora. The major role of FL in conidiation prompted us to investigate the possible regulation by light of fluffy gene transcription. The transcription of gene fluffy is activated by light. Wild type mycelia exposed to 15 min of light increased fluffy mRNA accumulation four-fold, but longer light exposures reduced fluffy photoactivation due to photoadaptation. The photoactivation of fluffy is observed with blue light, but not with red light, as in other light responses of Neurospora. The threshold for fluffy photoactivation is similar to the threshold for other Neurospora light activated genes. The activation of fluffy by light was observed in other conidiation mutants of Neurospora, suggesting that light-dependent activation of fluffy does not require an active conidiation pathway. As expected, strains with mutations in wc-1 or wc-2 did not accumulate fluffy mRNA after light exposure. The promoter of fluffy contains a putative site for WCC binding at position -560 (from the transcription initiation site). We propose that light-dependent binding of WCC to the promoter of fluffy will trigger light-dependent gene transcription. The increased accumulation of FL will induce conidiophore development resulting in an increased production of conidia after light exposure. This simplified model for light-dependent conidiation in Neurospora could help to understand light-dependent conidiation in other Ascomycetes fungi.

41 A Genetic Screen for Negative Feedback Loss in *Neurospora* Revealed Dual Roles of FRH (FRQ Interacting RNA Helicase) in Circadian Regulation.

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A genetic screen was developed to identify clock components involved in FRQ-based negative feedback in *Neurospora crassa*. SNP (single nucleotide polymorphism) mapping revealed a chromosomal translocation and a missense *frh* mutation (named as frh^{LN}) in an arrhythmic strain. Mutant FRH^{LN}protein binds tightly with FRQ, but not with WCC (white collar complex), which results in a loss of negative feedback and a dominant negative effect on the circadian clock. WCC activity is upregulated in the mutant with a constantly high expression of *frq*. Interestingly, WCC levels are extremely low in the mutant strain. This result indicates that FRH together with FRQ has dual roles in both negative feedback to inhibit WCC activity and positive feedback to maintain WCC stability.

42 Genome-wide characterization of light-inducible responses reveals a hierarchical light-sensing cascade in *Neurospora crassa*

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To better understand the roles of WC-1, WC-2 and other putative photoreceptors in mediation of light signals and to identify new components in light sensing cascades, we used microarrays with genome-wide coverage to characterize light-inducible transcriptional changes in Neurospora crassa. Several questions in Neurospora photobiology were addressed. First, which genes display altered expression in response to light? Second, how can we categorize light responses in general, and how are they temporarily coordinated? Third, are wc-1 and wc-2 strains truly blind to all light-inducible transcriptional changes? Are there other putative photoreceptors involved in the control of any light response at the level of transcript abundance? Previous studies have demonstrated that Neurospora is very sensitive to light stimuli and can display dramatic transcriptional changes within 15 min or less. Therefore, we sampled more intensively within the first 30 min after exposure to light, namely at 0, 5, 10, 15, 30, 60, 90, 120, 180 and 240 min after lights-on. To eliminate intrinsic systemic variation between arrays and have a direct comparison between experiments, we adopted an unsupervised hierarchical clustering approach to identify bona fide light- responsive genes. An assumption made here is that genes that are truly responsive to light should behave more similar to each other across sequential time points and different strains. Before clustering, the data were normalized so that each measurement reflects the abundance of each transcript relative to the mean value across all arrays. Genes missing more than 25% of their measurements from 90 microarrays were all excluded, which results in 5588 valid spots, providing approximately 53.8% coverage of all 10372 spots on the array. In addition, only genes have at least 4 fold changes (maximum - minimum) across 90 microarrays were selected for further clustering. Totally, 2864 spots passed these selection criteria. After clustering, 316 genes, which are about 5.7% of the total detectable genes, were identified as light-responsive due to their robust and consistent light-responsive pattern. Functional analysis further suggests that there is correspondence between the timing of transcript up regulation and gene function. Moreover, the differential regulation of early and late light responses was further elucidated by the identification of a dedicated transcription factor and motif that specifically control a subset of late light responses. Meanwhile, by comparing light responses in different photoreceptor knockout strains, our data show that most light-induced transcriptional changes in Neurospora are controlled by WC-1 and WC-2, independent of PHY-1, PHY-2, CRY and OPSIN. Intriguingly, the photoadaptation defect noted in vvd strain was observed for nearly all of the early and late-light responsive genes, providing us a unique opportunity for a completely independent validation to the genes we identified as "light responsive".

43 Regulation of the RNAi pathway in *Neurospora*

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RNA interference (RNAi) is a post-transcriptional gene silencing (PTGS) mechanism conserved from fungi to humans. The RNAi pathway is initiated by recognition of double-stranded RNAs (dsRNAs) by Dicer, which cleaves dsRNA into siRNAs. The siRNA duplexes are then loaded onto the Argonaute protein whereby one strand will be used as the template to guide the Argonaute protein to cleave the complementary mRNA sequences. We showed that the conversion of duplex siRNA into single-stranded siRNA requires the catalyitic activity of the *Neurospora*

Argonaute protein QDE-2. We biochemically purified the argonaute protein QDE-2 protein from *Neurospora*and identified QIP, an exonuclease domain-containing protein, as a QDE-2 interacting protein. The gene silencing triggered by dsRNA is greatly impaired in *qip* mutant. Furthermore, the exonuclease activity of QIP is required for efficiently removing passenger strand from siRNA duplex *in vivo*. Therefore, QIP acts as a positive regulator of RNAi pathway most likely by rapidly degrading the passenger strand. dsRNA, in addition for being the substrate of dicers, can initiate transcription-based interferon response in mammals. In *Neurospora*, we found that the expression of *qde-2* and *dcl-2*, two central components of the RNAi pathway in *Neurospora*, are highly induced by dsRNAs. The dsRNA-induced *qde-2* expression is required for normal RNAi efficiency. Genome-wide search revealed that additional RNAi components and homologs of antiviral and interferon-stimulated genes are also dsRNA-activated genes in *Neurospora*. These results uncovered a novel dsRNA response program in fungi and suggest that RNAi is part of a broad spectrum and ancient host defense response system against viral and transposon infections. Taken together, our data show that the gene silencing by RNAi pathway in *Neurospora* is tightly controlled through multiple mechanisms.

44 ATF-1 transcription factor regulates the catalase *cat-1* expression through CRE-element in *Neurospora crassa*.

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CAT-1 catalase activity increases during the stationary growth phase in hyphae, and CAT-1 is highly accumulated in conidia in *Neurospora crassa*. The *cat-1* gene expression and the enzyme activity were induced by osmotic stress and fludioxonil in the wild type, but not in the *os-2* MAP kinase mutant and *atf-1* transcription factor disruptant hyphae. Although CAT-1 activity in the *os-2* conidia was slightly lower than that in the wild-type strain, the *atf-1* conidia lacked CAT- 1 activity, suggesting that production of CAT-1 was largely dependent on the *atf-1* gene. To identify its binding sequence, ATF-1 was expressed in *Escherichia coli*as a NusA-His-tag fusion protein. The fusion protein was purified by affinity chromatography and used for the electrophoretic mobility shift assay (EMSA). ATF-1 bound strongly to a cAMP response (CRE) element of the *cat-1* promoter located in the region 174 bp upstream of the initiation ATG. ATF-1 also bound to CRE in the promoter region of the *ccg-1*, which is regulated by OS-2 MAP kinase. These results suggest that ATF-1 regulates *cat-1* expression via OS-2 activation in the stress response and also via OS-2-independent signaling during conidiation.

45 Identification of *Neurospora crassa* Transcription Factors Involved in Glycogen Metabolism Regulation

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In living cells, transcription factors dynamically interact with DNA playing an essential role in gene expression, therefore regulating many cellular functions. With the availability of the *Neurospora crassa*genome sequence and the knowledge of the transcriptions factors encoded by the fungus genome, a collection of transcription factors knockout strains became available to the scientific community, which allows studying regulation of specific aspects. In our laboratory we have undertook a systematic search for transcription factors that affect glycogen accumulation by taking advantage of the N. crassa knockout strains collection available from the FGSC. It is already known that in*N. crassa* glycogen content reaches maximal level at the end of the exponential growth phase. However, under heat shock, glycogen content rapidly drops. Such glycogen decrease might result from the regulation at transcriptional level of enzymes involved in the carbohydrate metabolism, since transcription of the gene encoding glycogen synthase (*gsn*) decreases under this condition. In our screening the glycogen content in the *N. crassa*mutant strains was quantified in the whole cellular extract from mycelium before and after exposure to heat shock (from 30ºC to 45ºC). Approximately 150 mutant strains belonging to different transcription factors classes were analyzed and less than 20 strains showed changes in glycogen accumulation when compared to the wild type (WT) strain. We identified transcription factors

mediating regulation of glycogen metabolism during normal conditions, that is mutant strains having hypo and hyperaccumulation of glycogen and transcription factors mediating regulation after heat shock. In this latter case, we identified mutant strains having similar glycogen content before and after heat shock. The majority of the transcription factors identified were annotated as hypothetical proteins and a few of them have been already characterized at the protein level in *N. crassa*. Supported by FAPESP, CAPES, and CNPq

46 Circadian Rhythm Promoters for an Optimized Bioluminescent Gene in *Neurospora crassa*. Julie Fox†, Luis F. Larrondo§, Van Gooch†, Jay Dunlap§, and Jennifer Loros§. †Division of Science and Math, University of Minnesota-Morris, Morris, MN 56267, §Department of Genetics, Dartmouth Medical School, Hanover, NH 03755. foxx0226@morris.umn.edu

The success of inserting the codon-optimized firefly bioluminescent gene, luciferase, into *Neurospora crassa* under the control of the circadian regulated frequency (, *frq*) promoter encouraged us to explore the use of other circadian promoters to control this luciferase reporter. Six new promoter-luciferase constructs were successfully created and studied in wild type, *ras- 1* ^{bd}, and *ras-1* ^{bd}, *frq* ^{7} Neurospora. The selected promoters included: *fluffy*, *vivid*, *con-6*, *con-10*, *ccg-13* and *ccg-4*. These clock-controlled genes were chosen based on their underlying biology and so that different peak times of their corresponding mRNA accumulation are represented. The circadian rhythms and bioluminescent outputs of these promoter-luciferase systems seem to correspondingly reflect the function of the individual clock genes. For example, the light regulated *con-10* and *con-6* reporters display high amplitudes of bioluminescence and appear to be extremely light responsive; in addition, the *ccg-4*construct is mating type specific. These new bioluminescent constructs may advance our understanding of the molecular mechanisms underlying the transcriptional regulation of these, and others, clock-controlled genes.

47 MAP kinase signaling pathways play crucial roles in fungal development and pathogenesis. Gyungsoon Park and Kathy Borkovich, University of California, Riverside.

In this study, we characterized three components of a MAPK cascade in *Neurospora crassa* (*mik-1*, MAPKKK; *mek-1*, MAPKK; *mak-1*, MAPK), homologous to that controlling cell wall integrity in *Saccharomyces cerevisiae*.

Growth of basal and aerial hyphae is significantly reduced in *mik-1*, *mek-1*, and *mak-1* deletion mutants on solid medium. Formation of macroconidia was almost abolished in *mek-1* and *mak-1* mutants and reduced in *mik- 1* strain. In contrast, the normally rare asexual spores, arthroconidia, were abundant in cultures of the three mutants. *mik- 1*, *mek- 1*, and *mak-1* mutants were unable to form protoperithecia or perithecia when used as females in a sexual cross. The MAK-1 MAP kinase was not phosphorylated in *mik-1* and *mek-1* mutants indicating signal cascade among the three kinases. Interestingly, we observed increased levels of mRNA and protein for tyrosinase, an enzyme catalyzing melanin biosynthesis, in the mutants under nitrogen starvation, a condition favoring sexual differentiation. These results implicate the cell MAK-1 pathway in regulation of development and secondary metabolism in filamentous fungi.

48 Investigation of functional domains of RRG-1, a response regulator that regulates the OS-2 MAPK cascade.

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Two component signaling pathways are found in bacteria, plants, Archaea, and fungi; however these signaling pathways are not found in humans, Drosophila or *C. elegans* and are believed to be absent from the animal kingdom as a whole. Two component regulatory systems contain proteins with histidine kinase (HK) and response regulator (RR) domains. Fungi contain hybrid histidine kinases (HHKs) with both histidine kinase and response regulator

domains in the same protein. A conserved histidine residue in the histidine kinase domain of the HHK is autophosphorylated in response to some environmental signal. That phosphate is then transferred intramolecularly to an aspartate in the response regulator domain of the HHK. From the RR domain in the HHK the phosphate is then transferred to a histidine in a histidine phosphotransferase (HPT) protein, and finally onto an aspartate on a separate RR protein. *Neurospora* is predicted to have eleven HHKs, one HPT and two RR proteins. Analysis of a mutant lacking the RR gene, *rrg-1*, showed that RRG-1 is involved in female fertility, osmotic stress response, fungicide resistance, and conidial integrity. NIK-1/OS-1, and at least one other HHK, function upstream of RRG-1. RRG-1 activates the OS-2 MAPK cascade in response to osmotic stress, fungicide treatment, and other stimuli. The predicted site of phosphorylation for RRG-1 is D921. *rrg-1*^{D921N} mutants exhibit phenotypes that differ from those of wild type and the *rrg-1*deletion mutant, in that these strains have delays in conidiation and maturation of female reproductive structures and have slowed apical extension of hyphae. Analysis of N-terminal-truncated versions of RRG-1 will be presented. The results suggest that the N-terminal region of RRG-1 has a specific function during sexual maturation. carol.cjones@gmail.com

POPULATION AND EVOLUTIONARY GENETICS

49 Conflict between *mat*-gene trees and species phylogeny of heterothallic and pseudohomothallic *Neurospora*
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Ten heterothallic and one pseudohomothallic taxa of *Neurospora* were used to estimate the phylogenetic relationships of the mating-type genes: *mat a-1*, *mat A-1*, *mat A-2* and *mat A-3*. We used parsimony heuristic searches (PAUP) and Bayesian analysis, and both methods resulted in similar, well-supported phylograms. The results showed that phylogenies of *mat A-1*, *mat A-2* and *mat A-3* were congruent. Comparison between *mat a* and *mat A* gene phylogenies revealed a conflict wherein the placement of the pseudohomothallic *N. tetrasperma*was incongruent in the different phylogenetic trees. We hypothesize that the origin of this species, and its unique life cycle with constant heterokaryosis for mating-type, may account for this result. In addition, a conflict was observed by comparing phylogenies of different mating-type genes with published species phylogenies derived from non-coding sequences (microsatellite- flanking regions). The placement of *N. crassa* subgroup C (NcC) and Phylogenetic species (PS2) differed between all *mat*-genes and the species tree. The reason for this is uncertain, however, the possibility for horizontal gene transfer or a hybridization event should not be excluded and will be discussed.

50 Multiple shifts in reproductive modes in the evolutionary history of Neurospora and Gelasinospora
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The closely related genera *Neurospora* and *Gelasinospora*cover a diverse set of reproductive modes, including heterothallic, homothallic as well as pseudohomothallic species and they are therefore suitable models for studying the evolution of sex and mating systems. Multiple convergent shifts in reproductive modes make it possible to study their genetic mechanisms and consequences. However, the degree to which such events have happened independently in these lineages is largely unknown and in order to infer convergent evolution it is essential to have a robust phylogeny of the whole group. This has proven to be problematic in this group of fungi.

Our aim is to construct a robust phylogeny containing many members of both genera. We here present a phylogeny based on partial sequences from four genes: β-tubulin, elongation factor 1-α, protein kinase C and 28S rDNA; collected

from 44 taxa representing most species of *Neurospora* and *Gelasinospora*. We use the phylogeny to infer shifts in reproductive modes and test for instance whether selfing (homothallic) lineages form a monophyletic clade. Our data indicate that homothallism evolved from heterothallism in multiple evolutionary events.

51 RFLP analysis reveals more isolates belonging to new Neurospora species
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Recent studies using phylogenetic species recognition (PSR) and biological species recognition (BSR) found three new Neurospora species, phylogenetic species (PS) 1, 2, and 3 (Dettman et al., Evolution 57: 2703-2720, 2003). However, in that study there were too few individuals found for each species to provide a confident formal description. A more comprehensive search of the existing Perkins culture collection was done to find more individuals of each of these species. A method using RFLPs of the markers used for PSR was devised to easily distinguish members of the new species (PS1-3) from the closely related species *N. crassa* and *N. intermedia*. The RFLP analysis was performed on isolates that were either not clearly identifiable by BSR (crosses with tester strains), or were from the same geographic locations as PS1-3. Additional members of all these species were identified: 1 new PS1, 9 new PS2, and 1 new PS3. BSR in and among PS1-3 was investigated by performing intraspecific and interspecific crosses. PS1 and PS2 appear reproductively isolated, successfully mating intraspecifically while not mating successfully with other species of Neurospora. The additional PS3 strain successfully crossed with other PS3 isolates, however, it also successfully crossed with *N. crassa* confirming PSR but not BSR between PS3 and *N. crassa* (Dettman et al., Evolution 57: 2721-2741, 2003). We are confident that PS1-3 are indeed new species, and they will be named *N. hispaniola* (PS1), *N. metzenbergi* (PS2), and *N. perkinsi* (PS3).

52 Evolution and diversity of a nonself recognition locus in Neurospora.
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Among filamentous fungi, hyphal fusion between individuals that differ in allelic specificity at any one of a number of heterokaryon incompatibility (HI) loci called *het*, results in the termination of heterokaryon formation and cell death. Alleles of some *het* genes in *Neurospora*are highly polymorphic and alleles of alternative specificity are equally frequent in populations and show trans-species polymorphisms. At the well studied *het-c, pin-c* HI locus, nonself recognition requires allelic (*het-c*) and non-allelic interactions (between *het-c* and it's flanking gene *pin-c*). The availability of a variety of isolates of *N. crassa, N. discreta,* and *N. tetrasperma* as well as genomic sequence from many species of filamentous fungi allow us ask fundamental questions about the evolution heterokaryon incompatibility, specifically at the *het- c, pin-c* locus. This data allows us to trace the adaptation of *het-c* and *pin-c*from independent ancestral genes, uninvolved in nonself-recognition to a functional HI locus. The comparison of alleles from multiple isolates combined with experimental determination of HI specificity allows us to study the evolution of new alleles. We will also examine the biological consequences of recombination within *het-c, pin-c* locus as well as the flanking DNA. Our analysis will broaden our understanding of the evolution of heteroincompatibility systems in fungi.

53 Circadian clock phenotype as a fitness trait; a test under ecologically relevant conditions
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Neurospora crassa has been a successful eukaryotic model system for studying many biological questions using biochemical, genetic, genomic, and molecular tools. However, most of knowledge that we have on Neurospora biology has been learned through experiments under (controlled) artificial laboratory conditions. I developed an experimental system for *N. crassa* to address how fungal development/behaviors affect fitness under ecologically relevant conditions; natural objects were used as substrates and experimental field conditions were recorded using portable sensors for temperature and light conditions. As a proof of concept for this experimental system, I performed experiments with specific objectives in mind; characterizing the life cycle of *N. crassa* in natural habitats and testing the hypothesis that circadian clock/light-perception phenotypes affect the fitness of an organism. I found that *N. crassa* forms abundant microconidia on *Ostrya virginiana* (Ironwood). On four other species of wood tested in this study, I found different proportions of microconidia/macroconidia. *N. crassa*is a poor competitor in these habitats unless the substrates have been autoclaved. Developmental behaviors, which were not observed on artificial media, were observed in natural objects. For example, sexual spores were released from the perithecia by oozing instead of shooting. I also performed experiments using wild type and a *wc-1* mutant to test the role of circadian clock/light-perception in nature. There were six times more conidia produced in the wild type in comparison to the *wc-1* mutant. We concluded that clock/light-perception plays a significant role in the fitness of *N. crassa* in nature.

54 Circadian clock phenotype as a fitness trait; a test by progressive selections

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Circadian clock behavior is a complex trait; there are multiple genes making contributions to different aspects of clock-controlled fungal behaviors. Studies in several model systems have proposed a crucial role of circadian clock in fitness of an organism. However, there was no systematic study on whether the clock plays a role in fitness for a fungal organism. To gain insights on the roles of circadian clock associated with organism's fitness, we relied on population/quantitative genetics approach using *Neurospora crassa*. First, we generated isogenic population by progressive inter-cross selection (not by mutation), as commonly used in the agricultural traits, e.g. crop yield or milk production. We had a hypothesis that the strains with extreme clock phenotype would show less fitness in comparison to those with ¡°normal¡+ clock phenotype. We focused our study on the circadian period phenotype. We performed four inter-crosses aimed to select for the *N. crassa* strains with an extreme clock periods, we noticed that 1) population mean values are shifted toward the selective direction, 2) population structure become deviant from the normal distribution, and 3) the interval of periods among inter-cross populations are not changing, between 20 and 26 hours in period. Our data suggest that circadian clock period is important phenotype for fitness. The change of the population composition and the implications of the artificial selection will be discussed.

55 Characterization of the *ric8* gene from *Neurospora crassa*

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Heterotrimeric G protein signaling is essential for eukaryotes to sense changes in their environment. The heterotrimer consists of G alpha, G beta, and G gamma subunits, which are bound to G protein coupled receptors (GPCRs). The heterotrimer binds the receptor when G alpha is bound to GDP. Receptor activation causes the G alpha to exchange GTP for GDP, causing dissociation of of G proteins from the receptor. *N. crassa*contains three G alpha (GNA-1,GNA-2,GNA-3) one G beta (GNB-1), one G gamma (GNG-1) and 10 predicted GPCRs. Another regulator of G proteins, RIC8, was found to play a significant role in asymmetrical cell division and synaptic vesicle priming in *C. elegans* and mice. RIC8 is thought to function as a GPCR independent guanine nucleotide exchange factor for G alpha. A homolog of RIC8 in *N. crassa*, RIC8, was identified and has not been characterized in fungi. The *ric8*gene deletion mutant exhibits severe growth inhibition, inappropriate conidiation in submerged culture, and is female sterile, similar

to strains lacking both the *gna-1* and *gna-3* genes. In addition, introducing constitutively activated alleles of *gna-1* and *gna-3* partially rescues the defects of *ric8* mutants. Localization studies using a GFP tagged allele of *ric8*show that RIC8 is cytosolic, and semi-quantitative RT-PCR results show that the gene is expressed highly in conidia. These results suggest that RIC8 may play a role in G alpha activation and cellular pathways regulated by G alpha subunits.

OTHER TOPICS

56 The effects of *prd* mutations on the FRQ-less oscillator (FLO).
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The circadian rhythm of conidiation in *Neurospora* is assumed to be driven by a FRQ/WC-based oscillator. However, strains carrying null mutations at the *frq* locus, such as frq^{10}, can show rhythmic conidiation under some conditions. This rhythmicity is driven by a FRQ-less oscillator (FLO), and we are interested in identifying the components of the FLO. We are using the *chol-1* mutant, which requires choline for normal growth and has a long conidiation period without choline. The double mutant *chol-1* frq^{10} is rhythmic with a long period, indicating that the long period of *chol-1* is driven by the FLO. We introduced the clock mutations *prd-1, prd-2, prd-3* and *prd-4* into this double mutant to look for interactions with FLO. Double mutants *chol-1 prd-1* and *chol-1 prd-2*, and triple mutants *chol-1* frq^{10} *prd-1* and *chol-1* frq^{10} *prd-2*, are arrhythmic without choline. Double mutants *chol-1 prd-3* and *chol-1 prd-4*, and triple mutants *chol-1* frq^{10} *prd-3* and *chol-1* frq^{10} *prd-4*, have long periods that are similar to *chol-1* frq^{10}. In entrainment experiments using temperature pulses at different T-cycles, the triple mutants *chol-1* frq^{10} *prd-1* and *chol-1* frq^{10} *prd-2* failed to entrain while *chol-1* frq^{10} *prd-3* and *chol-1* frq^{10} *prd-4* demonstrated entrainment similar to the *chol-1* frq^{10} strain. These results indicate that *prd-1* and *prd-2* have larger effects on rhythmicity in *chol-1* frq^{10} than *prd- 3* and *prd-4* and may be candidates for components of the FLO.

57 The Role of Coronin in Polarized Growth in *Neurospora crassa*
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Coronin is an actin binding protein that plays a major role regulating actin patches formation. The mutation of the *coronin*gene affects locomotion, growth and cytoskeleton organization. Actin is very important for polarized growth and assisting the Spitzenkörper (Spk). To determine whether the lack of coronin can affect the presence and features of the Spk and the polarized growth, we characterize the *coronin* null mutant in *Neurospora crassa* and compare it with a wild type (wt) strain. The growth rate of *coronin* was around 40% of the rate for wt ($p<0.05$). The Spk size and shape of *coronin*mutant was smaller with a half moon shape. Mitochondria had a spherical shape instead of the wt elongated mitochondria. Hyphal apical growth was affected showing a bumping and zig-zag morphology. Branching rate was 5-folds higher than the wt ($p<0.05$). *Coronin* mutant produced half of the conidia than the wt ($p<0.05$). The biomass production per day was 40 % less than the wt. The mutation of the coronin gene in *N. crassa* is not essential but strongly reduces the growth rate, affects the direction of polarized growth, increased the frequency of branching and defects in septal construction.

58 Characterization of base excision repair mutants in *Neurospora crassa*
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There is no report on the base excision repair (BER) in *Neurospora crassa*. In mutants isolated as mutagen-sensitive mutants, BER-deficient mutants could not be found. From results of genome DNA sequencing and annotation, we identified some BER-homologous genes. In this experiment, 3 homologue genes, *mag-1*, *apn-1*, *apn-2*, were disrupted by homologous gene targeting. And their phenotypes were investigated. They are all sensitive to alkylating agents such as methyl metahnesulfonate (MMS), ethyl methanesulfonate (EMS) and N-methyl N'-nitro N-nitrosoguanidine (MNNG), but not to UV, hydrogen peroxide (H2O2), hydroxylurea (HU). Epistasis analysis showed that *apn-1* and *apn-2* belong to different group. The relationship between *apn-1* and *apn-2* looks similar to that between *mus-18* and *mus-38* in nucleotide excision repair.

59 Redundancy of *vib-1* and its paralog during the sexual cycle reveals additional functions of a heterokaryon incompatibility suppressor gene.

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Neurospora crassa hyphae of genetically identical individuals can undergo fusion during vegetative growth, forming a heterokaryon. However, individuals different at any of 11 heterokaryon (*het*) loci are heterokaryon incompatible; fusion of these strains results in rapid compartmentalization and cell death. *vib-1*is a suppressor of heterokayon incompatibility (HI) and has two paralogs, NCU04729 and NCU09915. All of these genes have homology to the DNA binding domain of *NDT80*, a known meiotic transcription factor in *S. cerevisiae*. Neither of the *vib-1*paralogs suppresses HI, and the NCU04729 knockout has no apparent phenotype. The NCU09915 knockout is female sterile, and trichogyne assays show that the knockout is not defective in trichogyne-microconidium fusion. Interestingly, the female sterility defect in the NCU09915 deletion strain is complemented by the helper strain (FGSC 4564), even in homozygous crosses. These data indicate that NCU09915 is required for perithecial development, but not karyogamy and meiosis. Consistent with these data, Q-RT-PCR showed that NCU09915 is highly upregulated in the perithecium. Surprisingly, sexual development is impaired in homozygous crosses between NCU09915 and *vib-1*double knockouts, and cannot be fully complemented by the helper strain. These data suggest that NCU09915 and VIB-1 have overlapping functions during sexual development.

60 Development of *neurospora* Host Strains with Reduced Codon Bias for Elevated Protein Expression
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The expression of functional proteins in heterologous hosts is a cornerstone of modern biotechnology. Unfortunately, proteins are often difficult to express outside their original host context. They might contain codons that are rarely used in the desired host, come from organisms that use non-canonical code or contain expression-limiting regulatory elements or secondary structure within their coding sequence. Codon optimization often results in expression improvement of poorly expressed genes in *Neurospora*. To test the hypothesis that codon-usage is limiting for heterologous protein expression, nine tRNA genes that are under- represented in *Neuropsora* and may be the source of the observed codon bias in this organism have been identified and were engineered into *Neurospora* strains. The ability to express genes with non-*Neurospora* codon representation in these strains at high levels is currently being examined.

61 Regulation of crossing over near *cog* is a complex process
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There seems to be a crossover hotspot near, but distinct from, the recombination hotspot *cog*. Heterozygous deletion of a 1.8 kb sequence between *his-3* and *cog* reduces crossing over between *his-3* and *ad-3* to about 65% of the normal level, while increasing the adjacent *his-3* to *lys-4* interval to 140%. Homozygous deletion of a 200 bp region, near the 3' end of *his-3*, in which an excess of crossovers was found during analysis of 150 octads, yields a similar but smaller effect (85% and 125% respectively). Interestingly, heterozygous replacement of the 1.8 kb region with a foreign DNA fragment has little or no additional effect on the *his-3* to *ad-3* interval, but invariably prevents the increase in the *his-3* to *lys-4*interval. In addition, homozygous replacement of the 1.8 kb region with a foreign sequence does not necessarily restore normal levels of crossing over, depending on the actual sequence used. In conclusion, although the frequency of recombination initiation near *his-3* is regulated by the alleles of *cog* and *rec-2*(discussed in the presentation by Bowring in session I) in the cross, the frequency and location of resultant crossovers also depends on additional sequences up to 2 kb away. This work was supported by a grant from the Australian Research Council.

62 Strain Improvement for Heterologous Protein Secretion; Exploiting the Power of the FGSC and the Neurospora Genomics Project.

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Neugenesis Corporation is using knock-out strains generated by the NGP to develop improved stains for production of biopharmaceutical proteins. Many monoclonal antibodies and glycoprotein antigens or antigen variants can be expressed and secreted from Neurospora, but are often degraded by proteolytic activities that are co-secreted. New production strains have been generated using KOs generated by the NGP, and morphological mutants from the Fungal Genetics Stock Center. Titers and quality of secreted antibody and Influenza hemaggutinin antigen of have been compared in various production strains . Data will be presented demonstrating dramatic differences in both titer and quality of secreted proteins in these new strains of Neurospora.

63 Relationship between G beta and G alpha Proteins in *Neurospora crassa*.
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Sexual and asexual development of *N. crassa* is regulated by heterotrimeric G-proteins. Three G alpha subunits (GNA-1, GNA-2 and GNA-3), one G beta (GNB-1) and one G gamma (GNG-1) have been identified in this organism. Previous work showed that loss of GNB-1 leads to extremely reduced levels of G alpha subunits in *N. crassa*. In this work, we analyze interactions between GNB-1 and G alpha proteins. Using a genetic approach, delta *gna-1* delta *gnb-1*, delta *gna-2* delta *gnb-1* and delta *gna-3* delta *gnb-1* strains, and strains containing constitutively activated G alpha genes in the delta *gnb-1*background were isolated and their phenotypes analyzed. Using biochemical approaches, Co-IP and pulse-chase assays were used to determine interactions between G protein subunits as well as G alpha turnover in the delta *gnb-1* background. The results support an interaction between GNB-1 and all three G alpha proteins in *N. crassa*.

64 The preparation and use of *Neurospora crassa in vitro* systems for studying translational regulation. Cheng Wu[1], Vladimir Zeenko[2], Kate Moore[2], Ryan Cleland[2] and Matthew Sachs[1]

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Cell-free translation systems recapitulate many important features of *in vivo* translation. They faithfully synthesize polypeptides when programmed with mRNA, therefore enabling biochemical determination of factors and mechanisms contributing to translational processes. For example, co-translational real-time measurement of luciferase enzyme activity arising from translation of a reporter transcript can be used to determine the optimal conditions for *in vitro* translation to study regulatory effects. Primer-extension inhibition (toeprinting) is used to map the positions of ribosomes on mRNA that are at rate-limiting steps of translation. Pulse-chase studies with [^{35}S]Met can track intermediates in polypeptide synthesis as well as providing qualitative and quantitative data on translation efficiency. Here we describe and compare the efficiency of several different approaches to prepare cell-free translation extracts from *N. crassa*, and the use of *N. crassa* extracts to assess the translational effects of several upstream open reading frames present in either *N. crassa* and *Cryptococcus neoformans* mRNAs.

65. Progress at the Fungal Genetics Stock Center: A culture collection for model organisms becomes a model for culture collections.

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The Fungal Genetics Stock Center has been closely connected to its community since it was founded in 1961. That close connection has allowed the FGSC to provide access to materials that were at the forefront of existing technology. First seen as the deposition of genetically marked strains for genetic mapping, this developed into multiply marked strains, populations for RFLP mapping, cloning vectors, gene libraries and ultimately the collection of targeted knockout strains of *Neurospora crassa*. Among microbial culture collections, the FGSC is unique in a number of ways including its close connection with its user community as well as its willingness to embrace new resources. The FGSC has also survived the retirement of two Directors. Many collections are not able to do this and it is the integration in the user community that has enabled the FGSC to do so. As much as the community depends on the resources in the FGSC, the FGSC depends on the user community for its continued success.

66. The Exocyst Components Sec5 & Sec15, Localize at the Apical Membrane in Hyphae of *Neurospora crassa*

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The last stage of the secretory pathway is exocytosis, a process that needs high fidelity protein-protein interactions and the spending of energy to fuse exocytic vesicles to the plasma membrane. The exocyst, first described in secretion termosensitive mutants of *Saccharomyces cerevisiae* (*sec*) is an octameric complex (SEC3, SEC5, SEC6, SEC8, SEC10, SEC15, EXO70 and EXO84) needed for the tethering of secretory vesicles to the plasma membrane, an event previous to the SNAREs assembly. Genome Analysis of *Neurospora crassa* allowed the identification of homologue genes for all components. We tagged with *gfp* two putative exocyst components *sec5* (NCU07698.3) and *sec15* (NCU00117.3), and expressed them in *N. crassa* FGSC #9717 strain (*mus51Δ::Bar;his-3*) deficient in Non-Homologous End Joining. We analyzed by Laser Scanning Confocal Microscopy the localization of SEC5::GFP and SEC15::GFP fusions in living hyphae of *N. crassa*. In both cases the accumulation of fluorescence was found at the very tip of fungal hyphae adjacent to the plasma membrane, just in front of the Spitzenkörper, which correlates with the predicted area of maximum exocytosis. SEC15::GFP occupied a larger area than SEC5::GFP. This work shows for the first time images of two of the eight components of the exocyst labeled with GFP in *N. crassa* as a way to understand secretion. Our results suggest the presence of an Exocyst in *N. crassa* and its possible involvement in the apical secretory process.

67. Regulation of Sporulation by G-protein Signaling Pathways in *Neurospora crassa*

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Neurospora crassa can sense change in the environment through heterotrimeric G-protein signaling. Heterotrimeric G-proteins couple to G protein coupled receptors and consist of Gα, Gβ, and Gγ subunits. Asexual reproduction (conidiation) occurs in response to nutrient deficiency and desiccation. The Gα protein, GNA-3, is involved in the conidiation process. Our lab showed that a *Δgna-3* mutant conidiates inappropriately in submerged culture.

Since conidiation can be a response to starvation, the possibility that GNA-3 might be involved in nutrient sensing was explored. A metabonomics approach was used to create a metabolite fingerprint by utilizing proton-NMR in order to compare amino acid levels and other metabolites that were present in differing levels between wild-type and *Δgna-3* mutant.

Principal Components Analysis showed that loss of *gna-3* does not significantly alter the metabolite fingerprint of *Neurospora crassa*. However, levels of trehalose decreased while glucose increased. Also, asparagine, histidine, and glutamate levels decreased while glutamine level increased, the latter a verification of conidiation. This suggests that GNA-3 may be involved in regulating metabolism and/or nutrient sensing in *Neurospora*.

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Neurospora 2008

Scientific Program

Thursday 27 March

3:00-6:00 p.m.	Registration, Administration	
6:00 p.m.	Dinner, Crocker	
7:00-8:00 p.m.	Metzenberg Memorial. Rowland Davis, Louise Glass, Patrick Shiu **Fred Farr Forum**	
8:00-10:00 p.m.	Mixer **Fred Farr Forum**	

Friday 28 March

7:30-8:30 a.m.	Breakfast, Crocker	
8:30-12:05	**Session I: Gene Silencing, Sex, Repair and Recombination.** Chair: David Catcheside	Chapel
8:30	Welcome and announcements	
8:35	Patrick Shiu **Beadle and Tatum Award Lecture**	Genetic dissection of Meiotic Silencing by Unpaired DNA
9:15	Mary Anne Nelson	Sexual development in *Neurospora crassa*: Elusive recessive mutations

9:40	Louise Glass	Getting connected: Formation and function of the hyphal network
10:05	Coffee break	
10:20	Eric Selker	Recent advances understanding the control of DNA methylation
10:45	Michael Freitag	Centromeres in filamentous fungi
11:05	Fred Bowring	Region specific regulation of recombination
11:25	Rodolfo Aramayo	Meiotic silencing in Neurospora
11:45	Hirokazu Inoue	DNA repair and cell death
12:05-2:00	Lunch, Crocker	Business meeting, presentation of Perkins and Dodge Awards
2:30-3:10	Jay Dunlap **Metzenberg Award Lecture**	Changing Times
3:10-5:50 p.m.	**Session II: Clocks and Light** Chair: Yi Liu	Chapel
3:15	Deb Bell Pedersen	Circadian output pathways in Neurospora
3:35	Michael Brunner	Functional characterization of the White Collar Complex
3:55	Jennifer Loros	Mechanism of the Neurospora circadian system
4:15	Coffee break	
4:30	Luis Corrochano	Neurospora mutants altered in their regulation of transcription by light
4:50	Kwangwon Lee	Does circadian clock matter for *Neurospora crassa* in nature?
5:10	Benedetto Grimaldi	White Collar Complex: acetylation and evolution

5:30	Patricia Lakin-Thomas	The effects of *prd* mutations on the FRQ-less oscillator (FLO)
6:00-7:00	Dinner, Crocker	
7:00-10:00	**Poster session Fred Farr Forum**	Presenters should be in front of their posters: odd numbers 7-8pm, even numbers 8-9 pm

Saturday 29 March

7:30-8:30	Breakfast, Crocker	
8:30-12:05	**Session III: Cell Growth, Morphogenesis and Populations** Chair: Nora Plesofsky	
8:30	John Taylor	Neurospora as a model for evolutionary biology
8:55	Barry Bowman	Where is calcium sequestered within cells, and how does it get there
9:20	Michael Plamann	Cytoplasmic dynein
9:45	Stephan Seiler	Regulation of polar tip extension by COT1 and Rho-type GTPases
10:10	Coffee break	
10:25	Frank Nargang	Mitochondrial biogenesis
10:50	Nick Read	Conidial sex tubes and phytochrome signalling
11:15	Alberto Rivetta	Genetic and functional characterization of three K+ transport systems in Neurospora
11:40	Stephen Free	Identification and partial characterization of Neurospora anastomosis mutants
12:05-2:30	Lunch, Crocker	

2:30-5:55	**Session IV Gene Regulation and Cell Signaling** Chair: Oded Yarden	
2:30	Carol Jones	Investigation of functional domains of RRG-1, a response regulator that regulates the OS-2 MAPK cascade
2:55	Matt Sachs	Post-transcriptional control of gene expression in Neurospora
3:20	Heng-Chi Lee	Regulation of the RNAi pathway in Neurospora
3:45	Alejandro Fernández Estrada	The *ylo-1* gene encodes an aldehyde dehydrogenase responsible for the last oxidative reaction in the Neurospora carotenoid pathway
4:10	Coffee break	
4:25	Robert P. Smith	Trans-species activity of the *Neurospora crassa* ribonucleotide reductase incompatibility domain in *Saccharomyces cerevisiae*
4:50	Jack Kennell	Plasmid-induced senescence in *Neurospora crassa* triggers mitochondrial-nuclear communication
5:15	Andrew Keeping	Characterization of the Neurospora VS ribozyme *in vivo*
5:35	Fabio Squina	New features over the expression of Neurospora crassa genes in response to extracellular phosphate levels and pH
6:00-7:30	**Banquet**, Crocker Chair, Oded Yarden	Presentation of the Beadle and Tatum Award
	Banquet Speech: Dorsey Stuart	Neurospora in the Corporate World; An overnight success story
7:30-10:30	**Poster session Fred Farr Forum**	

Sunday 30 March

7:30-8:30	Breakfast, Crocker	
8:30-11:50	**Session V: Genomics and Methodology** Chair: Jay Dunlap	Chapel
8:30-10:10	<u>Neurospora Functional Genomic projects</u>	
8:30	Jay Dunlap	Overview
8:40	Kathy Borkovich and Gloria Turner	Knockouts
9:10	James Galagan	Fungal Genomics
9:30	Louise Glass and Jeff Townsend	Microarray Analyses
9:50	Jennifer Loros, Meray Basturkmen	cDNA libraries and SNPs
10:10	Coffee Break	
10:25	Matt Sachs, Heather Hood	Annotation
10:45	Van Gooch	Neurospora Lights Up!
11:05	Ernestina Castro-Longoria	Expression of the red fluorescent protein mCherry During the circadian rhythm of *Neurospora crassa*
11:25	Christina Chung	Development of Neurospora host strains with reduced codon bias for elevated protein expression
12:00	Lunch and Checkout	

Summary of the four different Neurospora Awards

1. METZENBERG AWARD

a. Criteria: This award was established in 2004 by the Neurospora community to recognize the contributions of Dr. Robert Metzenberg to science, and to promote the work of others in the spirit of Metzenberg. The award will be given to a Neurospora worker, at any stage of career development, whose innovative achievements/contributions have significantly advanced our understanding of biology (Neurospora and beyond). The critical work would preferably, but not necessarily, have been done within the previous four years.

b. Venue of award: The award is given to one researcher every two to four years at the International Fungal Genetics Meeting.

c. Nature of prize: Memorabilium plus up to $1000 towards travel/meeting expenses if required. The awardee will be invited to give a special lecture at the following Neurospora meeting.

d. Nominations: For the first year, nominations will come from the NPC. Subsequently, nominations will be solicited from the community. Candidates will require one nomination letter and a CV. These will be sent to the chair of the NPC and appropriate candidate applications will be forwarded to the selection committee. All materials will need to be submitted to the NPC by Dec 1.

d. Selection committee: Eight prominent scientists, including an equal number of Neurospora and non-Neurospora researchers. One Neurospora member will include the chair of the NPC. Selection committee members will be chosen every 4 years by the NPC. One goal is to have several members serve for at least two consecutive terms. Nominations will be given to the selection committee by the NPC by Jan 1. A decision will be required by Feb 1 for announcement in the FG program.

e. Frequency: The Award will be made every 2 – 4 year at the discretion of the NPC

f. Past recipients:

2005: Jay Dunlap
2009: Hirokazu Inoue

2. BEADLE AND TATUM AWARD

a. Criteria: This award was established in 2002 by the community of Neurospora scientists in recognition of outstanding and original research using Neurospora as a model organism. This award is intended for young investigators whereby the award will help to advance their careers and to give more visibility to Neurospora as a research organism.

b. Venue of the award: The award is given to one researcher every two years at the Neurospora conference in Asilomar. The recipient is asked to give the Beadle and Tatum award lecture at the meeting.

c. Nature of the prize: Reproductions of four original drawings prepared by George Beadle are given to the recipient. The originals are kept at FGSC.

d. Nominations: These are solicited from the community using the Neurospora e-newsletter. Nominations should include a letter of nomination and a CV of the candidate and be sent to the NPC chair by Dec 1. The NPC will review the nominations and forward these to the Selection Committee by Jan1.

e. Selection Committee: A group of up to 8 distinguished scientists review the nominations forwarded by the NPC and select the recipient by Feb 1. This committee is arranged by the NPC and includes one member of the NPC. The awardee will be notified prior to the meeting.

f. Past recipients:

2002: Mike Plamann
2004: Yi Liu
2006: Carlo Cogoni
2008: Patrick Shiu
2010: Stephan Seiler
2012: Greg Jedd
2014: Michael Freitag
2016: Hanna Johannesson

3. PERKINS FUND AWARD

a. Criteria: This award was established in honor of our colleague, Dr. David Perkins, to support outstanding students and postdocs attending the Neurospora and Fungal Genetics Conferences.

b. Venue of the award: The award will be presented at either the Fungal Genetics Conference or the Neurospora Conference, preferably at the Neurospora conference banquet or at the Neurospora business lunch at the Fungal Genetics Conference. Each awardee will be asked to present their research findings at the meeting.

c. Nature of the prize: Awards of up to $400 to one graduate student and one postdoctoral scientist each year.

d. Nominations: Nominations are solicited from the Neurospora community via the Neurospora e-newsletter. Nominations for Perkins Fund Awardees should be made in the form of a one-page letter from the candidate's research advisor. This letter should include a 200 word or less description of why the individual's work is exceptional. Up to two re-prints or pre-prints of published research may also be included with the nomination. Nominations need to be received by Dec 1.

e. Selection Committee: Evaluation of the nominations are overseen by the Neurospora Policy Committee. Award decisions will be made by Feb 1. The awardees will be notified prior to the meeting.

f. Contributions to the Perkins fund may be made via the Genetics Society of America or via the FGSC e-commerce page (Please use "Perkins Award" as the invoice number).

g. History of Neurospora Awards from the David D. Perkins Scholarship Fund.

2016 Brad Bartholomai	**2015**	**2014** Jonathan Galazka, Andrew Klocko
2013 Eddy Sánchez-León	**2012** Chris Ellison and Charles Hall	**2011** Abby Leeder Alexander Lichius
2010 Shinji Honda Renato DePaula	**2009** Bill Alexander Keyur Adhvaryu Kristina Smith	**2008** Heng-Chi Lee Carol Jones
2007 Carmit Ziv	**2006** Fernanda Freitas Elizabeth Turner	**2005** Michael Vitalini Hyojeong Kim
2004 Carolyn Rasmussen	**2003** Jeremy Dettman	**2002** Qijun Xiang Rashmi Krishnapuram
2001 Stephen Seiler Alka Pandit Ann Kays Jennifer Bieszke	**1999** Frederick Bowring Patrick Shiu Jennifer Wu	**1997** Carlo Cogoni Stacie Schwartz Kelly Howe

4.THE B. O. DODGE COMMUNITY AWARDS

a Criteria. These awards are made in recognition of the fact that a significant part of the success of Neurospora as a model research organism is due to the collaborative nature of its research community. Certain individuals who have made exceptional contributions towards community will be acknowledged by the awards.

b Venue: At each Neurospora meeting, two awards will be made to individuals of any rank who have made sustained exceptional contributions to the community.

c Nature of the Award: Recipients will be presented with an appropriate trophy.

d Nominations: Nominations will be solicited in the E-news before each Neurospora meeting,

e Selection: The two winners will be selected by the Neurospora Policy Committee.

f. Past Recipients

2006 – Gloria Turner and Dick Weiss

2008 – Rowland Davis and Namburi Raju

2010 – Mary Case, Katherine Borkovich and Kevin McCluskey

A brief history of the Neurospora Conference

The first Neurospora Information Conference was held in La Jolla, California, March 2-4 1961. This meeting was attended by 93 scientists primarily from the US and Canada. One scientist came from England. Sessions were held on Cytology, Techniques, Recombination, Complementation, Genetic Fine Structure, Reversion, and Genetic Interaction in Enzyme Synthesis. Eleven more conferences were held at varying intervals.

In 1986 the meeting became the first Fungal Genetics Conference. It was held in Columbia, South Carolina, and there were 140 attendees from 9 countries. This meeting grew with the growth in research on filamentous fungi and now boasts over 700 attendees from all over the world. To reclaim the informal, focused atmosphere of the original Neurospora Information Conferences, the Neurospora Policy Committee has re-launched the Neurospora meetings with great success. The first new Neurospora conference in 1998 included over 175 scientists from 8 countries. The 5th new Neurospora Conference included scientists from 12 countries.

Past organizers

2006 Oded Yarden, Nora Plesofsky
2004 Anthony Griffiths, Deborah Bell-Pedersen
2002 Gloria Turner, Barry Bowman
2000 Dan Ebbole, Eric Selker
1998 Mike Plamann, Mary Anne Nelson
Kathy Borkovich, Matt Sachs

Banquet speakers

1998 Jay Dunlap, Dartmouth
2002 Rowland Davis, University of California, Irvine
2004 Stuart Brody, University of California, San Diego
2006 Guiseppe Macino, Università di Roma "La Sapienza"