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## Abstracts from the Neurospora 2006 Conference


Neurospora Conference



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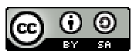

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# Abstracts from the Neurospora 2006 Conference


## Abstract

Plenary and poster session abstracts from the Neurospora 2006 Conference


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# NEUROSPORA 2006 PLENARY SESSION ABSTRACTS

## Session I: From Genes to Populations

Tony Griffiths, Chair

### Control of DNA Methylation in Neurospora

Eric Selker, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403-1229

Most methylated regions of Neurospora are products of RIP (repeat-induced point mutation), a premeiotic homology-based genome defense system that litters duplicated sequences with C:G to T:A mutations and typically leaves them methylated at remaining cytosines. I will present our current understanding about how A:T-rich DNA, such as that resulting from RIP, triggers methylation. Our efforts to elucidate the control of DNA methylation in vegetative cells have revealed mechanistic ties between modifications of DNA and histones. The DIM-2 DNA methyltransferase is directed by heterochromatin protein 1 (HP1), which in turn recognizes trimethyl-lysine 9 on histone H3, placed by the DIM-5 histone H3 methyltransferase. Results of *in vitro* and *in vivo* studies indicate that DIM-5 recognizes at least residues 8-12 of histone H3 and is sensitive to methylation of lysine 4 and phosphorylation of serine 10 in histone H3, supporting our suggestion that histones serve to integrate diverse signals to control DNA methylation. Additional support for this notion comes from our studies on mutants defective in other histone modification enzymes. DNA methylation and HP1 localization do not depend on RNAi machinery in Neurospora but do depend, in part, on deacetylation and dephosphorylation of histones. Conversely, DNA methylation can lead to deacetylation of histones, which may aid in propagation of DNA methylation and the associated silenced chromatin state.

### Structural studies of protein (histone) methylation

Xiaodong Cheng. Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia 30322;

There is a rapidly growing appreciation that the study of covalent modification to proteins and transcriptional regulation will likely dominate the research headlines in the next decade. Protein (de)methylation plays a central role in both of these fields, as several different residues (Arg, Lys) are methylated in cells and methylation plays a central role in the "histone code" that regulates chromatin structure, impacts transcription, and responds to DNA damage. In some cases, a single lysine can be mono-, di-, or trimethylated, with different functional consequences for each of the three forms. I will review structural aspects of methylation of histone lysine residues by two enzyme families with entirely different structural scaffolding (the SET proteins and Dot1p) and methylation of protein arginine residues by PRMTs, and discuss, somewhat speculatively, their mechanisms.

**Meiotic Recombination initiated by the *cog* hotspot in Neurospora.**

Frederick Bowring, Jane Yeadon, Hui-Yin Lee, Sue Conway and David Catcheside. School of Biological Sciences, Flinders University, PO Box 2100, Adelaide, SA 5001. Australia.

Meiotic recombination in *Neurospora crassa* is initiated at hotspots regulated by transacting genes. Our current focus is on recombination initiated by the hotspot *cog,* occurring within the *his-3* locus and in flanking regions stretching proximally to *lys-4* and distally to *ad-3.* We have analysed recombination by measuring the frequency of $His^+$ progeny from crosses heterozygous for auxotrophic *his-3* alleles, and by following segregation in octads heterozygous for *lys-4, his-3, ad-3, cot-1, am,* snp markers between *his-3* and *cog* and additional snps both proximal and distal of *his-3.* Octad analysis revealed additional hotspots that, like *cog,* appear to be particularly active in initiating recombination, and showed that all reciprocal exchanges whose location could be determined also experienced conversion nearby. We have constructed knockouts of *spo11, msh-2, msh4* and *ku70*. Each mutant shows disturbance of chromosome behavior during meiosis. Although chromosome pairing is severely affected in crosses homozygous *spo11* and aneuploidy rife amongst the progeny, we were surprised to find recombination events initiated by the *cog* hotspot are somewhat elevated over normal amongst those infrequent spores that are sufficiently genetically balanced to be viable. One interpretation of these data is that Neurospora is able to initiate recombination, at least at *cog*, by mechanisms other than *Spo11*-induced double strand breaks.

**DNA repair and genomic instability in Neurospora**

Hirokazu Inoue (Saitama University, Saitama, Japan)

Mutants which show high sensitivity to mutagen(s) have been isolated and characterized in Neurospora. Based on spectra of mutagen sensitivity and epistatic relationship, they were classified into 5 groups; excision repair, recombination repair, post replication repair, damage-checkpoint and mismatch repair. Some of those mutants have mutator phenotype and/or growth defect phenotype. Majority of spontaneous mutation is generated in replication process. Recently 2 different *recQ* genes encoding proteins with 3’-5’ helicase motif were identified in Neurospora. A double *recQ* mutant showed genomic instability. Mutator or growth defect phenotype of a *recQ* double mutant was suppressed by mutation of either of nonhomologous end joining (NHEJ) or homologous recombination (HR), respectively. Roles of HR and NHEJ in double-strand breaks repair, gene targeting and spontaneous mutagenesis are discussed.

**Neurospora comparative biology is enabled by phylogenetics and species recognition.**

John Taylor[1], Dave Jacobson[1,2], Elizabeth Turner[1], Luz Beatrice Gilbert,[1] Jeremy Dettman[3]. [1]UC, Berkeley, [2]Stanford U. [3]U. Toronto, Mississauga.

Comparative biology depends on accuracy in recognition of genetically isolated groups of organisms, in analysis of relationships among these groups, and in dating of evolutionary events that create modern groups. In Neurospora, outbreeding species form a single evolutionary lineage of 15 phylogenetic species in two lineages, biological and phylogenetic species recognition are nearly equivalent, and genetic isolation precedes reproductive isolation. Recognition and comparison of Neurospora species is unmatched in its scope in fungi and represents a tool to enable careful comparative biology. Research using this tool began with an

examination of the evolution of microsatellites. Current projects include: 1) Phylogenomics, where outbreeding Neurospora are providing a means of testing the claims made for this approach in studies of yeast. 2) Evolution of reproductive isolation, where previous knowledge of phylogenetic relationships and reproductive isolation among clades facilitated the choice of strains for QTL analysis of reproductive isolation in hybrid matings. 3) Intraspecific or intraclade variation, where studies of variation in fecundity and phenotypes associated with biological clocks or reproduction are being used to dissect genetic control of variable phenotype.

**Evolutionary genetics of reproductive isolation barriers separating *Neurospora crassa* and *N. intermedia.***

Elizabeth Turner & John W. Taylor, Plant & Microbial Biology, University of California, Berkeley, CA 94720

Reproductive isolation (RI) barriers between different pairs of *N. crassa* and *N. intermedia* strains range from mild (reduced numbers of viable progeny) to severe (failure to develop perithecia). For the *N. crassa* NcC clade, which is endemic in southern India, the severity of RI from *N. intermedia* is biogeographically structured: crosses to sympatric *N. intermedia* strains show more severe barriers. This pattern is consistent with reinforcement, the evolution of stronger RI barriers by natural selection against hybridization. The potential fitness advantage of the putative reinforcement barrier, the early abortion of hybrid perithecia, was demonstrated in experiments showing that early abortion can dramatically increase the overall fecundity of NcC females when they have additional opportunities to mate with conspecific males. We are undertaking quantitative trait locus (QTL) mapping to study the evolutionary genetics of RI barriers separating *N. crassa* and *N. intermedia* using a mapping population derived from a cross between strains of the *N. crassa* NcA and NcC clades. We have identified loci that are significantly associated with the strength of RI in mating assays between these $f_1$ hybrids and *N. intermedia* tester strains from different geographic regions, including a QTL on linkage group VI responsible for about 30% of the variance in development of hybrid perithecia fertilized by *N. intermedia* sympatric to NcC.

**Artificial selection for ascospore size in *Neurospora crassa***

Heather H. Wilkinson, Department of Plant Pathology, Program for the Biology of Filamentous Fungi, Texas A&M University.

We are interested in ecological and evolutionary genetic bases for life-history trait variation in natural *Neurospora crassa* populations. Studies thus far have focused on breeding well-characterized isolates from a Louisiana sugar cane field and discerning the patterns of heritability associated with variation in a wide variety of developmental phenotypes. As a test of concept in the present study, crosses that yielded the smallest or the largest ascospores in that F1 population were used to artificially select, in both directions, for ascospore size. In total, selection on both large and small ascospores lineages has been carried out to the F4 generation. We are exploring 1) the degree to which evolution of the mean of one trait influences the evolution of the mean of another (e.g. ascospore shape); and 2) the degree to which selection on the mean of a trait influences the shape of the distribution around the mean (e.g. standard deviation, skewness, kurtosis). The implications to the functional ecology of a trait will be discussed.

**Workshop: How to utilize new tools and resources for Neurospora developed in the Program Project**

Jay Dunlap, Organizer

**Knockouts Workshop**
Hildur Colot and Patrick Collopy.

This workshop will present protocols and guidelines for making your own knockout strains in *Neurospora*. In order to achieve high-throughput production of knockout strains as part of the Program Project, we have created novel procedures and software tools, as well as adapting, simplifying and streamlining existing techniques.
We will briefly describe the overall scheme and then elaborate on certain details, including primer design, yeast recombinational cloning for assembling the deletion cassettes, the use of magnetic beads for isolation of yeast DNA, the creation of *mus-51* and *mus-52* strains, 96-well electroporation into *Neurospora*, a modified transformation medium, mini-slants for spot-testing, the use of magnetic beads for *Neurospora* DNA preps, and a custom-written application for designing Southern blots.
We have performed significant portions of the work on a pipetting robot. However, the protocols were first developed manually and can be done without the robot with small numbers of samples or in a 96-well format. We will provide protocols for performing all the procedures without the need for any specialized equipment, along with information on more expensive options suitable for high throughput.
Finally, we will give a brief tour of the relevant web-based resources, including lists of primers used, lists of knockout strains completed and in progress (including access to the LIMS we use to track our work), updated protocols, and the programs for both primer design and Southern design. The web sites include:

http://www.dartmouth.edu/~neurosporagenome/primers.html
http://www.dartmouth.edu/~neurosporagenome/protocols.html
http://www.dartmouth.edu/~neurosporagenome/knockouts_completed.html
http://borkovichlims.ucr.edu/php/sLIMS.php
http://borkovichlims.ucr.edu/southern/
http://borkovichlims.ucr.edu/primer/primerDesign.py

**Introduction to Neurospora Microarray Methods**
Jeffrey Townsend, Takao Kasuga, Baikang Pei

The detection of large and small yet statistically significant differences in gene expression in spotted DNA microarray studies is an ongoing challenge. We will begin with discussion of experimental protocols that are designed for investigations of differential gene expression using resources available in the Neurospora community. We have recently developed Neurospora microarrays comprising predicted 10,526 Neurospora genes and made them publicly available through FGSC. Since the release of the arrays and accompanying protocols at
http://web.uconn.edu/townsend/Links/ffdatabase/downloads.html
we have received feedback from the Neurospora community. We will discuss growth conditions,

RNA extraction, cDNA synthesis, hybridization and acquisition of microarray data. We will provide protocols, alternative methods and troubleshooting methodology. From there we will explore ways to design experiments. Multifactorial experimental designs using DNA microarrays are becoming increasingly common. We will highlight experimental design criteria that will maximize inferential and statistical power. In experimental design, opportunities for transitive inference should be exploited, while always ensuring that comparisons of greatest interest comprise direct hybridizations. We will briefly overview productive methods for analysis for completed datasets, including Bayesian (BAGEL) and ANOVA methods. Understanding the difference in gene expression that is detectable as significant is a vital component of experimental design and evaluation. The gene expression level at which there is an empirical 50% probability of a significant call is presented as a summary statistic for the power to detect small differences in gene expression. Lastly, we will provide an introduction to a filamentous fungal microarray database in construction where data may be deposited, examined, and analyzed. http://web.uconn.edu/townsend/Links/ffdatabase/introduction.htm

## Session II: Genomics and Program Project Report

Kathy Borkovich, Chair

### Enabling a community to dissect an organism: Functional analysis of *Neurospora* as a model filamentous fungus

Jay Dunlap[1], Hildur Colot[1], Kathy Borkovich[2], Gloria Turner[3], Dick Weiss[3], Mike Plamann[4], Bruce Birren[5], Matt Sachs[6], Louise Glass[7], Jeffrey Townsend[9], Mary Anne Nelson[8], Jennifer Loros[1] [1]Dept. Genetics, Dartmouth Medical School, Hanover, NH 03755 [2]Dept. Plant Pathology, UC Riverside, Riverside, CA [3]Dept. Microbiology, UCLA, Los Angeles, CA [4]Dept. Biology, Univ. Missouri, Kansas City, MO [5] MIT Center for Genome Research, Cambridge, MA [6] Oregon Health Sciences University, Portland, OR [7] Dept. Plant and Microbial Biology, UC Berkeley, Berkeley, CA [8] Dept. Biology, Univ New Mexico, Albuquerque, NM [9] Dept. Molec. Cell. Biology, Univ. of Connecticut, Storrs, CT

The overall goal of the four interdependent projects in this Program Project is to carry out functional genomics, annotation, and expression analyses of Neurospora crassa, the filamentous fungus that has become a model for the assemblage of over 250,000 species of non-yeast fungi. The timeline for this effort envisioned periodic repots to the community and public assessment of progress towards our goals and benchmarks. Building from the completely sequenced 43 Mb Neurospora genome the first Project is pursuing the systematic disruption of genes through targeted gene replacements, preliminary phenotyping of these strains, and their distribution to the scientific community at large. Project #2, through a primary focus in Annotation and Genomics, has developed a platform for electronically capturing community feedback and data about the existing annotation, while building and maintaining a database to capture and display information about phenotypes. Oligonucleotide-based microarrays created in Project #3 will be used to collect baseline expression data the nearly 11,000 distinguishable transcripts in Neurospora under various conditions of growth and development, and eventually to begin to analyze the global effects of loss of novel genes in strains created by Project #1. cDNA libraries

generated in Project #4 will illuminate alternative splicing, alternative promoters, antisense transcripts and help to document the overall complexity of expressed sequences in Neurospora, as well as driving the assembly of a SNP map.

**Genome informatics and Neurospora crassa functional studies**

Matthew R. Henn[1], Dave DeCaprio[1], Heather M. Hood[2], Steve Rounsley[1], Matthew Crawford[1], Phil Montgomery[1], Gloria E. Turner[3], Chad Nusbaum[1], Matthew S. Sachs[2], James E. Galagan[1], Bruce W. Birren[1]. [1]Broad Institute of MIT & Harvard, Cambridge, MA 02141, [2]Oregon Health & Science University, Beaverton, OR 97006, [3]Department of Chemistry and Biochemistry, University of California, Los Angeles California 90095

The next challenges for understanding the *Neurospora crassa* genome sequence are to refine the genome annotation and to actively capture, improve, and integrate with the sequence the wealth of information that exists within the research community. To this end, the Broad Institute as part of the NIH Program Project, "Functional Analysis of a Model Filamentous Fungus," has constructed community annotation and phenotype ontology infrastructures that for the first time provide the research community the ability to link information about genetic features with the Neurospora genome, to refine gene structures, and to curate all entries in a searchable database. To increase the accuracy of the gene model, gene calling using EST-based algorithms was also implemented. To maximize the value of EST sequencing, the Broad's Neighborhood Quality Score algorithm was adapted to work with fungal EST sequences and we have begun comparing Mauriceville strain cDNAs with the Oak Ridge strain genomic sequence to identify single nucleotide polymorphisms (SNPs) for genetic mapping. The Broad Institute is also improving the quality of the *N. crassa* genome for subsequent release. One barrier to finishing the genome is regions recalcitrant to cloning. Initial results from pyrosequencing using 454 technology has improved coverage of uncaptured regions. In addition, an optical map, which represents a restriction map of the entire genome, has helped anchor significant portions of the genome leading to better representation of complete chromosomes.

**Addition by subtraction: Novel insights into *Neurospora* biology obtained from transcription factor knockouts**

Gyungsoon Park. University of California, Riverside.

We have developed a high-throughput method for creating *Neurospora* knockout mutants. In this procedure, yeast recombinational cloning is used to create constructs that are then transformed into *Neurospora* strains deficient in nonhomologous end-joining DNA repair (*mus-51* and *mus-52* mutants). Here we present the results of our initial application of the procedure, with mutational analysis of 103 transcription factor-encoding genes. The methodology is robust, with a >90% success rate for producing the desired knockout mutant. The resulting mutants were screened for a variety of phenotypes and 43% exhibited discernible defects. The genes producing phenotypes are variously involved in growth of basal hyphae (25 genes), aerial hyphae height and/or macroconidiation (27 genes), and differentiation of protoperithecia or perithecia (15 genes). This analysis demonstrated roles for many uncharacterized transcription factors and also revealed novel functions for genes that had been previously studied. Several transcription factors are required for than one aspect of growth or development, suggesting roles in integration of multiple upstream signals. The observation that half of the genes did not produce obvious defects


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when mutated may result from functional redundancy, which has been reported for other transcription factor genes. The availability of this collection of *Neurospora* transcription factor mutants will enable future investigations aimed at elucidating the complexity of gene regulation in filamentous fungi.

**A High-Density SNP Map for *Neurospora crassa.***

Randy Lambreghts[1], Mi Shi[1], David DeCaprio[2], James E. Galagan[2], Bruce W. Birren[2], Jay C. Dunlap[1], and Jennifer J. Loros[1]. [1]Department of Genetics, Dartmouth Medical School, Hanover, NH 03755; [2] Broad Institute, Cambridge, MA 02141

A collection of SNPs (single nucleotide polymorphisms) in the Mauriceville strain of *Neurospora crassa*, relative to the Oak Ridge standard, was established, randomly distributed over the entire genome with an average density of one every 100 kb. To this end, an EST library was constructed from Mauriceville germinating conidia and sequenced. By alignment with the published Oak Ridge genomic sequence, a set of putative SNPs was formulated, out of which a subset was selected based on quality of the sequencing information as well as amenability to CAPS (cleaved amplified polymorphic sequence), some of which were experimentally validated by PCR amplification of genomic DNA followed by differential restriction digest. We present a map containing confirmed SNPs, each one supplemented by a primer pair and a restriction enzyme that has been shown to consistently distinguish between the Oak Ridge and Mauriceville version. Given the possibility of high-throughput assignment of a large number of SNP markers to individual progeny, we expect this information to be invaluable for the rapid mapping of conventionally derived mutations, as well as in the further assembly and orientation of the genomic sequence and in the analysis of complex traits such as QTLs.

**Exploring regulatory networks in Neurospora**

Chaoguang Tian, Takao Kasuga and N. Louise Glass
Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720

Exploring regulatory networks is one of the main functions of post-genomics research. As part of the Neurospora Functional Genomics Program Project grant, we have constructed full genome oligonucleotide microarrays for the filamentous fungus *Neurospora crassa* using gene annotation provided by The Broad Institute and Munich Information for Protein Sequences (MIPS). Our goal is to define transcriptional regulatory networks in *N. crassa*, as a model organism for filamentous fungi. By the genome-wide microarrays, we can define transcription factor targets by profiling transcription factor mutant strains. There are at least five predicted DNA-binding transcription factors families in *N. crassa*: BHLH, BZIP, C2H2, GATA and ZnII(Cys)6. Using phylogenetic analyses of the transcription factor gene families within ascomycete fungi, we have chosen to profile mutants in transcription factor genes that are phylogenetically diverse, plus their closest paralogs; the vast majority of these transcription factors and their regulatory networks are completely uncharacterized. Thirty-five transcription factor mutant strains (generated by the Neurospora Functional Genomics Program Project Grant) have been initially selected for transcriptional profiling. We compare the gene expression profiles of transcription factor knock out strains to wild type across a fungal colony and under different stress conditions. Putative target genes of the transcription factors are subsequently subjected to cis-element analysis. By performing comprehensive transcriptional profiling, cis-element analysis and

chromatin immunoprecipitation, the transcription regulatory network of a model filamentous fungus can be constructed.

## Session III: Cell Morphogenesis and Assembly

Oded Yarden,Chair

**Functio Variabilis: Unravelling the diverse roles of ion transport in hyphal morphogenesis.**

Roger R. Lew Biology Department, York University, Toronto, Canada. planters@yorku.ca

During tip growth (hyphal extension), Neurospora relies upon an internally generated $Ca^{2+}$ gradient to maintain polar growth of hyphae. The tip-high $Ca^{2+}$ gradient is created by the action of a stretch-activated phospholipase C which produces inositol trisphosphate ($IP_3$) that then activates a $Ca^{2+}$ channel to cause $Ca^{2+}$ release at the tip. $Ca^{2+}$ sequestration behind the tip occurs into endoplasmic reticulum and into a unique population of tip-localized mitochondria. As a walled cell, Neurospora usually relies upon hydrostatic pressure (turgor) to cause tip extension. Upon hyperosmotic stress, an ensemble of ion transporters (the $H^+$ ATPase, and $K^+$ and $Cl^-$ transporters) are activated by a MAP kinase cascade to maintain turgor at about 500 kiloPascals. Besides turgor, there are intrahyphal osmotic differences that cause mass flow of cytoplasm which is normally directed towards the growing tips. Thus, ion transport at a number of organelles plays key roles in multiple functions during hyphal growth: $Ca^{2+}$ gradients mediate tip polarity, trans plasma membrane ion gradients generate the turgor driving force, and intrahyphal ionic gradients mediate cytoplasm migration toward the growing tips.

**How is calcium sequestered and does vacuolar calcium play a role in morphogenesis?**

Barry Bowman

See poster # 10

**The cellular and genetic determination of Woronin body formation in apical hyphal compartments of *Neurospora crassa***

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

Woronin bodies are peroxisome-derived organelles that are centered on a crystalline core of the HEX-1 protein and function as emergency patches of the septal pore. To fully exploit this function, all hyphal compartments need to inherit Woronin bodies and my group has been studying the cellular and genetic control of this process. Time-lapse confocal microscopy has shown that Woronin bodies form in the apical hyphal compartment where they are generally transported in an apically directed manner. These vesicles undergo maturation entailing membrane fission and associate with the cell cortex roughly coinciding with septum formation. Cortical Woronin bodies are immobilized and thereby retained in sub-apical compartments, and by the continuous execution of this process all vegetative compartments are endowed with a


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roughly uniform number of Woronin bodies. To investigate the genetic control of this process, we determined the requirements for hex-1 gene expression and defined an important role for intron splicing in hex1 mRNA export or stability. We next examined the localization of YFP expressed from hex-1 regulatory sequences and observed a tip-high fluorescent gradient that diminishes towards sub-apical compartments. To directly assess the spatial distribution of various species of mRNA, we developed a method to fractionate the fungal colony into a series of zones corresponding to apical and increasingly sub-apical compartments. Examining RNA from these regions we found that hex-1 mRNA is highly enriched in apical hyphal compartments, whereas other transcripts accumulated in sub-apical compartments. When the hex-1 structural gene was expressed from regulatory sequences of an abundant, sub-apically localized transcript, Woronin body formation was re-determined to this region of the colony. Together, these results define the genetic differentiation of apical hyphal compartments and show that polarized gene expression is a key determinant of apically localized Woronin body-genesis[1].

[1]Tey, W.K., North, A.J., Reyes, J.L., Lu, Y.F., Jedd, G. (2005) Polarized gene expression determines Woronin body formation at the leading edge of the fungal colony. Mol. Biol. Cell. 16, 2651-2659.

**Glycosphingolipid structure and biosynthesis in *Neurospora crassa*.**

Steven B. Levery[1], Chaeho Park[2], Beau Bennion[1], Elizabeth Owuor[1], Isabelle E.J.A. François[3], Kathelijne K.A. Ferket[3], Bruno P.A. Cammue[3], Karin Thevissen[3]. [1]Department of Chemistry, University of New Hampshire, Durham, NH, USA; [2]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, USA; [3]Centre of Microbial and Plant Genetics (CMPG), Katholieke Universiteit Leuven, Heverlee-Leuven, Belgium.

Glycosphingolipids (GSLs) have been implicated in a number of studies as targets of plant defensin binding to the fungal membrane. For example, sensitivity of the yeasts *Pichia pastoris* and *Candida albicans* toward RsAFP2, a defensin isolated from seeds of *Raphanus sativus* (radish), was found to be dependent on GCS, the gene encoding glucosylceramide synthase (UDP-Glc:ceramide beta- glucosyltransferase). Although *Neurospora crassa* is not a phytopathogen, it has been used as a system to investigate plant defensin-phytopathogen interactions. Chemically mutagenized *N. crassa* strains selected for resistance to RsAFP2 were found to have dramatically altered glycolipid expression profiles. Characterizing the true nature of these alterations has required detailed structure elucidation of GSL components isolated from wild type and defensin-resistant mutant *N. crassa* strains. Key general characteristics of fungal GSL expression at the gene and metabolic levels will be discussed and compared with what we have learned so far from studies of GSL expression in *N. crassa*.

**Biogenesis of mitochondria: Pathways and machineries involved in the import of proteins**

Walter Neupert, Institute of Physiological Chemistry, University of Muenchen, Germany

The life of almost all proteins of the mitochondrion begins at ribosomes in the cytoplasm of the cell. In order to reach their active states as constituents of protein assemblies in one of the various mitochondrial subcompartments (the outer membrane, the intermembrane space, the inner membrane or the matrix) they interact with protein translocases of the mitochondria. So far six such molecular machines have been identified: two in the outer membrane (the TOM and

TOB complexes), one in the intermembrane space (the Mia1/Erv1 machinery), and three in the inner membrane (the Tim23, TIM22 and OXA1 complexes). In addition, a number of chaperones and co-chaperones in the matrix are required for folding in the matrix space as well as a series of assembly factors for the formation of cofactor containing supramolecular protein complexes of the mitochondria.
The two most recently identified protein translocases are the TOB complex and the Mia40/Erv1 machinery. The TOB complex is responsible for the membrane integration of β-barrel proteins of the mitochondrial outer membrane, such as Tom40 and porin. The TOB complex interacts with the precursors of these proteins during or after their translocation across the outer membrane by the TOM complex. The Mia40/Erv1 machinery is essential for the import into the intermembrane space of members of a family of small proteins with CX3C and CX9C motifs. Oxidative folding leading to formation of disulfide bonds appears to be a step which provides a driving force for translocation across the outer membrane via the TOM complex.
A further focus of our interest is the ATP driven import motor of the mitochondria which is coupled to the TIM23 translocase. Tim44 is a central component which is peripherally associated with the membrane integrated components Tim23 and Tim17, and which organizes the import motor. Tim44 recruits mtHsp70 which binds and, by a ratchet-like mechanism, takes into the matrix unfolded precursor polypeptides that have passed the TOM complex and the channel of the TIM23 translocase. The import motor contains two further Tim44 associated essential proteins, the J-protein, Tim14, and J-like protein, Tim16. The way how they control the activity of the import motor appears to be key to the understanding of the function of the TIM23 complex which is responsible for the import of the vast majority of nuclear encoded inner membrane and matrix proteins of mitochondria.

**Function and expression of Tob55/Sam50 in *Neurospora crassa*: An essential protein for assembly of beta-barrels into the mitochondrial outer membrane**
Dr. Frank Nargang, Dept. of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

The majority of mitochondrial proteins are encoded by nuclear genes, synthesized in the cytosol as mitochondrial precursors, and imported into the organelle. The translocase of the outer mitochondrial membrane (the TOM complex) recognizes and imports all classes of mitochondrial precursors either into, or across, the outer membrane. Further sorting of precursors to the correct mitochondrial sub-compartment is achieved via additional translocase complexes housed in the membranes of the organelle. One of these translocases is The TOB (Topogenesis of Outer Membrane Beta-barrel proteins) or SAM (Syntheses and Assembly Machinery) complex. The major protein of the TOB complex is Tob55. We have constructed a knockout of *N. crassa* tob55 and have shown that it is essential for the viability of the organism. Reduced levels of Tob55 result in a specific deficiency in the assembly of beta-barrel proteins into the outer membrane. Our studies have also revealed that the *N. crassa* Tob55 exists in at least three isoforms which appear to be generated via alternative splicing. We are currently investigating the ability of different isoforms to rescue Tob55 function.

**The NAD(P)H dehydrogenases of *Neurospora crassa* mitochondria**
Arnaldo Videira. Instituto de Biologia Molecular e Celular (IBMC) and Instituto de Ci ncias Biomédicas de Abel Salazar (ICBAS), University of Porto, Portugal

The proton-pumping NADH dehydrogenase or complex I is a major entry point of electrons into the mitochondrial respiratory chain. It catalyses electron transfer from NADH to ubiquinone through a series of protein-bound prosthetic groups. Complex I deficiencies have been implicated in various mitochondrial diseases. Complex I from the filamentous fungus *Neurospora crassa* contains at least 39 polypeptide subunits of dual genetic origin, mostly conserved in mammals, suggesting that the enzyme is involved in other cellular processes beyond bioenergetics. Mutations in different subunits have been generated in the last years, including mutations of conserved amino acid residues of iron-sulphur proteins as found in human diseases, in order to reveal the role of the proteins in complex I assembly and function. Complex I is likely regulated by transitions between active (A) and de-activated (D) forms and one of the proteins involved in this phenomenon has been identified. In addition to complex I and depending on the organism, several non-proton-pumping alternative NAD(P)H dehydrogenases may also be present in the inner mitochondrial membrane. The fungus *N. crassa* contains four alternative NAD(P)H dehydrogenases: the main external NAD(P)H dehydrogenase, an external calcium-dependent NADPH dehydrogenase, a third external enzyme and the single internal NADH dehydrogenase. These proteins appear to have both alternative and complementary functions. Overall, mitochondrial respiratory chain NAD(P)H dehydrogenases have important roles in fungal development.

## Session IV: Cell Signaling and Gene Regulation

Nora Plesofsky, Chair

**The arginine attenuator peptide: a regulator of translation and mRNA levels.**

Matt Sachs, OGI School of Science and Engineering, Oregon Health & Science University, Beaverton, OR

The *Neurospora crassa arg-2* gene and its fungal homologs encode the arginine-specific carbamoyl-phosphate synthetase (CPS-A) small subunit. Excess arginine decreases translation of *arg-2* through the action of an evolutionarily conserved upstream open reading frame (uORF) in the mRNA. This uORF encodes a cis-regulatory element, the arginine attenuator peptide (AAP), which stalls ribosomes in the presence of arginine, thereby decreasing ribosome access to the downstream CPS-A reading frame. We performed an extensive analysis of the sequence requirements for AAP-mediated translational control of ribosome stalling using the *N. crassa* cell free translation system. The results showed that some but not all of the evolutionarily conserved residues in the AAP sequence were crucial for regulation. Furthermore, we showed using yeast that AAP-mediated ribosome stalling at the uORF stop codon causes the mRNA to be destabilized by inducing the nonsense mediated mRNA decay (NMD) pathway. Preliminary evidence using an *N. crassa* mutant defective in NMD indicates that the amount of cellular arg 2 mRNA is also controlled at the level of mRNA stability by NMD.

**Involvement of the *Neursopora* mitochondrial tyrosyl-tRNA synthetase and DEAD-box proteins in splicing group I and group II introns.**

Alan Lambowitz, Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin TX 78712.

Group I and group II introns are commonly found inserted in fungal mtDNA genes. These introns self-splice *in vitro*, but require proteins for efficient splicing *in vivo* to help fold the intron RNA into the catalytically active structure. Protein factors that function in splicing group I introns were first identified by mutational analysis in *Neurospora crassa*. The *Neurospora* mitochondrial tyrosyl-tRNA synthetase (CYT-18) binds specifically to group I introns RNAs and promotes RNA splicing by stabilizing the catalytically active RNA structure. The *N. crassa* CYT-19 protein functions in conjunction with CYT-18 and is DEAD-box protein that acts as an ATP-dependent RNA chaperone to disrupt stable inactive structures that are kinetic traps during CYT-18-assisted RNA folding. Studies with CYT-18 and CYT-19 have suggested general paradigms for how proteins function in RNA folding and facilitate RNA-catalyzed reactions. They also show how cellular RNA bindings proteins can evolve to function in RNA splicing, and more generally, how essential proteins can acquire new functions.

**G proteins and histidine kinases differentially regulate sexual development**

Katherine A. Borkovich, Department of Plant Pathology, University of California, Riverside.

Heterotrimeric G protein pathways and two-component regulatory systems control environmental responses in fungi. Our laboratory has demonstrated roles for both of these pathways in regulation of sexual development in *Neurospora*. The G protein coupled receptors (GPCRs) PRE-1 and PRE-2, along with their cognate pheromone ligands, MFA-1 and CCG-4, are essential for initial recognition between opposite mating type females and males during mating. The heterotrimeric Gα protein GNA-1 and the Gβγ dimer GNB-1/GNG-1 are also necessary for female fertility, but in a mating type independent manner. Loss of the G protein subunits or the receptor leads to trichogyne "blindness" in females, while males that do not produce pheromone are unable to attract trichogynes. A change in identity can be accomplished by heterologous expression of the receptor or pheromone in cells of opposite mating type and co-expression of a cognate receptor-pheromone pair leads to self-stimulation. The GPCR GPR-1 is required for proper formation of beaks and ostioles during perithecial development; the coupled Gα is not currently known, but available genetic data points to GNA-1 as the most likely candidate. We have begun analysis of two-component regulatory systems with characterization of the response regulator RRG-1. *rrg-1* mutants have an early block in female fertility, in that they do not produce protoperithecia. This defect correlates with loss of stimulation of a downstream mitogen-activated protein kinase pathway in Neurospora

**Cytology of conidial anastomosis induction, homing and fusion in Neurospora crassa**

M. Gabriela Roca and Nick D. Read. Institute of Cell Biology, University of Edinburgh, Rutherford Building, Edinburgh, EH9 3JH, UK.

Conidia of Neurospora crassa form conidial anastomosis tubes (CATs) which are morphologically and physiologically distinct from germ tubes, and under separate genetic control. The dynamic behaviour of nuclei, mitochondria and microtubules during macroconidial germination and fusion was analysed using a range of vital dyes and GFP labelling. We found that: (1) mitosis occurs much more rapidly in germ tubes (~ 15 min) than in ungerminated

macroconidia (~ 3.5 h); (2) mitochondria are concentrated within germ tube and CAT tips; (3) both nuclei and mitochondria are physically connected to microtubules; (5) CAT fusion occurs after mitosis has been undergone in ungerminated macroconidia or germ tubes; (6) nuclei do not enter CATs prior to fusion; (7) the nuclei within germ tubes or CATs do not exhibit any special localization which can be associated with the pattern of CAT induction, homing or fusion; (8) microtubules extend through fused CATs from both conidial germlings which have fused with each other; (9) microtubules pass through fused CATs prior to mitochondria which are then followed by nuclei; and (10) organelle fluxes between fused conidial germlings are typically several orders of magnitude slower than those between fused hyphae in the mature colony.

**Glycogen metabolism and stress response in Neurospora crassa. Regulation of the gsn gene.**

Prof. Dr. Maria Célia Bertolini. Instituto de Química, UNESP, Departamento de Bioquímica e Tecnologia Química R. Prof. Francisco Degni, s/n 14800-900, Araraquara, SP Brazi

The synthesis and degradation of glycogen molecules are carried out by the concerted action of a set of enzymes, the main control being of the activities of glycogen synthase and glycogen phosphorylase, respectively. Glycogen synthase catalyzes the formation of the α-1,4-glycosidic linkages of glycogen by addition of UDP-glucose units into glycogen, and is considered to be the limiting-rate step of the glycogen synthesis. This enzyme is regulated both by allosteric modulation, and by reversible phosphorylation. In addition to reversible changes in the glycogen synthase activity, glycogen levels are also correlated with physiological conditions. In *Saccharomyces cerevisiae*, glycogen accumulation is induced by conditions that stress the cells, such as heat shock. Such condition induces transcription of the gene encoding glycogen synthase (GSY2), which explains the glycogen accumulation. We have isolated the gene encoding the *Neurospora crassa* glycogen synthase (gsn) and demonstrated that the gene transcription is repressed when cells are exposed to temperatures varying from 30 to 45ºC (heat shock). In addition, glycogen levels rapidly decrease in the same growth condition. Analysis of the gsn promoter region allowed us to identify multiple regulatory elements, including many HSE (Heat Shock Element) and two STRE (STress Responsive Element), which are usually found in promoters of genes responsive to stress conditions. Gel shift assays (EMSA) using nuclear extract prepared from *N. crassa* heat shocked mycelia showed the presence of protein(s) that bind specifically to the STRE elements of the gsn promoter region. Our main purpose is to identify the protein(s) that bind to the STRE elements in order to study how these elements function in the regulation of gene transcription. Approaches coupling EMSA and mass spectrometry have been used to reach the goal. Results will be presented concerning the strategies we have used to identify the protein(s).

**Heterokaryon incompatibility**

N. Louise Glass, Karine Dementhon, Isao Kaneko and Qijun Xiang. Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102

Nonself recognition in filamentous fungi is conferred by genetic differences at *het* (heterokaryon incompatibility) loci. When individuals that differ in *het* specificity undergo hyphal fusion, the heterokaryon undergoes a programmed cell death reaction or is highly unstable. In *Neurospora crassa*, three allelic specificities at the *het-c* locus are conferred by a highly polymorphic

domain. This domain shows trans-species polymorphisms indicative of balancing selection, consistent with the role of *het* loci in nonself recognition. We determined that a locus closely linked to *het-c,* called *pin-c* (partner for incompatibility with *het-c*) was required for *het-c* nonself recognition and heterokaryon incompatibility (HI). The *pin-c* alleles in isolates that differ in *het-c* specificity were extremely polymorphic. Heterokaryon and transformation tests showed that nonself recognition was mediated by synergistic non-allelic interactions between *het-c* and *pin-c,* while allelic interactions at *het-c* increased the severity of the HI phenotype. The *pin-c* locus encodes a protein containing a HET domain. These data suggest non-allelic interactions may be important in nonself recognition in filamentous fungi and that proteins containing a HET domain may be a key factor in these interactions. Functional VIB-1, which is a putative transcription factor, is required for expression of *pin-c, het-6* and *tol*, all of which encode HET domain proteins. These observations explain why mutations at *vib-1* suppress *het-c, het-6* and *mat* incompatibility.

**MAK-2 MAP Kinase and cAMP signaling pathways interact to control aerial growth and conidiophore development**
Dan Ebbole, Texas A & M University.

The *mak-2* and *pp-1* mutants have reduced growth rate, produce short aerial hyphae, and fail to develop protoperithecia. In addition, ascospores carrying null mutations of either gene are inviable. Subtractive cloning was used to isolate genes having reduced expression in the *mak-2* mutant.
Expression of some of these genes is protoperithecia specific and three of them are part of a gene cluster potentially involved in the production of a polyketide secondary metabolite. Microarray analysis was used to extend the analysis of gene expression in *mak-2* and *pp-1* mutants.

**Session V: Clocks, Light, and Oxygen**

Deborah Bell-Pedersen, Chair

**Molecular mechanism of light responses in *Neurospora***
Qiyang He, and Yi Liu. University of Texas Southwestern Medical Center, Dallas, TX.

Blue light regulates many molecular and physiological activities in a large number of organisms. In *Neurospora crassa,* a eukaryotic model system for studying blue-light responses, the transcription factor and blue-light photoreceptor WHITE COLLAR-1 (WC-1) and its partner WC-2 are central to blue-light sensing. *Neurospora's* light responses are transient, i.e. following an initial acute phase of induction, light-regulated processes are down-regulated under continuous illumination, a phenomenon called photoadaptation. The molecular mechanism(s) of photoadaptation are not well understood. Here we show that a common mechanism controls the light-induced transcription of immediate early genes (such as *frq*, *al-3*, and *vvd*) in *Neurospora*, in which light induces the binding of an identical large WC-1/WC-2 complex (L-WCC) to the light response elements (LREs) in their promoters. Using recombinant proteins, we show that the WC complexes are functional without the requirement of additional factors. *In vivo*, WCC has a

long period photocycle, indicating that it cannot be efficiently used for repeated light activation. Contrary to previous expectations, we demonstrate that the light-induced hyperphosphorylation of WC proteins inhibits bindings of the L-WCC to the LREs. We show that, *in vivo*, due to its rapid hyperphosphorylation, L-WCC can only bind transiently to LREs, indicating that WCC hyperphosphorylation is a critical process for photoadaptation. Finally, phosphorylation was also shown to inhibit the LRE-binding activity of D-WCC (dark WC complex), suggesting that it plays an important role in the circadian negative feedback loop.

**Clocks and Light and Oxygen! Oh My!**

Luis Larrondo, Bill Belden, Allan Froehlich, Jay Dunlap and Jennifer Loros. Dept. of Biochemistry, Dartmouth Medical School, jennifer.loros@dartmouth.edu

The ascomycete Neurospora crassa has a long standing as a model organism for investigating both circadian rhythms and photoreception (J. J. Loros and J. C. Dunlap, Circadian Rhythms, Photobiology and Functional Genomics in Neurospora. Ch.4, pp 53- 74 in Volume XIII The Mycota. "Fungal Genomics". Editor Alistair J P Brown, 2005). The circadian system of N. crassa involves a number of interlocked molecular feedback loops that regulate the time-of-day-specific expression of a number of output genes, thereby generating distinct phenotypes, including the clock-dependent rhythm of macroconidiation. Light is a major entraining signal to the clock, coordinating the organism with the diurnal environment. Known players involved in circadian and light regulation of these processes include the products of the frq, wc-1, wc-2, and vvd genes. Both VVD and WC-1 have been shown to be blue light photoreceptors; WC-1 is required for circadian entrainment as well as the autoregulatory feedback loop involving the frq gene and VVD has been shown to participate in light signaling to the clock. The recent cloning of a mutation, bd, that allows the easily visualized rhythm in conidiation suggests an imbalance of reactive oxygen species in this mutant strain. Recent work on the clock, light signaling and Ras will be discussed. This work was supported by grants from the National Institutes of Health MH44651 AND NIGMS1P01 GM 068087-01 to J.C.D. and J.J.L. and R37GM34985 to J.C.D., the National Science Foundation MCB-0084509 to J.J.L., and the Norris Cotton Cancer Centre core grant at Dartmouth Medical School.

**Coordination of negative and positive functions of FREQUENCY in the circadian clock**

Michael Brunner. Biochemie-Zentrum Heidelberg, Im Neuenheimer Feld 328, D 69120 Heidelberg

The Neurospora circadian clock protein FREQUENCY (FRQ) has a function in the negative and the positive limb of interconnected feedback loops. In the negative feedback loop FRQ inhibits its transcription factor White Collar Complex (WCC), and in the positive loop it supports accumulation of WCC. These contradictory functions of FRQ are confined to distinct subcellular compartments and coordinated in temporal fashion. Negative feedback occurs early after the onset of FRQ expression and requires nuclear FRQ. Nuclear FRQ promotes hyperphosphorylation of WCC leading to its inactivation. Support of WCC accumulation depends on cytosolic FRQ and occurs about 8 h after the onset of FRQ expression when high amounts of FRQ have accumulated. The transcriptional function of FRQ in the negative limb and its posttranslational function in the positive limb are independent and associated with distinct

regions of FRQ. Phosphorylation of serine residues within the PEST-2 region triggers the maturation FRQ of toward a cytoplasmic activator.

**Quelling in Neurospora: an overview**

Carlo Cogoni, Dip. Biotecnologie Cellulari ed Ematologia, Sez. Genetica Molecolare, Policlinico Umberto I Universita' degli Studi di Roma 'La Sapienza'
Roma, Italy

The introduction of transgenes or double-strand RNAs (dsRNAs) into a variety of eukaryotic cells can trigger a series of post-transcriptional gene silencing mechanisms in which dsRNA intermediate molecules, after being processed into short interfering RNA molecules (siRNA), were identified as strong elicitors of mRNA degradation. Two PTGS mechanisms have been identified in Neurospora: quelling and MSUD (meiotic silencing by unpaired DNA). Quellling occurs during the vegetative phase of the life cycle and was the first PTGS mechanism characterized in this organism.
Several components of the quelling machinery have been identified by using either forward or reverse genetic approaches. The identification of genes required in the silencing process together with findings from other organisms has led to a current model for quelling. In Neurospora, as in other organisms, it would seem that quelling is serving to limit the expansion of transposons since an introduced Tad element, a LINE-1-like retrotransposon, has an elevated expansion in the absence of the quelling components QDE2 and DICER.

**Natural variation and the multigenic nature of circadian behavior in Neurospora**

Kwangwon Lee. Department of Plant Pathology Cornell University

We studied two under-explored areas in Neurospora biology, natural variation and quantitative trait loci (QTL) analysis. Natural variation in circadian period, phase and temperature compensation optimizes the ability of an organism to synchronize its biological processes to a local environment. Among a world-wide collection of 144 *Neurospora crassa* accessions circadian rhythms of asexual conidiation revealed significant variation centered on a 22 hour period and morning specific phase. Consistent with the phenotypic variation, there is significant genotypic variation among circadian components, WHITE COLLAR-1 (WC-1), WHITE COLLAR-2, FREQUENCY, and VIVID. Furthermore, we found significant association between circadian parameters and molecular variation in the circadian genes. WC-1 mediates interactions between the circadian clock and the environment, acting both as a core clock component and a blue light photoreceptor. Our data show that a putative activation domain in WC-1 is highly polymorphic in length, revealing a significant association between circadian period, latitude of origin and *wc-1* genotype. We suggest that environment specific natural variation at WC-1 fine-tunes circadian period.
In an attempt to characterize the polygenic nature of the circadian clock, we performed QTL analyses in three mapping populations, which were generated by crossing natural accessions, with 188 F1 progenies. At least 80 loci were determined by simple sequence repeat markers for their map position in each population, covering the genome with about 1000 cM. The clock QTLs identified from three experimental populations will be discussed.

**Chromatin-Remodeling Enzymes and Circadian Rhythms**.

William J. Belden, Jennifer J. Loros, and Jay C. Dunlap.

*Neurospora crassa* contains a circadian feedback loop that is controlled by daily oscillations in transcription of the *frequency* gene. The transcription factors White Collar-1 (WC-1) and White Collar-2 (WC-2) activate *frq* expression in a circadian and light dependent manner, and are thought to act together as heteromeric complex. To better understand regulated events at the *frq* promoter, we deleted genes homologous to the *swi/snf* family of ATP-dependent chromatin-remodeling enzymes. The 19 putative chromatin-remodeling factors were knocked out by gene replacement and characterized; one was essential for growth, another was ascospore lethal and a third had a defect in circadian regulated spore formation, and is now designated *clockswitch (csw-1)*. To further define events at *frq*, we used ChIP and nuclease accessibility assays to examine how nucleosome modifications might regulate circadian relevant transcription. The WC proteins do not act solely as an obligate complex because *in vivo* binding of WC-2 to the *frq* promoter occurs in a rhythmic fashion with the peak in binding occurring coincident with the peak in *frq* transcription. A basal level of WC-1 is associated with the promoter at all circadian times and only slight increases are observed when *frq* is transcribed. There is marked reduction in the level of acetylated histone H3 upon light induced transcription. Chromatin rearrangements at *frq* are seen when the gene is expressed and ChIP assays indicate that CSW-1 is localized to this region, suggesting a direct role for this chromatin-remodeling enzyme in regulating *frq* expression.

**Cell differentiation as a response to oxidative stress**

Leonardo Peraza[1], Nallely Cano[2], Mauricio Rios[1], Jesús Aguirre[2], and Wilhelm Hansberg[1]
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Different stress conditions in *Neurospora* lead to an unstable state in which formation of reactive oxygen species surpass the antioxidant capacity of the cell. Cell differentiation is one possible response to this inevitably transient hyperoxidant state. The hypothesis predicts that disruption of anti-oxidant enzymes should intensify cell differentiation processes; deletion of pro-oxidant enzymes should inhibit them. Catalase and NADPH oxidase genes were disrupted to find that *cat-3* mutant increased asexual and sexual sporulation, *cat-2* mutant increased submerged conidiation, *nox-1* decreased asexual and inhibited sexual sporulation, and *nox-2* inhibited ascospore germination (1). *sod-1* deletion mutant presented circadian conidiation, enhanced aerial and submerged conidiation, and diminished protoperithecia formation. *sod-1* phenocopies *bd*, which is a dominant mutation probably in *ras-1*. Both strains seem to produce a cyclic oxidative stress that leads to cyclic conidiation. 1) Aguirre J; Rios-Momberg M; Hewitt D; Hansberg W (2005) Reactive oxygen species and development in microbial eukaryotes. Trends in Microbiology **13**:111-118.

Please note, some talks were selected from posters and their abstracts appear here.

# NEUROSPORA 2006 POSTER ABSTRACTS

## BIOCHEMISTRY AND SECONDARY METABOLISM

### 1. Using metabolomics to identify the molecule that causes efflux of basic amino acids from the vacuole of *Neurospora crassa.*

Kelly A. Keenan, Michael Coords and Mia Carraturra, Richard Stockton College, PO Box 195, Pomona, NJ 08240, keenank@stockton.edu

The goal of this project is to both begin the identification of metabolites found in Neurospora crassa and to identify the metabolite that causes efflux of basic amino acids from the vacuole. Under conditions of nitrogen starvation, arginine is released from the vacuole and it has been shown that a metabolite is responsible for this. Metabolite extracts have been prepared from N. crassa grown under normal as well as nitrogen starvation conditions, derivitatized to protect reactive groups and then passed through the gas chromotograph-mass spectrometer (GC-MS)using the Feihn method which has yielded information on metabolites in several other organisms. Peaks from the GC are selected and the mass spectra are compared to the NIST library to identify the metabolites. Results so far indicate that there are peaks unique to the nitrogen starvation extract and the identification awaits confirmation by running standards. Such peaks are thought to contain the metabolite that causes efflux. Such metabolites will be tested in the efflux assay. Other metabolites found in both extracts are also being identified and should provide a starting point for list of metabolites.

### 2. The Mechanism of Ammonium Transport via Amt Proteins in Neurospora

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On the basis of physiological data plus high-resolution X-ray data from bacterial proteins, several laboratories have concluded that ammonium transporters homologous with the E. coli AmtB protein actually function as channels for ammonia: so that each transit requires NH4+ to shed a proton into solution on the cis side of the membrane and NH3 to retrieve a proton from solution on the trans side of the membrane (P.N.A.S. 95:7030, 1998; Science 305:1587, 2004; P.N.A.S. 101:17090, 2004). In low-pH solutions (free NH3 scarce), then, this ammonia-channel mechanism should mimic ammonium/proton antiport: being electroneutral overall and counterflowing one proton for each NH3 transit. Electrophysiological data from Neurospora contradict these inferences*. During ammonium starvation, the Neurospora plasmalemma becomes increasingly susceptible to depolarization by test pulses of ammonium (0.01-0.1 mM). Susceptibility increases with a half time of ~15 minutes and yields maximal depolarization (from -200 mV to about –50 mV) with ~10 mM NH4+. The system is only about 10% as sensitive to methyl ammonium as to ammonium itself, and is unresponsive to K+ and to lipid-soluble cations such as TPMP+. Critical unanswered questions are which of the Amt homologues in Neurospora are synthesized/activated during ammonium starvation? which mediate the observed currents? and how do their "pore" structures differ from AmtB? There are four putative Amt genes in Neurospora: NCU03257.1, NCU05843.1 (MEPA), NCU06613.1, and B14A6.240. *Slayman,

C.L., 1977. Energetics and control of transport in Neurospora. In Jungreis, A.M., et al., eds., "Water Relations in Membrane Transport in Plants and Animals." Academic Pr. (New York), pp. 69-86.

## CELL BIOLOGY

### 3. Live-cell imaging of trichogyne-macroconidium interactions in *Neurospora crassa*.

Hsiao-Che Kuo and Nick D. Read. Institute of Cell Biology, University of Edinburgh, Rutherford Building, Edinburgh, EH8 3JH, UK

The initial stages of Neurospora crassa sexual reproduction involve a specialized 'female' receptive hypha (the trichogyne) which grows out from the ascogonium of the protoperithecium, and fuses with a 'male' cell (a conidium). We have analysed this process using live-cell imaging. On water agar medium, trichogynes first emerged from protoperithecia which were 20-25 um in diameter. Fringe hyphae grew out from the protoperithecial wall soon after. Trichogynes first responded chemotropically to macroconidia of opposite mating type ~6 h after adding macroconidia; macroconidium-trichogyne fusion took place ~4 h later. At 24°C trichogynes grew more slowly (0.25-0.63 um/min) than vegetative hyphae (5-20 um/min). More than one trichogyne grew out from a single protoperithecium and all of these trichogynes could home towards different or the same macroconidia. Even if a macroconidium had germinated it still attracted trichogynes although conidial germ tubes often stopped growing when trichogynes were in their vicinity. Furthermore, the trichogyne was sometimes attracted to the conidial germ tube rather than the conidium itself. This suggested that chemoattracting sex pheromone can be produced from germ tubes as well as from macroconidia. Male macroconidial nuclei migrated into the trichogyne and mitotic division of both male and female nuclei in the trichogyne was inhibited during at least the first 5 h following fusion. In addition, the female nuclei in the trichogyne immediately became immobilized following fusion and only the male macroconidial nuclei moved through the trichogyne towards and into the ascogonium.

### 4. Ontogeny of the Spitzenkörper in germlings of *Neurospora crassa*

C. L. Araujo-Palomares, E. Castro-Longoria, and M. Riquelme. Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada. Ensenada, Baja California, México. ecastro@cicese.mx

The Spitzenkörper (Spk) is a highly dynamic and pleomorphic complex located in the cell apex of filamentous fungi. In previous studies the structure and dynamics of the Spk have been analyzed in mature hyphae of filamentous fungi, both in main leading hyphae and branches. By enhanced phase-contrast high resolution video-microscopy we have analyzed the intracellular changes prior to the appearance of the Spk in germlings of *Neurospora crassa*. Observations were done from the initial stages of spore germination until a conspicuous Spk was observed in the apex of germ tubes. Before a Spk could be observed, the cell showed a uniform distribution of organelles such as nuclei, mitochondria and cytoplasmic granules. Once the germlings of *N. crassa* reached a length of approximately 150 µm the organelles were displaced towards the subapical region of the cell and a small exclusion zone (0.6 ± 0.3 µm) was formed at the apex. The position of this exclusion zone within the apex seemed to direct the germ tube growth direction, which was highly erratic. Thirty minutes after it first appeared, the exclusion zone

started to become occupied by a dark-phase material that gradually concentrated into a light gray body that we called immature Spk. During this phase the presence of the Spk in the apical dome was not constant. Approximately 20 minutes later the Spk became more robust until acquiring its typical dark-phase appearance, at which point the growth direction of the germ tube became more unwavering

**5. Characterization and transcriptional profiling of *vib-1* in *Neurospora crassa*.**

Karine Dementhon, Chaoguang Tian, Elizabeth Hutchison, and N. Louise Glass. Plant and Microbial Biology department, UC Berkeley, California, 94720

During vegetative growth, filamentous fungi produce heterokaryons by undergoing hyphal fusion. However, differences at the *het-c* haplotype, whose specificities are categorized into one of three major types, cause heterokaryon incompatibility (HI), resulting in programmed cell death and repression of conidiation and growth. Non-allelic interactions of polymorphic *het-c* and *pin-c* alleles are essential for nonself recognition and HI. HI is suppressed by mutations in *vib-1*, a putative transcription factor. Though mutations in *vib-1* suppress the HI phenotype, *vib-1* mutants exhibit irregular conidiation. GFP-tagged *vib-1* has been shown to localize to the nucleus, and the signal intensity of *vib-1*::GFP increases in incompatible vs. compatible cells. These results indicate that *vib-1* has an important role in HI. In order to investigate the function of *vib-1*, transcriptional profiling experiments were performed to identify targets of *vib-1* regulation. When expression patterns of a *vib-1* knockout strain were compared to those of a wildtype strain, more than 100 genes exhibited altered expression. Transcriptional profiling will also be used to compare a *vib- 1* overexpression strain to a wildtype strain. Finally, the expression patterns of a *vib-1* knockout in a compatible heterokaryon will be compared to those in an incompatible heterokaryon. By comparing *vib-1* knockout strains in compatible vs. incompatible situations, differences in expression patterns can be attributed to the effects of the *het-c* and *pin-c* interactions. Transcriptional profiling experiments, in conjunction with data on the characterization of *vib-1* mutants, will give important insights into the role of *vib-1* during vegetative growth and HI.

**6. Modifications of PCNA and DNA repair in *Neurospora crassa*.**

Tsuyoshi Kawabata, Akihiro Kato, Keiichiro Suzuki, Hirokazu Inoue. Department of Regulation Biology, Faculty of Science, Saitama University, Saitama City, Japan.

Proliferating cell nuclear antigen (PCNA) is an auxiliary factor for DNA polymerases and is essential for DNA replication. It forms a heterotrimeric clamp of DNA. Some yeast PCNA mutant alleles exhibited sensitivity to mutagens such as UV and MMS, indicating that PCNA is related to DNA repair. Recently, it has been elucidated that PCNA is modified by ubiquitin in response to DNA damage and by SUMO at S- phase. In yeast, PCNA is monoubiquitinated by Rad6/Rad18 and polyubiquitinated by Rad5/Ubc13/Mms2. However, there are some differences between yeast and human in these modifications. Because of insufficiency of genetic studies, these precise molecular events remain unclear in higher eukaryote. Therefore, in order to clear how PCNA is modified in higher eukaryote, we analyzed modifications of PCNA in *N. crassa*. In this study, we made disruptant of Neurospora homologs of *RAD5* and *UBC13* (*mus-41* and *mus-46*, respectively) and investigated roles of these genes in modifications of PCNA. These mutants were sensitive to mutagens as observed in yeast, but epistasis analysis showed that *mus-*


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*41* and *mus-46* have roles different from yeast. We created strains that express FLAG- or HA-tagged PCNA and observed PCNA modifications by western blotting. It was shown that PCNA is polyubiquitinated independently from *mus-46* and *mus-41* in *N. crassa*. It suggests existence of some unknown factors required for PCNA polyubiqiutination in *N. crassa*.

**7. SO, a protein involved in hyphal fusion, localizes to septal plugs.**
Andre Fleissner and N. Louise Glass. Department of Plant and Microbial Biology, University of California, Berkeley, CA

The *so* mutant of *Neurospora crassa* exhibits a pleiotropic phenotype, including lack of anastomosis, shortened aerial hyphae, and female sterility (1). The *so* gene is highly conserved in filamentous ascomycetes, but not present in ascomycete yeast species. The encoded protein contains a conserved WW-domain possibly involved in protein-protein interactions. In this study we show that SO-GFP fusion proteins localize to septal plugs. In filamentous ascomycetes septa get plugged in injured hyphae and at different stages during the fungal development, such as protoperithecia formation, aging, or cell death during vegetative incompatibility. While injured hyphae are sealed by Woronin bodies, other plugs seem not to contain this organelle. In contrast SO-GFP localizes to the different types of plugs mentioned above. To prove that this localization is Woronin body independent, we expressed *so-gfp* in the *hex-1* mutant, which lacks Woronin bodies (2,3). Although *hex-1* hyphae extensively bleed cytoplasm after injury they are eventually sealed in a Woronin body independent manner. SO-GFP localizes to these late forming plugs. However overexpression of *so- gfp* does not complement the *hex-1* phenotype. The *so* mutant has no bleeding phenotype, and a double mutant of *hex-1* and *so* does not show a more severe bleeding phenotype than *hex-1* alone. We conclude that SO is part of septal plugs, that its localization to the plugs is Woronin body independent, and that its role is different from simply preventing the loss of cytoplasm.
(1) Fleissner et al., Euk. Cell 2005 (2) Jedd and Chua, Nature Cell Biol. 2000 (3)Tenney et al., Fungal Genet. Biol. 2001

**8. Antibiotic Binding Sites and the Structure of the Vacuolar ATPase.**
Emma Jean Bowman, Marija Draskovic, Molly McCall, and Barry Bowman. Dept. of MCD Biology, University of California, Santa Cruz, CA

The V-ATPase is a complex enzyme that functions as a rotary motor, transporting protons across membranes. Changes in V-ATPase activity appear to have a significant role in many human diseases, such as kidney malfunction, osteoporosis, and cancer. The macrolide antibiotics bafilomycin and concanamycin are potent inhibitors of V-ATPases. To determine where the drugs bind and how they inhibit, we selected mutant strains of *Neurospora crassa* that are resistant to these antibiotics. We also developed a method for site-directed mutagenesis to generate additional mutant forms of the enzyme. Approximately 20 amino acid residues were identified that, when mutated, resulted in a drug-resistant enzyme. All of the selected mutations were in subunit c, a small hydrophobic protein. Multiple copies of subunit c associate to form part of the “rotor,” which is embedded in the membrane. Using a prokaryotic homolog of the V-ATPase, a high-resolution structure for this sector of the enzyme was recently reported (T. Murata *et al.* Sci. 308:654-659). We used these data to construct a model of subunit c and the rotor of the *N. crassa* V-ATPase. The amino acid sequence of the *N. crassa* protein fit

remarkably well to the model based on the *E. hirae* structure. Furthermore, the residues implicated in binding antibiotics were located in a pocket formed at the interface between two c subunits. The model appears to be an excellent representation of the structure of this part of the V-ATPase in *N. crassa* and other eukaryotes. Furthermore, the data strongly support the conclusion that the antibiotics inhibit by blocking the rotation of the c subunits.

**9. A functional approach to the study of cellular morphogenesis in filamentous fungi.**
Aleksandra Virag and Steven D. Harris, Plant Science Initiative, University of Nebraska, N234 Beadle Center, Lincoln, NE 68588- 0660 (sharri1@unlnotes.unl.edu)

Polarized hyphal growth is a defining feature of the filamentous fungi. Despite its importance to the fungal lifestyle, the molecular mechanisms underlying the establishment and maintenance of hyphal polarity remain poorly understood. Moreover, during asexual development, fungi such as *Neurospora crassa* switch to a budding mode of growth that may involve dramatic switches in the spatial and temporal patterns of cellular morphogenesis. Previous genetic screens have identified several genes required for cellular morphogenesis in *N. crassa*, including many novel genes that are unique to fungi. We reasoned that a systematic survey of the *N. crassa* knockout collection would be the most effective approach to identify the complete set of genes involved in cellular morphogenesis. As a preliminary study, we have screened a set of ~100 knockout mutants for defects in hyphal morphogenesis, branching patterns, and conidial morphogenesis. Despite the small size of our sample, we have identified at least two genes (NCU08741.2, related to Striatin/Pro11; NCU03043.2, related to krueppel protein) with previously unknown roles in hyphal morphogenesis, as well as others whose role is far more important than previously suspected (i.e., NCU00406.2, CHM1). We will describe our screening format and present results for each of the mutants we have analyzed.

**10. Calcium and polar growth: The role of calcium transport proteins in organelles.**
Barry Bowman, Marija Draskovic, Emilio Margolles-Clark, and Stephen Abreu. Dept. of MCD Biology, University of California, Santa Cruz, CA

Calcium, a key signaling molecule in all organisms, is often sequestered in intracellular compartments. In filamentous fungi a calcium gradient has been proposed to play a key role in polar grow. We identified five genes in *Neurospora crassa* that may encode organellar calcium transporters. Using information from yeast homologs, calcium is predicted to be transported into the vacuole by NCA-2 and NCA-3 (P-type ATPases) and CAX (a Ca+2/H+ exchanger). In the Golgi, calcium and/or manganese are transported by PMR (a P-type ATPase). Unlike *S. cerevisiae, N. crassa* has a homolog of the mammalian SERCA ATPase (P-type), named NCA-1; it may reside in the ER. We generated null-mutant strains for each transporter. In the wild-type strain the vacuoles contained high concentrations of calcium, at least 20 mM. *Nca-2* and *nca-3* mutant strains maintain high levels of calcium, but inactivation of the *cax* gene caused the complete loss of calcium from the vacuole. Wild type, *nca-3* and *cax* strains grew normally in media with high concentrations of calcium (50-200 mM), but growth of the *nca-2* mutant and of the *nca-2 cax* double mutant was strongly inhibited. The data were not consistent with a localization of NCA-2 in the vacuole, but instead suggested that in *N. crassa* NCA-2 may pump calcium out of the cell. Polar growth was affected only in the *pmr* mutant strain. Because the loss of PMR affects the function of the Golgi, it cannot be concluded that the polar growth defect is


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directly caused by mis-localized calcium. Thus, the results indicate that the vacuole is a major storage site for calcium; however, loss of vacuolar calcium does not affect polar growth or the ability to tolerate high concentrations of calcium in the medium.

**11. Circadian Rhythms in Neurospora crassa: “after-effects” of the vvd mutation.**
Stuart Brody, Kevin Schneider, Sabrina Perrino Division of Biological Sciences-0116, UCSD, La Jolla, CA. 92093

Vvd mutant strains, which over produce carotenoids, are known to be deficient in photo-adaptation. On certain media, strains containing vvd mutations and the band (bd) mutation will exhibit short (6 – 14 hours) periods in constant light (L/L) but relatively normal (22-23 hours) periods in constant darkness (D/D). After four days of growth in D/D, these strains were switched from D/D to L/L. The subsequent periods were 17 hours, 9 hours, 9 hours, etc., i.e. a relatively quick adaptation to the light. However, when these strains were switched from L/L to D/D, the periods were 8 hours (in L/L), then 9 hours,10,12,12,15, and finally 20 hours, i.e. a slow adaptation to the dark. Control (bd) strains immediately showed normal (22 hour) periods upon transfer from L/L to D/D or loss of rhythmicity from D/D to L/L. One possible explanation is that the “after-effects” could be due to some key clock component which becomes elevated in L/L (in vvd) and therefore drives the conidiation rhythm to a high frequency. It then decreases slowly (in D/D) and eventually the rhythm returns to a 23 hr period. Another possible explanation may be analogous to the removal of hamsters from L/L to D/D and the slow loss of “splitting” seen in L/L of their rhythms. The hamster splitting has been postulated to occur due to two oscillators, the E and M oscillators.

**12. The response regulator, RRG-1, functions upstream of the OS-2 MAPK cascade.**
Carol A. Jones, Suzanne Phillips, and Katherine A. Borkovich. Department of Plant Pathology, University of California, Riverside, 92521, carol.cjones@gmail.com

Two-component signaling pathways are found in a variety of organisms including bacteria, plants, and fungi. Two-component regulator systems regulate phosphotransfer between two components: a histidine kinase (HK) at a histidine residue and a response regulator (RR) at an aspartate residue. In Neurospora there are eleven predicted hybrid histidine kinases (HHKs), which contain a histidine kinase domain and a response regulator domain within the same protein. Phosphotransfer is believed to occur as follows. A conserved histidine residue in the histidine kinase domain is autophosphorylated in response to some environmental signal. That phosphate is then transferred intramolecularly to the aspartate residue in the response regulator domain of the HHK. From the RR domain in the HHK the phosphate is then transferred to a histidine residue in the histidine phosphotransferase (HPT) protein, and finally onto an aspartate residue on a separate RR protein (RRG-1 or RRG-2). The RR are involved in the activation of downstream signaling pathways such as mitogen-activated protein kinase (MAPK) cascades and/or in transcriptional regulation. Environmental signals that may activate two-component cascades include light, osmolarity, nutrient availability, and many other factors. Analysis of the *rrg-1* gene replacement mutation shows that RRG-1 is involved in osmotic stress response, fungicide resistance, conidial integrity, and other factors. The *rrg-1* deletion mutant and mutants in the downstream *os-2* MAPK cascade have a reduced growth rate on salts and have resistance to certain fungicides. Also the phosphorylation status of OS-2, a downstream MAPK, is greatly

increased by exposure of wild type Neurospora to salts or fungicide. The working model is that NIK-1/OS-1 and at least one other HHK function upstream of RRG-1 through the HPT protein. RRG-1 can then activate the OS-2 MAPK cascade in response to osmotic stress, fungicide treatment, and/or other stimuli.

**13. Apical microtubule dynamic instability in *Neurospora crassa* hyphae**

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*Neurospora crassa* exhibits one of the fastest hyphal growth rates among filamentous fungi. To assess the probable role of microtubules (MTs) in such rapid extension rate, we monitored the dynamic instability (DI) of GFP-tagged apical MTs using live-cell imaging methods. Results obtained in mature hyphae revealed that MT polymerization rates were twice as fast as those reported in *Aspergillus nidulans*, while MT depolymerization rates in both species were similar. Furthermore, MT polymerization rates in *Neurospora* were much faster than in other organisms thus far reported, including plant and mammalian cells. In order to address the influence of motor protein mutations on DI, GFP-tagged MTs were examined in ropy 1 and nkin strains. In these strains, MT polymerization rates were reduced by one half relative to the wild type. Significance of these results will be discussed relative to hyphal growth and regulation of MT dynamics.

**14. Optical tweezer micromanipulation of *Neurospora crassa*.**

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Optical tweezers were first described in the 1980’s and have been implemented in a wide variety of research across many disciplines; however, the attention received from fungal biologists has been limited. In an attempt to correct the balance we have evaluated what can be achieved using optical tweezers to micromanipulate cells of the model filamentous fungus *Neurospora crassa*. Utilizing the properties of laser light, tweezers permit the non-invasive trapping and movement of cells and organelles. We have built a simple, safe and user-friendly optical tweezer system that can be mounted on a commercial microscope and is, importantly, easy to use for biologists who lack optics experience. The tweezers can be used synchronously with differential interference contrast, phase contrast and fluorescence microscopy to enable enhanced visualization and manipulation of living cells and organelles. The position and orientation of whole cells with respect to one another can be controlled and a variety of organelles (e.g. vacuoles and Woronin bodies) can be manipulated within cells. Inert beads can be used as tools for the measurement of forces generated by *Neurospora* hyphae, for the localized mechano-stimulation of cells or for the localized delivery of chemicals. We are presently using the optical tweezers as a powerful experimental tool to address a range of novel questions, using *Neurospora* as a model system.

**15. Control of circadian clock gene expression by a *frq*-less oscillator in *Neurospora crassa*.**
Sanshu Li and Patricia L. Lakin-Thomas. Department of Biology, York University, Toronto, Ontario, Canada. sanshuli@yorku.ca

The fungus *Neurospora crassa* expresses a circadian rhythm of conidiation when growing on agar medium. Assays of rhythmic gene expression in *Neurospora* using liquid culture systems have been used previously to construct a model for the circadian oscillator involving the *frq* and *wc* genes. We have now assayed the expression of circadian clock-associated genes using the same agar medium on which the function of the clock is assayed. We have found synchronous peaks of FRQ and WC- 1 proteins, in contrast to the out-of-phase rhythms found in liquid, and we have found out-of-phase RNA rhythms of the clock-controlled genes *ccg-2* and *ccg-7*, in contrast to the synchronous peaks in liquid. We have also assayed gene expression in the long-period choline-requiring mutant *chol-1*, and have found long-period rhythms in FRQ and WC-1 proteins and *frq* RNA, but circa-24 h rhythms in ccgs. Because the long-period *chol-1* conidiation rhythm continues in *frq* and *wc* null mutants, we conclude that long-period *frq* and *wc* expression rhythms must be driven by a *frq*-less oscillator. Regulation of rhythmic gene expression depends on metabolic conditions, and in the more physiologically relevant conditions of solid agar medium, the FRQ/WC feedback loop may not be operating as proposed. This research is supported by Discovery Grant 250133-02 from NSERC.

**16. Karyotyping of *Neurospora crassa* using synaptonemal complex spreads of translocation quadrivalents.**
Benjamin C. Lu, University of Guelph, Department of Molecular and Cellular Biology, blu@uoguelph.ca

The purpose of the present research is (1) to establish the karyotype of *Neurospora crassa* using visualization of kinetochores in the synaptonemal complex (SC) spreads, (2) to assign each chromosome to a linkage group, and (3) to examine chromosome pairing and recombination nodules in quadrivalents. Two strains containing reciprocal translocations were used: T(I;II)4637, which involves linkage groups I and II, and *alcoy*, which contains three independent translocations involving I and II, IV and V, and III and VI. Visualization of kinetochores in the spreads requires the use of freshly prepared fixatives. Kinetochore locations and arm ratios were documented in all seven *N. crassa* chromosomes. The new information, based on kinetochore position, arm ratios, chromosome length, and quadrivalent analyses, enabled unequivocal confirmation of chromosome assignments to genetic linkage groups. Chromosome pairing in a translocation quadrivalent starts at the four terminal regions, and proceeds right up to the translocation break point. Recombination nodules are found in all four arms of quadrivalents. The ability to identify a specific chromosome to a genetic linkage group together with the ability to visualize recombination nodules and their locations will allow future cytological analysis of recombination events.

**17. Mutational analysis of two components of the GPI-anchor transamidase complex.**
Shaun M. Bowman, Amy Piwowar, Eric D. Arnone, and Stephen J. Free, Department of Biological Sciences, University at Buffalo, Buffalo, NY 14260, email: free@buffalo.edu

We have used the RIP process to generate temperature-sensitive mutants in the Neurospora *gpit-1* and *gpit-2* genes. These two genes encode auxiliary components of the GPI-anchor transamidase, a multiprotein complex found in the ER membrane that transfers the GPI-anchor onto GPI-anchored proteins. It has been difficult to obtain structural data on the organization of the complex and how the different proteins function and interact with each other because the components are multipass transmembrane proteins and haven't been individually purified. We have used a RIP mutational analysis to define regions within each of these two proteins that can be mutated without loss of the protein's function at a permissive temperature. The analysis helps to identify regions within each of these two proteins that are critical for the activity of the proteins. For the most part, the sequences we define as being critical are sequences that are conserved during evolution.

**18. GPI-anchored proteins are required for cell wall biosynthesis.**
Shaun M. Bowman, Amy Piwowar, Mash'el Al Dabbous, John Vierula*, and Stephen J. Free, Department of Biological Sciences, University at Buffalo, Buffalo, NY 14260 and *Department of Biology, Carleton University, Ottawa, Ontario, Canada, K1S 5B6 email: free@buffalo.edu

We have isolated and characterized Neurospora mutants affected in four of the genes involved in the biosynthesis of the GPI-anchor structure and demonstrated that the production of GPI-anchored proteins is required for normal cell wall biosynthesis. We show that mutants affected in GPI-anchoring produce a cell wall that has an altered protein and carbohydrate composition and grow in a tight colonial manner. The mutant cell walls are weak, as assessed by cell lysis assays, and are sensitive to the presence of calcofluor white and congo red, reagents that disrupt chitin biosynthesis. Proteomic analysis of the cell walls demonstrated the loss of a number of GPI-anchored proteins.

**19. The STE20/germinal center kinase POD6 interacts with the NDR kinase COT1 to coordinate polar tip extension in a dynein/kinesin-dependent manner in *Neurospora crassa***
Stephan Seiler†, Nico Vogt†, Carmit Ziv§, Rena Gorovits§, and Oded Yarden§.
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Members of the Ste20 and Ndr protein kinase families are important for normal cell differentiation and morphogenesis in various organisms. We characterized POD6 (NCU02537.2), a novel member of the GCK family of Ste20 kinases that is essential for hyphal tip extension and coordinated branch formation in the filamentous fungus *Neurospora crassa*. pod-6 and cot-1 (an Ndr kinase mutant) exhibit indistinguishable growth defects, characterized by cessation of cell elongation, hyperbranching and altered cell-wall composition. We suggest that POD6 and COT1 act in the same genetic pathway, based on the fact that both pod-6 and cot-1 can be suppressed by (i) environmental stresses, (ii) altering PKA activity and (iii) common extragenic suppressors (ropy, as well as gul-1, which is characterized here as the ortholog of the budding and fission yeasts SSD1 and Sts5, respectively). Unlinked non-complementation of cot-1/pod-6 alleles indicates a potential physical interaction between the two kinases, which is further supported by co-immunoprecipitation analyses, partial co-localization of both proteins in wild-type cells and their common mislocalization in dynein/kinesin mutants. We conclude that

POD6 acts together with COT1 and is essential for polar cell extension in a motor-protein-dependent manner in *N. crassa*.

**20. Action of a Salivary Antimicrobial Peptide, Histatin 5, on Neurospora**

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Human salivary glands make histidine-rich fungicidal peptides known as histatins, of which the best studied is histatin 5 (Hst5): DSHAKRHHGYKRKFHEKHHSHRGY. The normal target for histatins is Candida albicans (oral thrush), which is killed via a process of non-lytic cytodialysis. The surface-bound stress proteins Ssa1p and Ssa2p function as receptors for Hst5 (J. Biol. Chem. 278:28553, 2003), and the subsequent loss of small metabolites (incl. ATP) is mediated by deranged channel-like behavior of Trk1p (J. Biol. Chem. 279:55060, 2004), the protein primarily responsible for potassium accumulation in Candida. The high density of cationic charge on Hst5 is presumed to be essential to its entry and/or its intracellular binding. Hst5 also kills Neurospora, and that organism is more convenient for studying the rapid effects of Hst5 at the plasmalemma: esp., its influence on membrane voltage and resistivity. Under standard conditions, 0.06 mM Hst5 depolarizes the Neurospora plasma membrane, from about –200 mV to about –50 mV, with a time-course similar to that observed in response to 1 mM cyanide (J. Membr. Biol. 14:305, 1973). Depolarization is accompanied by a high-voltage leak that is consistent with the deranged channel behavior of Trk1p in Candida, and is kinetically consistent with deactivation of the plasma-membrane proton pump (Pma1p) by depletion of cytoplasmic ATP. The two most likely intracellular actions of Hst5 in Neurospora are thus i) disruption of the potassium transporter, Trk1p, and ii) uncoupling of mitochondrial oxidative phosphorylation. We are soliciting the collaboration of genetics laboratories interested in constructing pertinent gene-deletion strains of Neurospora.

**21. The Ribosomes of *Neurospora crassa*.**

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This describes the results of data-mining of the *Neurospora crassa* genome for the genes encoding the structural ribosomal RNA components and the polypeptide components of the cytosolic and mitochondrial ribosomes. For the rRNA genes, homologous Blast probes with known *N. crassa* sequences was used to identify the multiple copies. For the ribosomal polypeptide genes, the probe sequences were predominatly from *Saccharomyces cerevisiae*. For cytosolic ribosomes, 33 small subunit polypeptide genes, 44 large subunit polypeptide genes and 3 acidic polypeptide genes, mainly by homology with yeast. For the 18S, 5.8S and 28S rRNA units, 43 copies spread over circa 40 assembly 7 contigs have been found, compared to an earlier Southern blotting estimate of 100-200 copies. It thus appears that the nucleolus organiser region is significantly under-represented in genome assembly 7. For the 5S rRNA genes, 87 copies (including some apparent pseudogenes) spread over 42 assembly 7 contigs have been identified, within the limits of an earlier estimate. For mitochondrial ribosomes, 18 small subunit polypeptide genes (all except 1 nuclear-encoded) and 33 large subunit polypeptide genes (all nuclear-encoded) were identified.

**22. Circadian Rhythms in Neurospora crassa: New fluorescent reporters.**

Michael Ferry†, Stuart Brody‡, Jeff Hasty† † Department of Bioengineering, UCSD; ‡Division of Biological Sciences, UCSD

We have made new, novel fluorescent constructs useful for studying the circadian oscillator in N. crassa. These are fluorescent proteins that are not excitable or emit light at wavelengths below 520 nm since the WC-1 light receptor responds to light in this range. We are in the process of creating a fusion protein between the FRQ protein and the mCherry protein (excitation max: 587nm, emission max 610 nm) linked by a sequence designed to separate the domains of the fusion protein. We have also made constructs between mCherry and the rhythmic ccg2 promoter, and the inducible qa2 promoter and are currently targeting them to the His-3 locus of the FGSC # 9717 _ mus-51::bar+; his-3 mat A strain. To target the frq locus we have generated a construct with both positive (hygromycin resistance) and negative (fluorodeoxyuridine sensitivity) markers and have transformed the N. crassa strain ?mus-51::bar+ FGSC # 9718. We are currently targeting our FRQ-mCherry construct to the native frq locus of this strain by growing them on media containing fluorodeoxyuridine and selecting against the untransformed cells. These reporters will allow us to: 1). Create a mathematical model for this oscillator which can be tested quantitatively; 2) obtain new information about this oscillator (amplitudes of components, production, degradation rates, etc) that would be of general interest to the study of circadian oscillators and of specific application to the modeling of this system.

**23. Genetic analysis of *POL32* homolog in *Neurospora crassa.***

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DNA translesion synthesis (TLS) is an important mechanism to avoid replication fork collapse by DNA damage. The *POL32* gene of *Saccharomyces cerevisiae* encodes a non-essential subunit of DNA polymerase *f*Â which is the major replicative DNA polymerase in eukaryotic cells. In *S. cerevisiae pol32* mutant is deficient in UV-induced mutagenesis, suggesting Pol32 act in TLS. In this study, we present identification and characterization of the *POL32*-homolog in *N. crassa*. A knockout mutant of *Nc POL32* is viable and sensitive to several DNA damaging agents. Epistasis analyses indicated that *Nc POL32* belong to same epistasis group with *mus-8* (*RAD6* homolog), *uvs-2* (*RAD18* homolog), *mus-41* (*RAD5* homolog), and *upr-1* (*REV3* homolog, catalytic subunit of Pol*f*Ä), suggesting a role for *Nc POL32* in post- replication repair (PRR). We also found that Nc pol32 mutant is deficient in UV-induced mutagenesis, indicating that Pol32 is required for UV-induced TLS. Further genetic analysis revealed that *pol32* is lethal in combination with mutation in *mus-9* (*MEC1*/ATR homolog), but not in combination with mutation in *mus-21* (*TEL1*/ATM homolog). Surprisingly, we also showed that *POL32* and *TEL1*/ATM belong to same epistasis group. These findings suggest that *Nc POL32* plays a role in both TLS and checkpoint pathways.

**24. Apical microtubule dynamics in *Neurospora crassa* and their role in rapid growth.**

Maho Uchida[1*], Rosa R. Mouriño-Pérez[2] and Robert W. Roberson[1] . [1]School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA [2]Departamento de Microbiología. Centro de Investigación Científica y Educación Superior de Ensenada. Ensenada, B. C. Mexico *Correspondence: maho.uchida@asu.edu


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*Neurospora crassa* exhibits one of the fastest hyphal growth rates among filamentous fungi. To assess the probable role of microtubules (MTs) in such rapid extension rate, we monitored the dynamic instability of GFP-tagged apical MTs using live-cell imaging methods. Results obtained in mature hyphae revealed that MT polymerization rates were twice as fast as those reported in *Aspergillus nidulans*, while MT depolymerization rates in both species were similar. Furthermore, MT polymerization rates in *Neurospora* were much faster than in any other system studied thus far, including plant and mammalian cells. In order to address the influence of motor protein mutations on dynamic instability, GFP- tagged MTs were examined in ropy 1 and nkin strains. In these strains, MT polymerization rates were reduced by one half relative to the wild type. These results will be further analyzed with impending data obtained in slow growing germlings.

**25. RHO-4 localization during conidial development**.

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Proteins in the Rho family are small monomeric GTPases primarily involved in polarization, control of cell division and reorganization of cytoskeletal elements. We show that *rho-4* loss-of-function mutants in *Neurospora crassa* lack septa. GFP-tagged RHO-4 was targeted to septa and to the plasma membrane. RHO-4 formed a ring at incipient septation sites that appeared to constrict with the formation of the septum. We studied RHO-4 localization during conidiation and in the conidial separation mutants *csp-1* and *csp-2*. Interestingly, RHO-4 disappears from the septum during or after the formation of the second septum and becomes cytoplasmically localized. We show that cytoplasmic localization of RHO-4 in conidia is dependent on the negative regulator RDI-1, a predicted Rho guanine dissociation inhibitor. Further, *rdi-1* mutants show greatly increased septation compared with a wild- type strain. Experiments to test direct interaction between RHO-4 and RDI-1 will be discussed.

**26. Nutrient sensing G protein coupled receptors in *Neurospora crassa.***

Liande Li and Katherine Borkovich, Plant Pathology Department, UC Riverside

*Neurospora crassa* is able to utilize a wide variety of carbon and nitrogen sources. The *N. crassa* predicted G protein coupled receptor (GPCR) GPR-4 is similar to a group of carbon-sensing GPCRs characterized in yeasts. Levels of *gpr-4* are greatly elevated during growth on a poor carbon source. *gpr-4* deletion mutants have reduced mass accumulation compared to wild type when starved for carbon or when cultured on high levels of poor carbon sources and also undergo inappropriate asexual sporulation in submerged cultures. Epistasis analyses and two hybrid assays support interaction of GPR-4 with GNA-1 in vivo. Analysis of various facets of cAMP metabolism are consistent with a downstream signaling pathway at least partially dependent on cAMP levels. Our results support the hypothesis that GPR-4 is coupled to GNA-1 in a pathway that senses and regulates the response to carbon starvation and/or poor carbon sources in *N. crassa*.

## DEVELOPMENTAL BIOLOGY

**27. Origin and Significance of Vacuolar Proliferation during Nutrient Restriction**
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Vacuoles in fungi, especially in mycelial fungi, have long been known as polyfunctional, highly pleiomorphic organelles, whose appearance is strongly influenced by age and by nutritional status. A particularly striking example of age- and nutrition-linked morphological change was noted in 1968 by Robertson and Rizvi (Ann. Bot. 32:279, who found that in mature hyphae, “cells” lying more than ~1 cm behind the growing tips are often stuffed with a vacuolar “foam”. Similar pictures are generated by several hours of carbon starvation; and these vacuoles accumulate a variety of “cytoplasmic” dye probes (e.g., BCECF) presented as neutral esters (J. Exp. Biol. 196:419, 1994), and greatly enhance intracellular proton buffer capacity (D. Sanders & C.L. Slayman, unpublished expts.). As viewed in the light microscope (phase contrast or interference contrast), vacuoles begin to proliferate within a few minutes of the onset of carbon starvation, first forming a “bubble-wrap” against the hyphal plasmalemma, and then crowding the interior, as the number and size of vacuoles increases. After several hours of carbon starvation, the vacuolar foam can occupy more than 80% of the apparent intrahyphal volume, and the foam can be fused into large vacuoles by mechanical shock. Upon re-presentation of a carbon source, the whole morphological process reverses, with a roughly symmetric time-course. We are beginning an investigation into the biogenesis of this vacuolar proliferation, by means of the newly available Neurospora genomic microarrays.

**29. Characterization of the *bd* mutation: filling the gap between ROS and RAS.**
Luis F. Larrondo, Bill Belden, Alan Froehlich, Mi Shi, Chen-Hui Chen, Jennifer Loros and Jay Dunlap. Department of Genetics, Dartmouth Medical School, Hanover, NH-03755. U.S.A.

The *bd* mutation in *Neurospora crassa* has been an integral tool, for the last 40 years, in the study of circadian rhythms. This mutation confers a semidominant phenotype that allows the clear visualization of the conidiation (banding) pattern, which is circadianly regulated by the *N. crassa* clock. Using a SNP mapping strategy, developed as part of the Program Project, we have identified *bd* as a single point mutation in the *ras1* gene, changing T79 to I. A genomic fragment generated from a bd strain and containing only the *ras1* gene was able to confer the bd phenotype when transformed into a WT strain. Based on the extensive literature for Ras biology and on the hypothesis that hyperoxidant states trigger cell differentiation events (Hansberg, W. and Aguirre, J. J. Theor. Biol. 142:201-221; 1990), we have postulated that the *bd* mutation leads to an imbalance in ROS (Reactive Oxygen Species) levels, which in return, is responsible for the banding phenotype observed on race tubes. A variety of genetic and biochemical data based on gene replacements and physiological analyses of strains are consistent with this hypothesis.
L.F.L. is a PEW Postdoctoral Fellow

**GENE REGULATION**

**30.**

**31. SAD-2 is required for Meiotic Silencing by Unpaired DNA and perinuclear localization of SAD-1 RNA- directed RNA polymerase.**
Patrick K.T. Shiu[1], Denise Zickler[2], Namboori B. Raju[3], Gwenael Ruprich-Robert[2], and Robert L. Metzenberg[4]. [1]University of Missouri, Columbia, Missouri. [2]Université Paris-Sud, Orsay Cedex, France. [3]Stanford University, Stanford, California. [4]California State University, Northridge, California.

A gene unpaired during the meiotic homolog pairing stage in Neurospora generates a sequence-specific signal that silences the expression of all copies of that gene. This process is called Meiotic Silencing by Unpaired DNA (MSUD). Previously, we have shown that SAD-1, an RNA-directed RNA polymerase (RdRP), is required for MSUD. We isolated a second gene involved in this process, *sad-2*. Mutated *Sad-2*$^{RIP}$ alleles, like those of *Sad-1*, are dominant and suppress MSUD. Crosses homozygous for *Sad-2* are blocked at meiotic prophase. SAD-2 colocalizes with SAD-1 in the perinuclear region, where siRNAs have been shown to reside in mammalian cells. A functional *sad- 2*$^{+}$ gene is necessary for SAD-1 localization, but the converse is not true. The data suggest that SAD-2 may function to recruit SAD-1 to the perinuclear region, and that the proper localization of SAD-1 is important for its activity.

**32. Investigation of two putative *Neurospora crassa* heat shock transcription factors.**
Seona Thompson, Antonis Sotiriou, Nirvana Croft, Ray O'Keefe and Susan Crosthwaite. Faculty of Life Sciences, University of Manchester, Oxford Road, Manchester M13 9PT. susan.k.crosthwaite@manchester.ac.uk

The heat shock response is a ubiquitous response to adverse high temperatures. At the onset of heat shock there is a rapid increase in the expression of heat shock proteins and inhibition of the production of normal non-stress proteins at both the transcriptional and post-transcriptional level. The induction of most heat shock genes is regulated by heat shock factor (HSF), a transcription factor displaying high functional conservation between organisms. Two open reading frames, NCU08512 and NCU08480, encoding putative HSFs are present in the *Neurospora* genome. We have deleted NCU08512 and preliminary data indicates that it is an essential gene. The single HSF encoded in the *S. cerevisiae* genome is also essential for viability. To find out if the putative *Neurospora* HSFs could function in yeast we transformed a diploid strain of *S. cerevisiae* heterozygous for the HSF gene with yeast expression vectors carrying NCU08512 and NCU08480 cDNA. Of note, several splice variants of NCU0840 are synthesized that encode altered forms of the predicted NCU0840 protein. The transformed yeast strain was induced to sporulate and our initial analysis of tetrads indicates that the *Neurospora* genes cannot functionally replace the yeast HSF. If NCU08512 and NCU0840 do indeed encode HSFs this result suggests that, like human HSF1, they may require different signals to their yeast counterpart for activation.

**33. Mutations in a gene repressor or a glucose transporter result in sustained gene photoactivation in Neurospora.**
Maria Olmedo, Laura Navarro-Sampedro and Luis M. Corrochano Departamento de Genetica, Universidad de Sevilla, Spain corrochano@us.es

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. The rco mutants were isolated by the abundant expression of con-10 in vegetative mycelia. 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. 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. The abundant photactivation of con-10 and con-6 in rco and vivid strains is best observed after five hours of light. The rco and vivid mutations do not alter the stability of the con-10 and con-6 mRNAs, suggesting that the sustained photoactivation is due to a high transcriptional rate that is not subjected to adaptation to light. The threshold of con-10 and con-6 photoactivation is significantly lowered in the rco-1 mutant, but remains unchanged in the rco-3 and vivid mutants. The circadian clock in these mutants does not seem to be altered. We propose that VVD, RCO-1, and RCO-3 participate in the mechanism responsible for transient gene photoactivation.

**34. The dominant suppressor of repeat-induced point mutation (RIP) in the Adiopodoume strain of *Neurospora crassa* is linked to an allele for a variant catalytic subunit of DNA polymerase zeta.**

Ranjan Tamuli, C. Ravindran and Durgadas P. Kasbekar Centre for Cellular and Molecular Biology, Hyderabad 500 007, India.

Crosses involving the Adiopodoume strain of *Neurospora crassa* are defective for repeat-induced point mutation (RIP), a genome defense mechanism of fungi. Mapping studies suggested that the Adiopodoume strain contains a dominant suppressor of RIP (Srp) located proximal to mat on linkage group (LG) IL. Srp strains can occasionally lose the dominant RIP suppressor phenotype and became phenotypically intermediate or non-suppressor. Localization of crossovers with respect to molecular markers further narrowed Srp to a ~34 kb genomic segment that is ~26 kb proximal to mat. This segment contains the upr-1 gene, which codes for the catalytic subunit of the translesion DNA polymerase zeta (Pol z). The upr-1 allele of Adiopodoume contains several synonymous and non-synonymous mutations relative to the standard Oak Ridge (OR) allele, suggesting that it has undergone rapid evolution. Another translesion polymerase, Rev1, is encoded by the mus-42 gene on LG IL and a Pol z regulatory subunit is encoded by the mus-26 gene on LG IVR. The mus-26 and mus-42 genes do not show evidence for rapid evolution in the Adiopodoume strain. The upr-1, mus-26 and mus-42 gene products are not required for RIP. We suggest that dominant RIP suppression might result from the variant Pol z catalytic subunit interfering with some other DNA polymerase that is required for RIP. The other genes in the candidate region have either very few or no mutation.

**35. Identification of Proteins Binding to the *cis* Regulatory STRE Element of the *gsn* Promoter. A Mass Approach.** Freitas, F. Z. ; Bertolini, M. C. Departamento de Bioquímica e Tecnologia Química, Instituto de Química, UNESP, Araraquara, SP, Brazil.

Cells respond and adapt to environmental stressing conditions such as heat shock by modulating metabolic responses. In the yeast *Saccharomyces cerevisiae*, heat shock activates multiple stress related genes by the binding of the *trans* acting elements to the *cis* elements HSE (Heat Shock Element) and STRE (STress Responsive Element) leading to transcription activation. The *gsn*

gene, which encodes the enzyme glycogen synthase in *Neurospora crassa* is repressed at transcriptional level when cells are exposed to high temperatures (heat shock). Analysis of the gsn 5'-flanking region showed the presence of multiple HSE elements and two STRE elements, which were demonstrated to be involved in the gene transcription regulation by EMSA (Electrophoretic Mobility Shift Assay). Our main purpose is to identify the protein(s) that bind to the STRE elements in order to study how these elements function in the regulation of gene transcription. Nuclear extract of heat-shocked mycelia was analyzed by SDS-PAGE and SouthWestern blotting, and fractionated by affinity chromatography. EMSA identified a protein fraction showing higher DNA-binding activity. To enrich this approach the crude nuclear extract was fractionated by SDS-PAGE, the fractions of molecular mass intervals were excised from the gel, crushed into a renaturation buffer and eluted from the gel. The fractions were analyzed by EMSA and the MW range was identified. In the same way, the nuclear proteins were fractionated by isoeletric focusing, and the pI of the active fractions was determined. All protein fractions were subjected to trypsin digestion, and the peptides were analyzed by mass spectrometry (MALDI). Supported by FAPESP, CAPES, CNPq and LNLS.

**36. Two Circadian Timing Circuits in *Neurospora crassa* Cells Share Components and Regulate Distinct Rhythmic Processes.** Zachary A. Lewis, Renato M. de Paula, Andrew V. Greene, Kyung Suk Seo, Louis W. Morgan, Michael W. Vitalini, and Deborah Bell-Pedersen Center for Research in Biological Clocks, Department of Biology, Texas A&M University, College Station, TX 77843

In *Neurospora crassa*, the *frequency (frq), white collar-1 (wc-1)*, and *wc-2* genes, and their corresponding proteins (FRQ, WC-1, and WC-2), comprise the core circadian FRQ-based oscillator that is directly responsive to light and drives daily rhythms in spore development and gene expression. However, physiological and biochemical data have suggested the existence of additional timing circuits in the cell that function in the absence of FRQ (collectively termed FRQ-less oscillators, FLOs). We previously identified an evening-peaking gene, W06H2 (now called *clock-controlled gene 16* (*ccg-16*)), which is expressed with a robust circadian rhythm in cells that lack FRQ protein. Here we demonstrate that *ccg-16* rhythms are generated by a temperature compensated FLO that, similar to the FRQ-based oscillator, requires functional WC-1 and WC-2 proteins for activity. Furthermore, we show that FRQ is not essential for rhythmic WC-1 levels. Our results are consistent with a model in which two circadian timing circuits exist within Neurospora cells, both of which require the WC proteins.

**37. VVD's role in entrainment of the *Neurospora crassa* circadian clock.**
Mark Elvin and Christian Heintzen. Faculty of Life Sciences, Michael Smith Building, The University of Manchester, Manchester, M13 9PT, UK

The PAS/LOV protein VVD is a modulator of light responses in Neurospora. We have recently shown that VVD modulates clock resetting at dawn and dusk with implications for entrainment of the Neurospora circadian clock to light-dark cycles (Elvin et al., 2005, Genes Dev 19, 2593-2605). In *vvd* knockout strains the phase of clock-controlled conidiation is altered when compared to wild-type and a second round of conidiation is initiated. The cause for this second peak of conidiation in vvd knockout strains is unclear but may be due (amongst other possibilities) to a reduction in the threshold for clock-independent light- induced condiation,

repression of conidiation, or unmasking of a second pacemaker that controls a second round of conidiation in light-dark cycles. Data will be presented that distinguish between these possibilities.

## GENOMICS AND PROTEOMICS

### 38. Systems Biology of the Biological Clock.

W Dong, Y. Yu, C. Altimus, J. Griffith, M. Morello, L. Dudek, H.-B. Schuttler, & J. Arnold. Genetics Department, University of Georgia. arnold@uga.edu.

Genetic networks as models for the biological clock have been developed and successfully fitted to published data in the literature. A series of microarray experiments were performed on N. crassa liquid cultures placed in the dark for 48 h to measure RNA levels of all 11,000 N. crassa genes every 4 hours (13 time points) to refine the fitted ensemble of models. A total of 3594 genes were found to be oscillatory with a period between 16 and 30 hours. Among these, 3594 oscillatory genes, 1,835 (16.6%) genes were found to have a WHITE-COLLAR complex consensus binding site for inclusion in the genetic networks. Microarray results were validated by real-time PCR. Using several different methods of analysis the clock and its associated genes was placed in the context of the metabolic web. The data are being used to guide additional microarray experiments to refine the specification of the genetic networks describing the clock. As a further test of the genetic networks, light entrainment experiments were performed using race tubes to test genetic network light response. Work is supported by NSF QSB-0425762.

### 39. The *Neurospora crassa* e-Compendium.

Alan Radford, IICB, Faculty of Biological Sciences, The University of Leeds. a.radford@leeds.ac.uk

The initial "gene list" has developed, in both the number of genes listed and the level of sophistication, to the point where it has been named the "e-Compendium". The URL is http://www.bioinf.leeds.ac.uk/~gen6ar/newgenelist/genes/gene_list.htm. The e-Compendium continues the development of the gene database from Barratt et al (1954) Adv in Genet., Perkins et al (1982) Micro Revs, and Perkins et al (2001) The Neurospora Compendium into the era of the WwW. It provides a browsable and searchable database of genetic and molecular data about individual genes (gene symbol and name, map location, phenotype, gene product, sequence data, contig data, references etc.) with cross-links to sequence databases, Exapasy Enzyme, Pubmed, graphic images of morphological mutants, biosynthetic pathway diagrams etc.. Recent additions to the e-Compendium include a Java-based utility for compiling linkage maps from the genetic and physical data in the e-Compendium, and another utility for the extraction into a spreadsheet of all the contig data on genes in the database. New genes are added to the database and additional data added as the curator finds it or as individual workers submit new information. The e-Compendium is as up to date as you, the Neurospora community, make it. Please use the in-built utilities to submit new and amended information.

### 40. Comparative Genomic Hybridizations using the Genus *Neurospora*.

Luz B Gilbert[1], Lee Chae[1], Takao Kasuga[1], Jeff Townsend [2], John W. Taylor[1], [1]Department of

Plant and Microbial Biology, UC Berkeley, [2]Department of Molecular and Cellular Biology, University of Connecticut, lgilbert@berkeley.edu

Comparative Genomic Hybridization (CGH) using DNA microarrays is becoming a popular method of determining phylogenetic relationships among individuals and even species. Although researchers praise the large-scale genome information output as an advantage of this technology for use in phylogenetic analysis, few have questioned the reliability of CGH to correctly determine evolutionary relationships. With the tools at my disposal for the filamentous fungus *Neurospora*: a genome sequence, a DNA microarray, and a well supported phylogeny for this genus, I had the ability to rigorously address the utility of CGH for phylogenetic studies using both experimental and simulated data.

**41. High throughput mutation procedure for *Neurospora* genes**

Luibov Litvinkova, Lorena Altamirano, Gyungsoon Park, John Jones, and Katherine Borkovich
Department of Plant Pathology, University of California, Riverside, CA

Deletion mutations have been made in annotated *Neurospora* genes using a high throughput procedure as part of an NIH-funded Program Project (P01). We have used *hph* marked KO cassettes created by yeast recombinational cloning techniques along with *mus-51* and *mus-52* deletion mutants as recipient strains for transformation. We have completed construction of of 225 *Neurospora* knockout mutants and the strains have been submitted to FGSC. Due to ascospore inviability, we were not able to generate homokaryotic deletion mutants for another 56 genes; heterokaryotic transformants for these 56 genes were submitted to FGSC. Homokaryons were isolated using the microconidiation procedure for seven of these genes; these strains are still in the *mus* deletion background. The list of submitted strains is available at the *Neurospora* genome project website (http://www.dartmouth.edu/%7Eneurosporagenome/knockouts_completed.html). At this time, 384 genes are in various stages of the knockout procedure. We have also developed informatics tools for tracking of genes during the knockout procedure. First, we have designed a program (http://borkovichlims.ucr.edu/southern/) that allows automated identification of the appropriate restriction enzyme to use during Southern analysis for confirmation of the gene replacement. Second, we have developed and implemented a Laboratory Information Management System (LIMS; www.borkovichlims.ucr.edu) for our gene knockout process. All plates and tubes used during the knockout procedure are labeled with barcodes and managed systematically. Use of these tools and our current progress in creating knockout mutants will be presented.

**42. Proteomic Approaches to Mitochondrial Function.** Richard A. Collins Department of Medical Genetics and Microbiology University of Toronto Toronto, Canada M5S 1A8

There are many examples of mitochondrial responses to changes in the functional or metabolic state of the cell. For example, inhibition of mitochondrial protein synthesis leads to the synthesis of alternative oxidase and to an increase in cytochrome c content. However, we have no global-scale information about the number and identity of proteins whose concentration changes in such a situation. We know anecdotally about the few proteins that we have chosen to examine, but does the cell respond by changing the amounts of a few proteins? Or a few dozen? Or a few hundred? To answer this question, I am determining what fraction of the mitochondrial proteome

can be resolved, detected and quantified using a variety of fractionation, 2D gel, and mass spectrometry approaches. This will provide an experimental platform with which to investigate how the cell compensates for a variety of alterations in mitochondrial function.

**43. Genetic analysis of cytoplasmic dynein in *Neurospora crassa***

Michael Plamann, David Madole, Robert Schnittker, and Elizabeth Wulff. University of Missouri-Kansas City, School of Biological Sciences, Division of Cell Biology and Biophysics, Kansas City, MO 64110

Cytoplasmic dynein is a large, microtubule-associated motor complex that facilitates minus-end-directed transport of various cargoes. Dynein heavy chain (DHC) is >4000 residues in length, with the last two-thirds of the heavy chain forming the motor head. Six domains within the dynein motor exhibit varying degrees of homology to the AAA+ superfamily of ATPases. These domains are followed by a distinct C-terminal domain and together form a ring-like structure from which a microtubule-binding domain protrudes. Using a genetic assay, we have isolated over 50 DHC mutants of *Neurospora* that produce full-length proteins that are defective in function. We have identified DHC point mutations in all domains within the dynein motor head. To help define the mechanism(s) by which specific mutations lead to loss of dynein activity we have isolated revertants for a subset of DHC mutants. We are now in the process of identifying the respective intragenic suppressor mutations in these revertants.

**44. Cytoplasmic dynein, cytoplasmic streaming and nuclear movement in *Neurospora crassa***

Michael Plamann, Robert Schnittker, Samuel Schowengerdt, and Omar Abdulla Almoghrabi. University of Missouri-Kansas City, School of Biological Sciences, Division of Cell Biology and Biophysics, Kansas City, MO 64110

Cytoplasmic dynein is a large, microtubule-associated motor complex that is required for nuclear distribution in nearly all eukaryotes. In germinated conidia of *Neurospora*, dynein null mutants have clusters of nuclei in distal regions while hyphal tips are often anucleate. Using a nuclear-localized GFP constructed by Dr. Michael Freitag, we examined nuclear distribution in mature colonies of dynein mutants. In contrast with germinated conidia, nuclear distribution is nearly wildtype in hyphae at the colony edge of dynein mutants. Examination of these colonies revealed that there is very rapid streaming of cytoplasm and nuclei (up to ~50 μm/sec) from the interior of the colony to growing tips at the colony periphery. Hyphal fusion is critical for the formation of interconnecting hyphal networks within the interior of colonies. We found that *Neurospora* soft (*so*) mutants, which are blocked in hyphal fusion, lack cytoplasmic streaming. Dynein, soft double mutants were constructed and found to grow very slowly and produce short aerial hyphae with no conidia. The results suggest that cytoplasmic dynein is required for movement of nuclei in newly formed hyphae from germinating spores and young colonies, but nuclear movement in mature colonies results primarily from the bulk flow of cytoplasm.

**45. withdrawn**


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## OTHER

**46. The mitochondrial ribosomal RNA genes are as identified earlier by other workers**. S.
Conway, P. J. Yeadon, F. J. Bowring and D. E. A. Catcheside
School of Biological Sciences, Flinders University, PO Box 2100, Adelaide, South Australia, Australia

While the Neurospora genome appears to lack an *msh4* orthologue, a TBlastn search, using *msh4* sequences from *Saccharomyces cerevisiae*, *Homo sapiens* and *Mus musculus*, identified an *msh4* candidate on contig 3.27. This putative gene, which has an ATPase domain and a DNA-binding mismatch repair domain typical of MutS family members, was deleted by split-marker replacement. In crosses of deletion strains, sporogenesis is delayed and there is a four- to six-fold reduction in fertility. These defects also occur in heterozygotes showing that the gene is subject to meiotic silencing. The frequency of crossing over in the intervals flanking *his-3* is halved and, as is the case for yeast *msh4* mutants, residual crossovers show no interference, suggesting that we have knocked out Neurospora *msh4*. Interestingly, allelic recombination at *his-3* appears to be elevated in the mutant, a result that was not predicted using the yeast *msh4*Δ phenotype as a model.

**47. Novel Linear Vector Imparts High Stability to "Unclonable" DNA Sequences.**
Ronald Godiska[1], Vinay Dhodda, Valerie Gilbert[1], Nikolai Ravin[2], and David Mead[1]. [1]Lucigen Corp., Middleton, WI. [2]Centre BioEngineering RAS, Moscow, Russia

We have developed a novel linear *E. coli* cloning vector incorporating transcription-free cloning capabilities. This vector showed unprecedented ability to maintain large AT-rich inserts (>10-20 kb), as well as di-, tri-, and tetra-nucleotide repeats. These inserts were not stable in conventional plasmids. Torsional strain inherent to supercoiled plasmids can induce localized melting and generate secondary structures, which are substrates for deletion or rearrangement by resolvases and replication enzymes. For example, tandem repeats and palindromic sequences are highly unstable, presumably due to cleavage of hairpin structures or to replication slippage across the secondary structures. Most plasmid vectors also induce strong transcription and translation of inserted fragments, and they allow transcription from cloned promoters to interfere with plasmid stability. As a result, many DNA sequences are deleterious or highly unstable, leading to sequence "stacking", clone gaps, or a complete inability to construct libraries, especially from AT-rich genomes or toxic cDNAs. These problems appear to be greatly diminished with the transcription-free, linear vector. This vector showed numerous other advantages in cloning, such as the ability to clone inverted repeats, a significantly lower bias in size of fragments cloned, a low background of non-recombinants, and simple construction of large-insert libraries.

**48. Nonself recognition and Programmed Cell Death in *Neurospora crassa***
Karine Dementhon, Isao Kaneko, Julie Welch and N. Louise Glass Plant and Microbial Biology department, UC Berkeley, California, 94720. email: karine@berkeley.edu

Filamentous fungi are capable of undergoing hyphal fusion with each other to form a vegetative heterokaryon (genetically different nuclei in a common cytoplasm). However, if individuals undergoing hyphal fusion differ in allelic specificity at any one of a number of heterokaryon

incompatibility loci called *het*, the heterokaryotic cell is rapidly compartmentalized and destroyed by a programmed cell death reaction. This phenomenon is called heterokaryon incompatibility (HI). This nonself recognition is believed to reduce the risk of transmission of infectious elements such as mycoviruses and debilitated organelles throughout fungal populations, and to restrict resource plundering between individuals. In *Neurospora crassa*, we determined that *het-c* HI requires non-allelic interactions with *pin-c*, a locus closely linked to *het-c*. HET-C is a plasma membrane protein and PIN-C encodes a HET domain protein. Isolates from populations fall into one of three allelic specificity groups for the *het-c* locus: *het-c(1)*, *het-c(2)* and *het-c(3)* (previously called Oakridge, Panama and Groveland, respectively). They differ by a highly variable domain (specificity domain) which determine allelic specificity. *Pin-c* alleles in isolates of alternate *het-c* specificity are extremely polymorphic. *Pin-c* alleles can also be classified into 3 groups: *pin-c(1)* , *pin-c(2)* and *pin-c(3)*, which are in severe linkage disequilibrium with *het-c*. Similar to *het-c*, *pin- c* alleles show trans-species polymorphisms. Together, our data suggest that balancing selection operates at *het-c/pin-c* haplotype to maintain allelic diversity for nonself recognition and HI in *N. crassa*.

**49. Systems Biology of the Quinic Acid Cluster in *Neurospora crassa***

Allison Koch and Dr. Jonathan Arnold of the University of Georgia

The quinic acid (*qa*) gene cluster, in *Neurospora crassa*, encodes the organism's pathway for utilizing quinic acid (QA) as a sole carbon source. When *N. crassa* is grown on sucrose medium, the quinic acid gene cluster is inactive. When the organism is shifted to a medium that has quinic acid instead of sucrose, the *qa* cluster genes are activated. To see the changes in expression of these genes, samples were taken at eight time points after the shift to quinic acid from 0 to 8 hours. The RNA samples from these time points were analyzed with micro array chips, which gave the precise measurements of the RNA levels of each of the 11,000 genes in the genome. A total of 549 genes respond to a shift to quinic acid, and 164 of these have a QA-1F binding site. These data are being used to evaluate a genetics network describing: (i) *qa* gene cluster regulations; (ii) QA metabolism; (iii) catabolic repression by glucose or sucrose

**50. Protein interaction studies of the *Neurospora crassa* $G_i$ alpha homolog, GNA-1.**

Sara Martinez and Katherine Borkovich. Department of Plant Pathology, University of California, Riverside, CA 92521

Heterotrimeric G protein signaling pathways are important for eukaryotic cells to respond to environmental stimuli. Heterotrimeric G proteins consist of a triad of proteins named G alpha, G beta, and G gamma. In pathogenic filamentous fungi, these proteins are important for virulence. In the non-pathogenic filamentous fungus, *Neurospora crassa*, there are three G alpha subunits (GNA-1, GNA-2, and GNA-3), one G beta subunit (GNB-1), and one G gamma subunit (GNG-1). It has been previously reported that GNA- 1 is involved in female fertility, vegetative growth, and stress response. Such a broad impact of this protein suggests that there are multiple proteins with which it interacts. A cDNA library was made using RT-PCR to make cDNA and yeast recombination to make a library in the yeast two-hybrid AD vector (Clontech). The RNA was obtained from 16hr submerged culture tissue and 6 day old SCM plates. This library was screened with GNA-1 using a mating strategy, and several interesting interactors were identified. This poster describes the techniques used for the screen and follow up experiments on some of

the interesting hits. Identifying novel downstream effectors of GNA-1 will give insight as to how eukaryotes respond to stimuli, and also give us a clue as to how GNA-1 affects such a broad range of tissues types in *Neurospora crassa*.

**51. Meiotic recombination in Neurospora**

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

Our knowledge of meiotic recombination is based upon detailed analysis of a small number of organisms. The genome project coupled with recent advances in methodology makes a comparable analysis of the process in Neurospora feasible. We are currently using fusion-PCR/split-marker deletion to examine the role of several genes in Neurospora recombination. We are also trying to develop a visual recombination reporter system using histone-GFP fusions targeted to a recombination hotspot.

**52. Activities and Research at the Fungal Genetics Stock Center.**

Kevin McCluskey, Sheera Walker and Mike Plamann. School of Biological Sciences, University of Missouri- Kansas City.

In 2005 the FGSC distributed nearly two times as many strains as ever before. We sent out 2125 fungal strains to 138 different recipients in thirty-one different countries. Of these, 1509 were Neurospora strains. We have also distributed significant numbers of cloned genes and library clones. Not including strains from the functional genomics program, we added 406 new strains to the collection. 277 of these were Neurospora strains. Six were other organisms that have been sequenced or will be soon.

We have also added a number of new resources to the FGSC web-site including all back issues of the Aspergillus newsletter. We also built an interface that allows us to link between online methods and existing indices.

In our continuing effort to identify the function of temperature sensitive unknown lesions, we have complemented two different unknown genes. One of these, *un-16*, had been previously identified. Nevertheless, we have attempted to develop this gene as a selectable marker for transformation in Neurospora.

The FGSC is supported by the US National Science Foundation, Grants 0235887 and 0603830

**53. The Neurospora crassa community genome annotation project.**

Heather M. Hood[1], Matthew R. Henn[2], Dave DeCaprio[2], James E. Galagan[2], Bruce W. Birren[2], Jay C. Dunlap[3], & Matthew S. Sachs[1]

[1]Oregon Health & Science University, Beaverton, OR 97006; [2]Broad Institute of MIT & Harvard, Cambridge, MA 02141; [3]Dartmouth Medical School, Hanover, NH 03755.

In order to produce a more accurate Neurospora protein-coding gene catalog, we need to include the wealth of information about gene structure and function that is contained within the scientific community. Automated gene calling predicts 10620 putative genes in Release 7. As of this conference, a web-based community annotation resource is released. Using this site, community annotators will be able to add a variety of information that automated annotation cannot capture to Release 7, which will be incorporated into the database. These data include gene symbol, gene name, and protein product. Incorrect gene models also can be fixed or alternative transcripts can

be appended to the predicted gene. Gene product function can be assigned using Gene Ontology terms along with the corresponding literature citation. Annotators can also indicate where the gene or its product is expressed using the Fungal Anatomy Ontology. To ensure accuracy, all community annotations will be manually inspected and curated. Integrating these community annotation data into the Neurospora genome annotation will greatly improve this resource.

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