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Volume 51 Article 16

Abstracts from the Neurospora 2004 conference

Neurospora Conference

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Abstract

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Volume 51 Article 16

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Neurospora Conference

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Abstracts from the Neurospora 2004 conference

Abstract
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Neurospora 2004

Neurospora 2004

March 25- 28, 2004

The group photo

The Neurospora 2004 photo gallery (courtesy of Matt Sachs)

Invited Talk Abstracts

Poster Abstracts

These abstracts are published online as a supplement to the Fungal Genetics Newsletter # 51.

Schedule of Activities

Thursday, March 25

3:00-6:00 pm, **Registration: Administration**
6:00-7:00 pm **Dinner: Crocker**
7:00-10:00 pm **Mixer: Merrill Hall**

Friday, March 26

7:30-8:30 am **Breakfast: Crocker**
8:30-12:00 noon: **Session I, Merrill Hall**

Postgenomics: how to capitalize on the genome sequence: Chair, Gloria Turner

8:30 - Welcome (Barry Bowman)
8:35 - **James Galagan** "Fungal Genomes at the Broad Institute: Neurospora and beyond"
9:00 - **Robert Metzenberg** "Where do we go from here? "
9:25 - **David Perkins** " *Neurospora* comes into its own "
9:50 - Break
10:15 -**Jay Dunlap** "Functional analysis of a model filamentous fungus "
10:40 - **Eric Selker** "Advances in genome defense and DNA methylation"
11:05 - **Kathy Borkovitch** "Impact of the genome sequence on analysis of signal transduction pathways in *Neurospora"*
11:30 - **Takao Kasuga** "mRNA profiling of conidial germination and hyphal growth in *Neurospora crassa* using oligomer microarrays "
11:45 - **Alan Radford** "Developing the *Neurospora* gene list"

12:00-2:00 pm **Lunch: Crocker**
Neurospora Business Meeting (Perkins award presentation, FGSC (Mike Plamann), Neurospora Newsletter (Matt Sachs), Methods Book (Deb Bell-Pedersen), Neurospora home page (Tony Griffiths)).

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2:00-5:30 pm: **Session II, Merrill Hall**

Organismal Biology: Chair, Tony Griffiths

2:00 - **Louise Glass "** Non-self recognition and programmed cell death in *Neurospora crassa* "
2:25 - **Myron Smith** " An incompatibility supergene at *het-6* in *Neurospora crassa*
2:50 - **John Taylor** "The genetic basis of adaptation: How good it's gonna be
3:15 - Break (vote for new policy committee members)
3:40 - **Dave Jacobson "** New findings of *Neurospora* in Europe and comparisons of diversity in temperate climates on continental scales "
4:05 - **Don Natvig "** What we know about local diversity in *Neurospora"*
4:30 - **Martha Merrow "** Photoperiodism in *Neurospora crassa"*
4:55 - **Heather Wilkinson "** Influence of parental genotype and mating type on quantitative traits in field isolates of *Neurospora crassa*"
5:10 - **Hirokazu Inoue** " Characterization of *ku-70* and *ku-80* knockout mutants in *Neurospora crassa"*

6:00-7:00 pm **Dinner: Crocker** 7:00-10:00 pm

Poster Session: Merrill Hall

Saturday, March 27

7:30-8:30 am **Breakfast: Crocker**

8:30-12:00 noon: **Session III, Merrill Hall**

Morphogenesis: Chair, Tony Griffiths
8:30 - **Salomon Bartnicki-Garcia "** The role of the Spitzenkörper in hyphal morphogenesis"
8:55 - **Oded Yarden** " Suppression of *N. crassa cot-1* morphology by environmental stresses and farnesol suggest that COT1 is involved in environmental stress response and quorum sensing"
9:20 - **Steve Free "** Identifying genes required for morphogenesis by a map and sequence strategy"
9:45 - Group Photo
10:00 - Break
10:20 - **Stephan Seiler** "Cellular morphogenesis in *Neurospora*"
10:45 - **Mike Plamann "** Genetic analysis of cytoplasmic dynein heavy chain"
11:10 - **Maho Uchida "** Mechanism of polarized growth and hyphal morphogenesis in *Neurospora crassa"*
11:25 - **Carolyn Rasmussen "** A novel rho-type GTPase required for septation in *Neurospora crassa"*
11:40 - **Greg Kothe** "Analysis of *Neurospora sirtuins*: evidence for control of telomeric silencing and homologous recombination"

12:00-1:00 pm **Lunch: Crocker**

2:00-5:30 pm: **Session IV, Merrill Hall**

Cell Biology: Chair, Barry Bowman
2:00 - **Barry Bowman "** Does transport of calcium into the vacuole affect hyphal morphology and tolerance of high external calcium?"
2:25 - **Roger Lew "** Hyphal ion currents, calcium and tip growth"
2:50 - **Holger Prokisch "** Integrative analysis of the mitochondiral proteome in yeast"
3:15- Break
3:40- **Frank Nargang "** Assembly of the mitochondrial TOM complex"
4:05 - **Nick Read "** Analyzing the cell biology of macroconidial germination and early colony development in *Neurospora crassa"*
4:30 - **Rick Collins "** Exceptionally fast RNA cleavage and ligation catalyzed by a Neurospora Varkud Satellite ribozyme"
4:55 - **John Kennell** "The use of mitochondrial plasmids to study nuclear-mitochondrial interactions in Neurospora"
5:10 - **Mary Anne Nelson** "EST evidence for alternative splicing in *Neurospora crassa*"

6:00-7:00 pm **Saturday Evening Banquet: Crocker**
6:50-6:55 pm David Perkins, Presentation of the Beadle and Tatum award
7:00 -7:30 pm **Plenary Lecture**, Stuart Brody Genetics and Kinetics

7:30-10:30 pm **Poster Session: Merrill Hall**

Sunday, March 28

7:30-8:30 pm **Breakfast: Crocker**

8:30-12:00 noon: **Session V, Merrill Hall**

Regulation: Chair, Deborah Bell-Pedersen
8:30 - **Jennifer Loros "** Molecular biology of the *Neurospora* circadian clock"
8:55 - **Cara Altimus "** Modeling and analysis of the biological clock in *Neurospora crassa"*
9:10 - **Patrick Shiu "** Meiotic silencing by unpaired DNA"
9:35 - **Giuseppe Macino** "The RNA-dependent RNA polymerase, QDE-1 is a rate-limiting factor in post-transcriptional gene silencing in *Neurospora crassa"*
10:00 - Break
10:20 - **Michael Freitag** "A *Neurospora* heterochromatin protein is essential for DNA methylation"
10:45 - **Matthew Sachs** "A *Neurospora* nascent polypeptide domain that regulates translation elongation"
11:10 - The Beadle and Tatum Award Lecture, **Yi Liu** Posttranslational and light regulation of the *Neurospora* circadian clock

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DOI: 10.4148/1941-4765.1146

12:00- **Check out**
12:00 - 1:00 pm, **Lunch: Crocker**

Invited Talk Abstracts

Postgenomics: how to capitalize on the genome sequence

Where do we go from here? Robert L. Metzenberg, Department of Chemistry, University of California, Los Angeles.

Our challenge is to grow our imagination and our taste fast enough to match the riches we have suddenly inherited. Like most of us here, I have my own wish-list of goals I would like to see reached -- goals that are no longer the stuff of science fiction. Foremost among these is to see Neurospora more fully developed as a powerful tool for understanding the biochemical architecture and dynamics of a macroscopic, coenocytic cell. This will require a much greater degree of cytochemical sophistication than most of us now possess. Second, I think Neurospora has great potential for shedding light on the role of introns. This may be straightforward, but laborious. Finally, I think that the very complexity of Neurospora in terms of signalling mechanisms, coupled with its simplicity of genetics, offers insights that cannot be duplicated in simpler organisms, and cannot be readily understood in more complex ones.

Neurospora comes into its own. David D. Perkins. Department of Biological Sciences, Stanford University, Stanford, CA 94025-5020

As the genome sequence invigorates research, we are led to ask what will be the role of genetics in future experimental work with Neurospora, what can be done to gain wider recognition of Neurospora among those outside the community, and what is needed to make it easier for newcomers to begin using Neurospora.

Functional Analysis of 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], James Galagan[5], Matt Sachs[6], Louise Glass[7], Mike Eisen[7], Mary Anne Nelson[8], Jennifer Loros[1]. [1]Dept. Genetics, Dartmouth Medical School, Havover,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

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. Most Neurospora genes have no homologs in yeasts and nearly 40% have no strong homologs in any organism to date, suggesting that examination of the functions of these genes will both novel and informative. Neurospora is an important model for basic research in eukaryotes, and fungi allied to Neurospora include significant animal and plant pathogens and industrial strains yielding antibiotics, chemicals, enzymes, and pharmaceuticals. The 43 Mb Neurospora genome is completely sequenced (>16 fold archival coverage), and automated annotation using programs trained on Neurospora genes predict 10,082 proteins. The first Project will pursue the systematic disruption of genes through targeted gene replacements, preliminary phenotyping of these strains, and their distribution to the scientific community at large. Project #1 will rely on bioinformatic support from Project #2. Through a primary focus in Annotation and Genomics, Project #2 will produce 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, relying on data from EST

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analyses in Project #4 to refine the gene structures. Oligonucleotide-based microarrays created in Project #3 will allow Transcriptional Profiling of the nearly 11,000 distinguishable transcripts in Neurospora. This effort will provide a baseline analysis of gene expression under a variety of growth conditions, and later begin to analyze the global effects of loss of novel genes in strains created by Project #1; these data will be made available through the web via structures created in Project #2. Since alternative splicing, alternative promoters, and long antisense transcripts contribute widely to the overall complexity of expressed sequences in Neurospora, in Project #4, cDNA libraries will be generated from wild type and related strains to document this complexity to aid in annotation in Project #2. Sequences from related strains will drive assembly of a SNP map. Overall this effort will help to anchor genomic exploration within the largely unexplored phylogenetic Kingdom of the Fungi.

Advances in genome defense and DNA methylation. Eric U. Selker and colleagues, Institute of Molecular Biology, University of Oregon, Eugene, OR

The availability of the Neurospora genome sequence has advanced numerous areas of study, including ours. I will give an overview of our work and highlight how we have capitalized on the genome sequence data. I will also outline our plans to further exploit the availability of this information. Genomic studies verified that 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. These relics of RIP consist mostly of a variety of inactivated transposon. A combination of classical genetic and genomic approaches have provided evidence that the mechanism of RIP involves modifications of both DNA and chromatin. Our efforts to elucidate the control of DNA methylation in vegetative cells have revealed clear ties between modifications of DNA and chromatin. In vegetative cells, 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. DIM-5 is sensitive to certain modifications of histones including acetylation and phosphorylation and is presumably also directed by one or more factors that recognize DNA mutated by RIP. DNA methylation can lead to deacetylation of histones, which may aid in propagation of DNA methylation and the associated silenced chromatin state.

Impact of the genome sequence on analysis of signal transduction pathways in Neurospora. Katherine A. Borkovich, Department of Plant Pathology, University of California, Riverside, CA

The availability of the complete genome sequence of *Neurospora crassa* has greatly impacted study of gene products involved in signal transduction pathways. Genes encoding signaling components have been identified and tentatively assigned to pathways, allowing the design of experiments to test both cellular function and epistatic relationships between gene products. In many cases, identification of these gene products was not feasible prior to the completion of the genome sequence. For example, cloning of seven transmembrane helix, G protein coupled receptors (GPCRs) is very difficult using degenerate PCR or low-stringency hybridization, as the regions of homology are usually confined to the transmembrane helices, leading to relatively low similarity. However, using the genome sequence, our laboratory has identified 10 putative GPCRs and has utilized a gene replacement strategy to create null mutations in the respective genes. Results will be presented for the analysis of two pheromone receptors, as well as a class of GPCRs not found in budding or fission yeasts.

Organismal Biology

Non-self recognition and programmed cell death in *Neurospora crassa*. N. Louise Glass, Gopal Iyer, Qijun Xiang, Isao Kaneko, Amita Pandey and Karine Dementhon. Department of Plant and Microbial Biology, The University of California, Berkeley, CA 94720-3102

Nonself recognition during vegetative growth in filamentous fungi is mediated by heterokaryon incompatibility (het) loci. In *Neurospora crassa*, *het-c* is one of eleven het loci. Three allelic specificity groups, termed *het-cOR*, *het-cPA* and *het-cGR*, exist in natural populations. Heterokaryons, partial diploids or transformants that contain *het-c* alleles of alternative specificity show severe growth inhibition, repression of conidiation and hyphal compartmentation and death (HCD). Nonself recognition is associated with presence of a plasma membrane associated heterocomplex composed of polypeptides encoded by *het-c* alleles of alternative specificity. Mutations in the *vib-1* locus suppress growth inhibition and conidiation repression by genetic differences at *het-c* and *mat* and reduce HCD. The *vib-1* locus encodes a nuclear localized putative transcriptional regulator. HET-C heterocomplex formation occurs in the *vib-1* mutant, indicating that VIB-1 functions downstream of HET-C heterocomplex formation and may mediate some of the phenotypic responses to heterokaryon incompatibility at the transcriptional level. Mutations at a second locus, *vib-2*, confer temperature-dependent *het-c* heterokarkyon incompatibility. Initial data suggests that *vib-2* may encode a protein related to TOL, which is required for *mat* incompatibility. Our finding is a step toward understanding nonself recognition mechanisms that operate during vegetative growth in filamentous fungi and provide a model for investigating relationships between recognition mechanisms and cell death.

An incompatibility supergene at *het-6* in *Neurospora crassa*. Myron Smith. Department of Biology, Carleton University, Ottawa, Canada.

We constructed a lambda DNA library of the "Panama" (PA) background strain FGSC 1131 to clone and characterize PA alleles of *het-6* and *un-24*, two linked genes that both have heterokaryon incompatibility function. In the process, we identified a ~19 kbp paracentric inversion, *In(het-6)*, near *het-6* on linkage group II that differentiates *het-6*^{OR} from *het-6*^{PA} strains. The structural features of this inversion explain our earlier observations that *het-6* and *un-24* are in severe linkage disequilibrium. For example, the PA and OR haplotypes are completely dissimilar in the region around breakpoint1 (*brk-1*) that is situated between *un-24* and *het-6* in thePA haplotype. This arrangement guarantees that the PA-OR and OR-PA combinations of *het-6-un-24* cannot arise through homologous recombination between OR-OR and PA-PA forms. We hypothesize this inversion created an incompatibility supergene (comprising *un-24*^{OR} *het-6*^{OR} or *un-24*^{PA} *het-6*^{PA}) that underwent a selective sweep because it has a "strong" incompatibility function. This hypothesis is based partly on the elegant structure of the inversion but also on our observations of the characteristics of the PA alleles. Whereas the OR alleles at both loci cause cell death of PA strains in transformation-based assays, transforming the PA forms of either gene into OR background cells results in the recovery of inhibited, but viable colonies. From these self-incompatible transformed strains we recovered escapes that are functionally PA-OR or OR-PA at *het-6-un-24*. These escape strains will be used to test predictions of the incompatibility supergene hypothesis.

The genetic basis of adaptation: How good it's gonna be. John W. Taylor[1], Takao Kasuga[1], Luz B. Gilbert[1], Jeff Townsend[1], Jeremy Dettman[1], David Jacobson[1, 2], Anne Pringle[1], Elizabeth Turner[1], Don Natvig[3], and Louise Glass[1]. [1]Department of Plant and Microbial Biology, University of California, Berkeley, CA, 94720-3102, USA. [2]Department of Biological Sciences, Stanford University, Stanford, CA, 94305, USA. [3]Department of Biology, University of New Mexico, Albuquerque, NM, 87131, USA.

Adaptation to novel environments is easily inferred by comparisons between species, for example, Darwin's finches. Characterizing adaptation within a species and its genetic basis, perhaps as a prelude to or simultaneous with speciation, is more difficult. The keys will be to study a confirmed single species that ranges across different environments and to concentrate on traits that contribute to fitness and for which genetic control can be assigned. *Neurospora discreta* will be our model species because it thrives in different environments over a tremendous

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DOI: 10.4148/1941-4765.1146

latitudinal gradient in western North America (Jacobson et al. 2004 Mycologia 96:66-74), a range that offers variation in abiotic factors (e.g., day length and temperature) and biotic factors (e.g., host tree and coexisting microbes). To ensure conspecificity, we are applying phylogenetic species recognition to the *N. discreta clade* as we have done for the other outbreeding *Neurospora* species (Dettman et al. Evolution 57:2703-2741). We will start with comparing a simple trait, mitospore germination, between *N. discreta* individuals in a common garden experiment, using strains collected at the extreme ends of the range and using temperature as the environmental variable. Eventually, we will measure other fitness components, such as growth and sporulation. To assay genome activity, we are profiling transcription using microarrays designed from the *N. crassa* genomic sequence. Hypotheses about the adaptive role of differentially expressed genes identified by transcriptional profiling (e.g., as compared to drift)combined with existing knowledge of *Neurospora* biology can be challenged by genetic investigation ranging from QTL analysis to molecular manipulation.

What we know about regional diversity in Neurospora. David Jacobson[1,5], Cornelia Boesl[2], Shahana Sultana[2], Till Roenneberg[2], Martha Merrow[2], Margarida Duarte[3], Isabel Marques[3], Alexandra Ushakova[3], Patricia Carneiro[3], ArnaldoVideira[3], Donald O. Natvig[4], and John Taylor[5]. [1]Dept. of Biological Sciences, Stanford University, California. [2]Institute for Medical Psychology, University of Munich, Germany [3]Instituto deBiologia Molecular e Celular, Porto, Portugal. [4]Dept. of Biology,University of New Mexico, Albuquerque. [5]Dept. of Plant and Microbial Biology, University of California, Berkeley.

Neurospora was previously considered primarily a tropical or subtropical genus. However, recent field surveys found *Neurospora* occupying an entirely new ecological niche under the bark of fire-damaged trees in dry and cold habitats within a new geographic range, western North America, from New Mexico (34°N) to Alaska (64°N) (Jacobson *et al.* 2004 Mycologia 96:55-74). Isolates from these sites were comprised predominantly (95%) of a single species, *N. discreta*, heretofore the least common species of *Neurospora* collected. In autumn 2003, a multinational effort surveyed southern Europe for *Neurospora* after unusually devastating wildfires. *Neurospora* was found from southern Portugal (37°N) to Switzerland (46°N). Species collected included *N. crassa, N. discreta, N. sitophila*, and *N. tetrasperma*. Although the latitude, climate and vegetation are similar to western North America, species distribution and spatial dynamics were quite different. Rather, these characteristics are more similar between southern Europe and semitropical Florida, where four different species are also present over very small spatial scales (Powell *et al.* 2003 Mycologia 95:809-819). These differences in regional diversity will form the basis of testable hypotheses, furthering the value of this model organism as a subject for studying fungal ecology.

What we know about local diversity in *Neurospora*. D.O. Natvig[1], D.J. Jacobson[2], G.S. Saenz[1], A.J. Powell[1], W.H. Dvorachek, Jr.[1] and C. Kitchen[1].[1]University of New Mexcio and [2]Stanford University.

Studies with natural isolates from diverse locations, pioneered by David Perkins, set the stage to make *Neurospora* a model for evolutionary, systematic and population biology. This foundation is strengthened by the recent discovery that species are observed predictably after fires in western US forests. In addressing questions in realms such as population structure and gene flow, it is important to know whether isolates collected from a given location exhibit diversity. Typically, *Neurospora* species are observed in local "blooms" with copious conidia. Perithecia have been observed in nature rarely. It could be assumed that a bloom represents dispersal of conidia from a few early colonizers, or even a single colony. This is far from the truth. The Perkins collections revealed that multiple mating types of a given species, and at least occasionally, different species can be collected from a single "colony." Recently, we have shown that many distinct individuals can be obtained over small scales. In surveys of *N. discreta*, we found 9 different genotypes among 24 isolates from a single tree in New Mexico and 4 genotypes among 9 isolates from a single site in Alaska. Similarly, a survey of 16 isolates from adjacent cane stems in the Everglades revealed three species and 8 genotypes; and among 4 isolates from a single shrub, we observed three species and four genotypes. These results confirm the value of *Neurospora* for population studies. In addition, they highlight a poor understanding of modes of colonization and dispersal that result in blooms that appear within a few days after fire.

Photoperiodism in *Neurospora crassa.* Ying Tan[1], Zdravko Dragovic[1], Cornelia Boesl[1], Shahana Sultana[1], David Jacobson[2], Till Roenneberg[1] and Martha Merrow[1]. [1]Institute for Medical Psychology, Munich, Germany. [2]Stanford University, Palo Alto, CA, USA

Seasonality is common in nature. It is manifest in migration, hibernation, reproduction and pelage in animals, in flowering time and seed production in plants, and even in human psychology and fertility. Clever experiments and mutant plants and animals demonstrate that this regulation is part of the circadian system. Yet, circadian model organisms in which photoperiodism has been defined are inherently complex. We therefore investigated regulation of propagation, reproduction and light-regulated physiology in the fungal clock model system, *Neurospora crassa*. Regulation of all three of these functions can be demonstrated as sensitive to day or night length. Enhanced responses in 12, 14 or 14 to 20 h photoperiods, respectively, are specific for light duration and do not represent simple irradiance responses, as cultures grown in constant light resemble those grown in constant darkness. Night break experiments suggest that at least part of the timing mechanism measures night length. Clock mutant strains confirm that, like in other model organisms, photoperiodism in *Neurospora* depends on an intact circadian system. Recent collections over a wide range of latitudes suggest an adaptive advantage to using night or day length to program reproductive or metabolic function to certain seasons. Preliminary experiments with these strains demonstrate phenotypic diversity – perhaps comparable to that in the human population – in *Neurospora* chronotypes.

Morphogenesis

The role of the Spitzenkörper in hyphal morphogenesis. S. Bartnicki-Garcia[1], Meritxell Riquelme[1] and Robert W. Roberson[2]. [1]División de Biología Experimental y Aplicada, CICESE, Ensenada, Baja California, México. [2]Department of Plant Biology, Arizona State University, Tempe, AZ.

Mathematical modeling of the secretory process led to the concept that the polarized growth and shape of a fungal hypha is generated by the movement of the Spitzenkörper (Spk) acting as a vesicle supply center (VSC). The VSC model provides the basis for explaining how a fungal cell generates and changes its morphology. Tested mainly by high resolution video microscopy and image analysis, the model provided an explanation of how the Spk controls growth direction in hyphae of *Neurospora crassa*. Experiments with cytoskeleton inhibitors and light- and electron-microscopic examination of two *ropy* mutants of *N. crassa* led to the conclusion that a fully functional microtubular cytoskeleton is essential to maintain a steady, well developed Spk, and thus regular (hyphoid) morphology and a vigorous elongation rate. Immunofluorescence microscopy of *ro-1* hyphae confirmed that the microtubule cytoskeleton was severely disrupted. A comparison of lateral vs. apical branching in hyphae of *N. crassa* disclosed a major difference in Spk ontogeny. Lateral branching occurs without affecting the behavior of the primary Spk or the shape and elongation rate of the primary hyphae. Lateral branching is probably triggered by the accumulation of a critical excess of wall precursors (mainly wall-building vesicles) in the subapical region. The trigger of apical branching may be traced to a sudden discrete disruption in cytoplasmic organization (cytoplasmic contraction) that causes the disappearance of the primary Spk, followed by the development of two new Spk.

Suppression of *N. crassa cot-1* morphology by environmental stresses and Farnesol suggest that COT1 is involved in environmental stress response and Quorum sensing. Oded Yarden, Nourit Cohen, Carmit Ziv, Haya Sandori, Leonid Chernin, Zohar Kerem and Rena Gorovits. Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot Israel.

https://newprairiepress.org/fgr/vol51/iss1/16
DOI: 10.4148/1941-4765.1146

cot-1 is a *Neurospora crassa* colonial temperature-sensitive mutant that ceases to elongate and produces multiple branches at the restrictive temperature. Various environmental stresses (e.g., NaCl, Sorbitol, H_2O_2, ethanol or reduced availability of fermentable carbon sources) significantly suppressed the *cot-1* phenotype. These effects are not dependent on *os-2* or on *mak-3* (inactivated by RIP) MAPK function, but are accompanied by alterations in PKA activity. When grown at 10-fold cell density (10^6 conidia/ml), *cot-1*exhibited near-wild type morphology (at restrictive temperatures), indicating that a biotic-derived environmental signal can phenocopy the effect of abiotic stresses. Replacing low-density cell medium with spent medium obtained from high-density cultures of *cot-1* or wild type abrogated the *cot-1* hyperbranching phenotype. Similarly, culturing *cot-1* in the presence of 40-70 micromolar farnesol (a compound shown to be involved in *Candida albicans* Quorum sensing) suppressed the *cot-1* phenotype. Chemical analysis confirmed that *N. crassa* produces and secretes farnesol in a cell density-dependent manner. Based on our results, we propose that COT1 is involved in environmental stress response and Quorum sensing. Use of transcription profiling has facilitated the identification of several genes differentially expressed in *cot-1* cultures shortly after being shifted to the restrictive temperature. The possible involvement of these genes in the cellular response to environmental changes is being analyzed.

Identifying genes required for morphogenesis by a map and sequence strategy. Stephen J.Free, Amy Piwowar, and Shaun Bowman. Dept. of Biological Sciences, University at Buffalo, Buffalo, NY 14260.

We have used a map and sequence approach to identify genes involved in regulating the morphogenesis of *Neurospora crassa*. We initially isolated a number of morphological mutants using UV mutagenesis and then mapped the mutations using standard mapping procedures. We then identified candidate genes at the mapped loci and used PCR to amplify and sequence the candidate genes from mutant genomes. Using this approach we have identified new genes that are required for normal morphogenesis. These include an O-mannosyltransferase involved in cell wall biosynthesis and a gene thought to be involved in generating GPI-anchored proteins.

Genetic analysis of cytoplasmic dynein heavy chain. D. Madole, D. Gandhe and M. Plamann, School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO 64110-2499.

The three major families of motor proteins operating in eukaryotic organisms are the actin-associated myosin motors and the microtubule-associated kinesin and dynein motors. The mechanisms controlling myosin and kinesin motor function are relatively well understood; however, the molecular details of dynein motor function are largely unknown. Difficulties in understanding the dynein motor are due in part to the large size of its motor domain (~350 kDa vs. ~35 kDa for kinesins) and the involvement of >15 additional subunits. In *Neurospora crassa*, cytoplasmic dynein is highly conserved relative to vertebrates and is required for many of the same cellular activities. However, Neurospora dynein is not essential for viability and this allows the isolation of mutants defective in dynein function including many dynein heavy chain mutants. From a collection of >300 dynein heavy chain mutants, we have identified >80 mutants that produce full-length, but defective, heavy chain. We have defined the mutations in many of these mutants and have identified specific regions of the heavy chain that are essential for function of the dynein motor. Interestingly, the dynein motor mutants show significant variation in their cellular phenotypes. These results represent the first large-scale genetic analysis of the dynein motor domain.

Cell Biology

Does transport of calcium into the vacuole affect hyphal morphology and tolerance of high external calcium? Barry Bowman and Stephen Abreu, Department of Molecular Cell and Developmental Biology, University of California, Santa Cruz CA 95064

The vacuole of *Neurospora crassa* contains a relatively high concentration of calcium, which has been implicated as an important effector of hyphal morphology. Null mutations in the vacuolar ATPase in *S. cerevisiae* disrupt the ability of the vacuole to sequester calcium. In *N. crassa* loss of the vacuolar ATPase causes severe changes in hyphal morphology. We have characterized strains with null mutations in *nca-2, nca-3*, and *cax*, genes predicted to encode proteins that transport calcium into the vacuole. All three genes are expressed in standard minimal medium and expression is quickly elevated if 50-200 mM calcium is added to the external medium. Growth in medium with high calcium is partially affected by inactivation of the *nca-2* gene, and severely affected if both *nca-2* and *cax* are mutated. Inactivation of *nca-3* causes no change in phenotype. The data suggest that *nca-2,cax* double mutants have lost the ability to sequester calcium in the vacuole, but surprisingly, these mutant strains have normal hyphal morphology. We also examined strains lacking *pmr*, the gene encoding a manganese or calcium transporter in the ER/golgi. These strains exhibit mild changes in hyphal morphology but growth is inhibited very little by high calcium in the medium.

Hyphal Ion Currents, Calcium and Tip Growth. Roger R. Lew. Department of Biology, York University, Toronto, Ontario Canada

Fungi (and specialized cells in other organisms) use polarized cellular extensions to explore new territory. At the growing tip, there can be assymetric distributions of ion fluxes, oxygen fluxes, and ionic gradients which may or may not be correlated spatially with cytological features, depending upon the organism and cell type. The highly coordinated process of tip extension always involves the presence of an internal tip-high gradient of calcium ions. By mapping channel distributions, extracellular ionic currents and cytosolic ion gradients along growing hyphal tips, we are characterizing the mechanisms responsible for generation and maintenance of the calcium gradient during hyphal growth in *Neurospora crassa*. The gradient is generated from internal stores by the activity of IP_3-activated calcium channel. We envisage a mechanism that begins with tip-localized IP_3 production by a stretch-activated phospholipase C, followed by IP_3activation of calcium channels on calcium-containing vesicles. Elevated calcium then mediates the vesicle fusion required for continued hyphal tip expansion. Calcium sequestration occurs behind the growing apex into endoplasmic reticulum.

The research was funded by NSERC.

Integrative Analysis of the Mitochondrial Proteome in Yeast. Holger Prokisch, Lars Steinmetz, Curt Scharfe, David Camp, Wenzhong Xiao, Peter Oefner, Richard Smith, Ronald Davis, Thomas Meitinger

We use yeast and Neurospora mitochondria as a system to apply, integrate and compare different functional genomic approaches to define an organellar proteome.We first applied liquid chromatography mass spectrometry on purified organelles of yeast and Neurospora to identify 546 and 200 mitochondrial proteins, respectively. We then integrated proteomic with genomic approaches including deletion phenotype screening, expression profiling, subcellular localization, protein interaction analysis and computational predictions to derive a more comprehensive identification of yeast mitochondrial proteins. The integrative analysis achieves an accuracy higher than that of any single approach, predicts 650 yeast mitochondria localized proteins, and allows an evaluation of the success of individual methods. We show that two complementary systematic studies in combination, like deletion phenotype screening and mass spectrometry, identify 76% of the known mitochondrial proteome. These findings have implications for approaching other cellular systems, such as organelles and pathways in various species, using a minimal number of optimal genome-wide approaches. A comprehensive view of mitochondrial function and biogenesis will accelerate the understanding of Mendelian and complex mitochondrial disorders.

https://newprairiepress.org/fgr/vol51/iss1/16
DOI: 10.4148/1941-4765.1146
http://newprairiepress.org/fgr/vol51/iss1/16
DOI: 10.4148/1941-4765.1146

Assembly of the mitochondrial TOM complex. Frank E. Nargang, Rebecca D. Taylor, and Suzanne C. Hoppins. Dept. of Biological Sciences, University of Alberta, Edmonton, Alberta.

Over 95% of proteins found in mitochondria are encoded by genes in the nucleus, synthesized as precursor proteins on cytosolic ribosomes, and imported into the organelle. Mitochondrial precursor proteins contain targeting information which is recognized by multicomponent translocases in the mitochondrial membranes. The TOM complex (translocase of the outer membrane) is responsible for recognition of all mitochondrial precursor proteins and for their transfer into or across the mitochondrial outer membrane. In recent years, my laboratory has been interested in the assembly of the TOM complex. The major component of the complex is the beta-barrel protein Tom40, which forms the actual translocation pore. Tom40 requires pre-existing TOM complex for its own import into mitochondria. The Tom40 preprotein is incorporated into the complex via a well-defined pathway of intermediates that can be detected by blue native gel electrophoresis following import of radiolabeled Tom40 precursor intoisolated mitochondria. The Tim8-Tim13 complex exists in the intermembrane space and is traditionally considered a factor required for the import of the Tim23 protein. We have shown that mitochondria lacking the Tim8-Tim13 complex are deficient in the import of Tom40 and porin, another beta-barrel protein of the outer membrane. Crosslinking studies demonstrate that the Tom40 precursor contacts the Tim8-Tim13 complex. The complex is involved at an early point in the Tom40 assembly pathway since crosslinks can only be detected during theinitial stages of Tom40 import.

Analysing the cell biology of macroconidial germination and early colony development in *Neurospora crassa*. Nick D. Read, Fungal Cell Biology Group, Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh EH9 3JH, Scotland

In recent years there have been important developments in live-cell analytical techniques (e.g. confocal microscopy, vital and GFP probes, and laser tweezer micromanipulation) applied to studies on filamentous fungi. Much of the work of my lab has focused on developing and optimising these powerful technologies in applications to provide novel insights into the biology and dynamics of living fungal cells. We are currently using these approaches to investigate different aspects of the cell biology of macroconidial germination and early colony development in Neurospora. The process of hyphal homing and fusion (anastomosis) between conidial germlings of labelled has been imaged using different vital dyes and GFP probes. Both germ tubes and another type of specialised, morphologically distinct hypha (called a conidial anastomosis tube [CAT]) are produced by macroconidia. In wild type strains, CATs are thinner than germ tubes and do not undergo branching. In contrast to germ tubes, conidial anastomosis tubes grow towards each other. We have developed a simple laser tweezer technique to optically manipulate whole spores and germlings. When homing germlings are moved relative to each other the CATs subsequently reorientate themselves and grow back towards each other. This provides clear evidence for the existence of, as yet unknown, diffusible chemotropic signals being involved in the homing response of CATs. This experimental manipulation of macroconidia is being used in assays to determine whether strains of different genetic backgrounds can home towards or fuse with each other. In this way we have shown that fusion between CATs is independent of mating type. Nuclear movement, and continuity of the microtubular cytoskeleton between fused germlings, have been imaged. Endocytosis, as indicated by the internalisation of the membrane-selective probe FM4-64, is initiated after spore hydration before the emergence of germ tubes or CATs. Interestingly, the Spitzenkörper in germ tubes is not stained by the dye as it is in vegetative hyphae suggesting differences in the pathways of vesicle trafficking in these different cell types.

Exceptionally fast RNA cleavage and ligation catalyzed by a Neurospora Varkud Satellite ribozyme. Rick Collins.

Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada

Small ribozymes that use an internal phosphoester transfer mechanism to catalyze site-specific RNA cleavage have been identified in a several organisms and in in vitro selection experiments. All of these have been thought to be rather poor catalysts, exhibiting apparent cleavage rate constants typically less than 2 min-1. We have identified variants of one of these, the Neurospora VS ribozyme, that self-cleave with experimentally-measured apparent rate constants of up to 10 s-1 (600 min-1), about two orders of magnitude faster than any previously-characterized self-cleaving RNA. We describe structural features of the cleavage-site loop and an adjacent helix that affect the apparent rate constants for cleavage and ligation, and the equilibrium between them. These data show that the phosphoester transfer ribozymes can catalyze reactions with rate constants much larger than previously appreciated, and in the range of those of protein enzymes that perform similar reactions. These observations provide support for the feasibility of the "RNA World" and for improving the performance of ribozyme-based therapeutic agents.

Regulation

Molecular Biology of the Neurospora Circadian Clock. Jennifer J. Loros, Hildur V. Colot, Jay C. Dunlap
Department of Genetics, Dartmouth Medical School, Hanover, NH 03755

Circadian rhythms are a subset of biological rhythms having specific characteristics: they display period lengths close to 24 hrs, are readily entrainable to environmental cues including light and temperature, and their period lengths are compensated such they are close to the same under different ambient temperatures and nutritional conditions. Although noncircadian rhythms, rhythms lacking some or all of these characteristics, have been described in Neurospora, the circadian clock is one of the major regulators of conidiation in this organism. In all genetically studied model organisms, a negative feedback loop of gene expression makes a major contribution to the circadian rhythm mechanism. In animals and fungi this core loop involves a transcription factor (a heterodimer of proteins that interact via PAS domains) that activates expression of genes encoding proteins that feed back to depress the activity of the heterodimer. Additional feedback loops link to and close around this central loop. In Neurospora, a heterodimer of WC-1 and WC-2 makes up the PAS/PAS transcriptional activator and it acts to regulate the daily cyclical expression of the frq gene whose rhythmic expression is essential for all true circadian rhythms in this organism. FRQ is seen in the cell in two forms, a long form of 989 amino acids and a shorter form of 890 amino acids; the total amount of protein and the ratio between the two forms are regulated by ambient temperature. Based on limited frq cDNA analyses that had identified no introns, we believed that the transcription unit was simple and that the origin of the forms therefore lay in translational control. To examine this regulation we looked at transcripts, and when strand-specific primers were used to examine specifically the sense strand (with anti-sense strand primers as controls) a number of surprises emerged: (1) primary transcripts of frq are multiply spliced in a complex manner; (2) subsequent 5'RACE has revealed use of multiple promoters; (3) choice of alternative splice site within the 5'UTR is regulated by temperature as is use of promoters. The existence of an antisense transcript was also confirmed and has since been studied extensively by Crosthwaite and colleagues (Kramer et al, Nature 421:848-952, 2003). This complex environmentally regulated use of alternative splicing and multiple promoters to regulate the expression of frq provides a venue for examining the molecular biology of these phenomena in a genetically and molecularly tractable system.

Meiotic Silencing by Unpaired DNA. Patrick Shiu, Namboori Raju, Denise Zickler, and Robert Metzenberg. Department of Chemistry and Biochemistry, University of California, Los Angeles, CA; Department of Biological Sciences, Stanford University, CA. Institut de Genetique et Microbiologie, Universite Paris-Sud, Orsay, France.

Recently we have reported a novel system of targeted gene silencing that operates after karyogamy, namely Meiotic Silencing by Unpaired DNA (MSUD). In the MSUD system, the diploid cell scans the genome and compares a gene with its homolog during pachytene pairing. A gene which fails to be paired with a homolog generates a signal that transiently silences all sequences homologous to it. We have isolated a class of mutants called Sad (Suppressor of

http://newprairiepress.org/fgr/vol51/iss1/16

DOI: 10.4148/1941-4765.1146

ascus dominance) that fail to perform MSUD. Sad mutants also suppress several classical "ascus-dominant" mutants, suggesting that these, too, owe their ascus dominance to the MSUD mechanism. MSUD is not restricted to a few ascus-dominant genes, but is applicable to virtually the entire genome. This can be seen from the fact that a variety of genes are meiotically silenced if they are manipulated so as to be unpaired during meiotic prophase. The *sad-1* gene encodes an RNA-directed RNA polymerase (RdRP). RdRP has been implicated in many post-transcriptional gene silencing systems, such as co-suppression in plants, RNA interference in animals, and quelling in fungi. Owing to its ability to compare the genomes of two mating partners, MSUD has implications not only for surveillance against invading sequences but also for reproductive behavior.

The RNA-dependent RNA Polymerase, QDE-1 is a Rate-Limiting Factor in Post-Transcriptional Gene Silencing in *Neurospora crassa*. Emma C Forrest, Carlo Cogoni, and Giuseppe Macino. Dipartimento di Biotecnologie Cellulari ed Ematologia, Sezione di Genetica Molecolare, di Roma La Sapienza, Viale Regina Elena, 324, 00161 Roma, Italy

The RNA-dependent RNA polymerase *qde-1* is an essential component of post-transcriptional gene silencing, termed “quelling” in the fungus *Neurospora crassa.* Here we show that over-expression of *qde-1* results in a dramatic both a substantial increase in the efficiency of quelling, with a concomitant net increase in the quantity of *al-1* siRNAs. Moreover, in over-expressed strains there is a significant reduction in the threshold number of transgenes required to induce quelling, and an increase in the phenotypic stability of quelling despite progressive loss of tandemly repeated transgenes which normally determines reversion of a silenced phenotype to wild-type. These data demonstrate that QDE-1 is the a rate-limiting factor of the silencing mechanism pathway, and suggests the existence of a mechanism able to detect and count transgenes, prompting a silencing response above a certain threshold. Moreover, the stability of quelling at the phenotypic level is increased in strains over-expressing *qde-1*, despite progressive loss of tandem arrays of transgenes during vegetative growth which normally determines reversion of a silenced phenotype to wild-type.

A Neurospora heterochromatin protein is essential for DNA methylation. Michael Freitag, Tamir K. Khlafallah, and Eric U. Selker, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

Methylation of cytosines silences transposable elements and certain cellular genes in mammals, plants and some fungi. Recent studies have advanced the understanding of what controls eukaryotic DNA methylation. All DNA methylation in Neurospora, and some in plants and animals, depends on histone H3 Lys9 methylation by DIM-5. Biochemical studies showed that methylated Lys9 is bound by the chromo domain of HP1, a heterochromatin protein originally identified in Drosophila and implicated in silencing in Drosophila, fission yeast and mammals. We therefore investigated the possibility that a Neurospora HP1 homologue reads the methyl-Lys9 mark to signal DNA methylation. We identified an HP1 homologue and showed that it is essential for DNA methylation, is localized to heterochromatic foci, and that this localizationis dependent on the catalytic activity of DIM-5. We conclude that HP1 serves as an adapter between methylated histone H3 Lys9 and the DNA methylation machinery. Unlike DNA methyltransferase mutants, HP1 mutants exhibit severe growth defects. This suggests that HP1 is required for processes besides DNA methylation. We identified eight additional Neurospora chromo domain proteins in the genome sequence. At least one of these also binds heterochromatic regions, partially co-localizing with HP1.

A Neurospora nascent polypeptide domain that regulates translation elongation. Peng Fang, Christina C. Spevak, Cheng Wu and Matthew S. Sachs. Oregon Health & Science University, Beaverton, OR 97006

The *Neurospora crassa* arginine attenuator peptide (AAP) specified in the 5'-leader of the arg 2 transcript acts as a nascent peptide to stall the translating ribosome in response to the presence of a high concentration of the amino acid

arginine. We examined whether the AAP maintains regulatory function in *N. crassa*, wheat germ and reticulocyte cell-free translation systems when placed as a domain near the N-terminus or internally within a large polypeptide. Pulse-chase analyses of radiolabeled polypeptides indicated that the wild-type AAP functions at either position to stall polypeptide synthesis in response to arginine. Toeprint analyses performed to map the positions of stalled ribosomes on transcripts in the *N. crassa* system showed that ribosome stalling required translation of the AAP coding sequence. The positions of the stalled ribosomes were consistent with the sizes of the radiolabeled polypeptide intermediates. These findings demonstrate that an internal polypeptide domain in a nascent chain can regulate eukaryotic translational elongation in response to a small molecule. Apparently the peptide sensing features are conserved in fungal, plant and animal ribosomes. These data provide precedence for translational strategies that would allow domains within nascent polypeptide chains to modulate gene expression.

Posttranslational and light regulation of the *Neurospora* circadian clock. Yi Liu, Ping Cheng, Yuhong Yang, Qiyang He, & Qun He. Department of Physiology, UT Southwestern Medical Center, Dallas, TX 75390

FREQUENCY (FRQ), WHITE COLLAR-1 (WC-1) and WC-2 proteins are three critical components forming the circadian negative feedback loop in *Neurospora*. FRQ is progressively phosphorylated over time, and its level decreases when it is extensively phosphorylated. To identify the kinase phosphorylating FRQ and to understand the function of FRQ phosphorylation, two FRQ phosphorylating kinase was purified and identified as casein kinase II (CKII) and a calcium/calmodulin-dependent kinase. Our data showed that CKII is an essential clock components, and that the phosphorylation of FRQ by CKII promotes FRQ degradation and is important for the closing of the circadian negative feedback loop. On the hand, two protein phosphatases, PP1 and PP2A, play distinct roles in the *Neurospora* clock: PP1 regulating FRQ stability while PP2A is important for the function of the circadian feedback loop. After FRQ is phosphorylated, it is degraded through the ubiquitin-proteasome pathway. Such degradation is mediated by a E3 ligase, FWD-1 (an F-box/WD-40 repeat-containing protein), which is the *Neurospora* homolog of the *Drosophila* Slimb protein. The conservation of the posttranslational regulators in the *Neurospora* and animal circadian systems suggests that the molecules mediating the posttranslational regulation of clock proteins may be the common evolutionary link among distinct eukaryotic circadian systems.

In addition to the critical role of WC-1 and WC-2 in the circadian feedback loop, both proteins are essential components for the light input of various blue light responses, including the light entrainment of the circadian clock. We showed that the putative flavin-binding domain of WC-1, its LOV domain, is required for light responses. By purifying the endogenous WC complex from *Neurospora*, we showed that it is associated with FAD, suggesting that WC-1 is the blue light photoreceptor mediating light responses in *Neurospora*. VVD is a LOV domain protein regulating photoadaptation in *Neurospora*. By creating a *Neurospora* strain in which the LOV domain of WC-1 is swapped with that of VVD, we showed that the LOV domain of VVD partially replaces the function of the WC-1 LOV domain, suggesting that VVD is another blue light photoreceptor in *Neurospora.*

Plenary Lecture

Genetics and Kinetics, Stuart Brody Molecular Biology Section, Division of Biological Sciences, University of California, San Diego

February of 1964 was the beginning of my research on *Neurospora*. In those forty years, my studies have ranged from morphological mutants to conidial germination to membrane lipid composition to mitochondria, and of course, to circadian rhythms. Through all of these topics, a common theme emerged: the effect of mutations on rates. After reviewing some of these subjects briefly, I will make the following predictions / "wish list" for *Neurospora* research.

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DOI: 10.4148/1941-4765.1146
http://newprairiepress.org/fgr/vol51/iss1/16

DOI: 10.4148/1941-4765.1146

1) More studies are needed as to how *Neurospora* interacts with / modifies its environment. On the biological side, the study of volatile signaling molecules has been neglected, as has "quorum sensing".

2) We need more and better ways of analyzing physical effects, such as pressure, charge fields, etc., on and in this organism, not just its biology and chemistry.

3) We should apply the techniques of "systems biology" to *Neurospora* studies so that we can progress from individual components to pathways to groups of pathways (nodes) to interactions between nodes.

4) We should start to look past the Y2H studies to describe the other ways that proteins communicate with each other and to develop methodology that allows a readout of large numbers of intermediates in a cell at onetime (metabolosome?) and under different conditions, etc.

5) For circadian rhythms, this area could use a big push of quantitation in terms of reaction rates, affinity constants, etc. We should also figure out how to measure quantitatively another important kinetic property of oscillators, i.e. their amplitude.

These are published as an online supplement to the Fungal Genetics Newsletter #51. You may cite them as Fungal Genet. Newsl. 51S

Poster Abstracts

Please cite Fungal Genet. Newsl. **51S**:#

Biochemistry and Secondary Metabolism

1) Drug Discovery and the V-ATPase. Emma Jean Bowman, Barry Bowman, and Collaborators, Department of Molecular Cell and Developmental Biology, University of California, Santa Cruz, CA 95064, USA

Vacuolar ATPases (V-ATPases) are abundant, ubiquitous ion pumps in eucaryotic cells. They are directly involved in several diseases, including osteoporosis and Alzheimer's, and play a role in the growth of tumor cells. Three classes of natural products, which give similar profiles in the NCI 60-cell screen for inhibition of tumor growth, act as potent inhibitors of V-ATPases. The bafilomycins and concanamycins inhibit all known eucaryotic V-ATPases. The salicylihalamides and lobatamides show remarkable specificity for mammalian V-ATPases, having no effect on fungal enzymes. Chondropsins preferentially inhibit the fungal V-ATPase. Because of the variety of processes and diseases associated with V-ATPases and the possibility of designing selective inhibitors, the V-ATPases are becoming attractive targets for drug therapy.

2) Characterization of the Arg-13 Mitochondrial Transport Protein. Rey Renato, G. David, Gloria Turner and Richard L. Weiss. University of California, Los Angeles, Los Angeles, California, USA.

Metabolic processes take place in different compartments in eukaryotic cells. Intracellular compartments, such as the mitochondria, harbor enzymes and substrates that participate in specific metabolic pathways. Arginine biosynthesis in *Neurospora crassa* is an accessible model system to understand compartmentation. In *N. crassa*, glutamate is converted into citrulline inside the mitochondria, and citrulline is exported into the cytosol to be converted into arginine. We are investigating the role of the Arg-13 mitochondrial transport protein in the transport of arginine pathway metabolites across the mitochondrial membrane. The *arg-13* gene has been cloned and inserted into a pET3a vector. Arg-13 has been overexpressed in BL21-SI cells and purified. Characterization of Arg-13 involves determining the submitochondrial localization of the protein using polyclonal antibodies and using proteoliposome transport assays to determine substrate(s) specificity, transport activity and mode of transport. Preliminary results suggest that Arg-13 is an ornithine transporter. Characterization of Arg-13 will help elucidate the role it plays in arginine metabolism and add another piece of information towards understanding the role of compartmentation in metabolic regulation.

3) Unusual Cys-Tyr covalent bond in a large catalase. Wilhelm Hansberg, Adelaida Díaz, Eduardo Horjales, Enrique Rudiño-Piñera, Rodrigo Arreola. Instituto de Fisiología Celular and Instituto de Biotecnología, Universidad Nacional Autónoma de México, México, D.F.

Catalase-1 is associated with non-growing cells and accumulates in conidia. It is a large, tetrameric protein and a highly efficient and durable enzyme that is active at molar concentrations of H_2O_2. Catalase-1 is oxidized at the heme by singlet oxygen without affecting enzyme activity. The crystal structure of catalase-1 at 1.75 Å resolution, compared to the structure of two other large catalases, revealed differences mainly at the carboxy-terminal domain. Heme is rotated 180° around the a-g–meso carbon axis with respect to clade III small catalases. There is no co-ordination bond of the ferric iron at the distal side of the heme. The structure exhibited a partial oxidation of heme b to heme d. Singlet oxygen hydroxylates C5 and C6 of pyrrole ring III with a subsequent formation of a hydroxyl-g-

http://newprairiepress.org/fgr/vol51/iss1/16

DOI: 10.4148/1941-4765.1146

spirolactone. The site of modification is probably related to the exit of dioxygen through the central channel. The structure reveled an unusual covalent bond between the sulfur of a cysteine and the beta-carbon of the essential tyrosine of theproximal side of the active site. A peptide with the predicted theoretical mass of the two bound tryptic peptides was detected. A mechanism for the Cys-Tyr covalent bond formation is proposed. The tyrosine bound to the cysteine would be less prone to donate electrons to compound I to form compound II, making the enzyme resistant to inhibition and inactivation by substrate. An apparent closure of the main channel lead us to propose a gate that opens when there is sufficient hydrogen peroxide in the small cavity before the gate. This mechanism would explain the increase in catalytic velocity as the hydrogen peroxide concentrations rises. Grants: CONACyT C01-40697, DGAPA/UNAM IN225402

4) **Efflux of basic amino acids from the vacuole of *Neurospora crassa.*** Kelly Keenan, Richard Stockton College of New Jersey, Pomona NJ

The goal of the project is to characterize what causes the increase in efflux of three basic amino acids--arginine, lysine and ornithine--from the vacuole of *N. crassa* . Efflux can be measured using cupric ion-treated *N. crassa*. Nitrogen starvation results in an increase in efflux. The efflux permeases have not been identified and it is not known how they are able to change their activity. The change could be due to an increase in amount of permease protein or an elevated activity as the permease protein(s) binds to some positive effector. An extract of metabolites was collected from *N. crassa* grown under nitrogen starvation conditions and tested for its ability to increase efflux . Compared to conditions of having no extract as well as minimal medium extract, it increased efflux of the amino acids. In addition, efflux of the amino acids under nitrogen starvation conditions was not stopped when protein synthesis inhibitor was added. These results suggest that the increase in efflux is due to the presence of small molecule(s) that act as effectors. Efforts are underway to indentify these molecules. A series of mutants have been isolated that show altered efflux of oneor more of these amino acids. Extracts were prepared from mutants and tested inthe efflux assay. A number of these extracts were able to produce an increase in efflux which suggests that these mutants have increased efflux due to presence of effector molecules. Efforts are underway to identify these effector molecules.

Cell Biology

5) The search for stretch-activated Ca^{2+} channels in *Neurospora crassa*. Marinela I. Anderca and Roger R. Lew. Department of Biology, York University, Toronto, Ontario Canada

The *Saccharomyces cerevisiae* Mid1 (mating pheromone- induced death) is an integral membrane protein required for theviability of differentiated cells and Ca^{2+} influx induced by mating pheromone, that functions as a Ca^{2+}-permeable, stretch-activated channel when expressed in mammalian cells (Kanzaki et al., 1999 Science 285:882-6). In the *Neurospora crassa* genome we found a gene whose predicted protein is 30% identical and 45% similar to the Mid1 channel, with homologies to the H3 and H4 transmembrane regions and the cysteine-rich metal-binding regions, both essential for function. The gDNA derived from this gene (tentatively designated Ncmid1) was isolated by PCR from the cosmid library available at FGSC. Northern blot analysis is being employed to confirm that the Ncmid1 gene is expressed in *N. crassa*, to be followed by subcloning it into a vector suitable for performing RIP mutagenesis. Activities for two stretch-activated channels in *N. crassa* were detected previously in our laboratory. It is possible that one of these channels is encoded by Ncmid1,and we will attempt to confirm this by patch clamping the Ncmid1 mutant.

6) **Identification of Antibiotic Binding Sites in the V-ATPase.** Barry Bowman, Marija Draskovic and Emma Jean Bowman, Department of Molecular Cell and Developmental Biology, University of California, Santa Cruz, CA 95064, USA

The macrolide antibiotics bafilomycin and concanamycin are potent inhibitors of V- ATPases. To identify the binding site of bafilomycin we selected mutant strains of *Neurospora crassa* (named bfr) that are resistant to this antibiotic. In one class of bfr strains the V-ATPase was resistant to inhibition invitro. These strains had seven different point mutations in the *vma-3* gene, which encodes the hydrophobic c subunit of the vacuolar ATPase. Most of the mutated sites appear to be on the outer face of the "rotor" sector of the enzyme, a region hypothesized to form an interface with the "a" subunit.

Surprisingly, the bfr strains had little resistance to concanamycin, which has a similar structure. By further mutagenizing one of the bfr strains we obtained four new strains that were resistant to both antibiotics. Each of these had two altered residues in the c subunit. Thus, concanamycin does appear to bind to the same region. The positions of four of the mutated residues correspond precisely to the positions of mutated residues in the homologous c subunit of the mitochondrial ATPase that confer resistance to oligomycin. These results suggest that vacuolarand mitochondrial ATPases have an ancient, conserved antibiotic binding site. As the sequences of the polypeptides have diverged new antibiotics that target the same vulnerable site in this family of enzymes have arisen. The data also suggest a model for the tertiary structure of the c subunit of the V-ATPase.

7) **Circadian Rhythms in *Neurospora crassa*: Some unusual features of a new mutant, *ult* .** Michelle Tsukamoto, Brian Chen, and Stuart Brody. Division of Biological Science, UCSD, La Jolla,CA, 92093-0116

The circadian rhythm of *Neurospora crassa* is expressed as bands of conidiating regions on the surface of agar medium with 22 hr. periods. A new clock mutant *ult* (ultradian) has been isolated and characterized that differs from the existing clock mutants in the following ways : 1) It has a 12 hour period which can be lengthened to 20 hrs.upon changing the nitrogen source from ammonia to nitrate; 2)under certain conditions, it shows a novel pattern of a wide band followed by a narrower band, with unequal spacing between them ; 3) it is a dominant mutation with respect to period, while most clock mutants are either recessive or co-dominant ; 4) the mutation maps to the center of LG I, and does not appear to be a mutation at any previously known clock gene. On the other hand, the mutant strain is similar to other clock mutants in that the period is temperature compensated, shows strong resetting (phase-shifting) to both light and temperature pulses, and entrains to a light/dark regime.When *ult* was introduced into the frq^{10} (clock null) strain the double mutant ($ult\text{-}frq^{10}$) surprisingly showed rhythmic banding. However, this rhythm was not temperature compensated nor light-sensitive.This finding is interpreted in the context of a two oscillator model for *N. crassa*.

8) **Characterization of NAD(P)H dehydrogenases from *Neurospora crassa* mitochondria.** Patrícia Carneiro, Margarida Duarte, Isabel Marques, Joana Assunção and Arnaldo Videira. Instituto de Biologia Molecular e Celular and Instituto de Ciências Biomédicas de Abel Salazar, Porto, Portugal

The mitochondrial respiratory chain usually contains the type I NADH:ubiquinone oxidoreductase or complex I, a multi-subunit enzyme with proton-pumping activity. In addition, depending on the organism concerned, it has a variable number of alternative non-proton-pumping NAD(P)H dehydrogenases, both in the matrix and cytosolic faces of the inner membrane. The filamentous fungus *Neurospora crassa* also contains a quite well-conserved eukaryotic complex I. Besides, we have recently characterized three alternative enzymes: the main external NAD(P)H dehydrogenase, another externalcalcium-dependent NADPH dehydrogenase and an internal NADH dehydrogenase. In the present work, we describe the characterization of a fourth alternative NAD(P)H

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DOI: 10.4148/1941-4765.1146

dehydrogenase. The respective gene was fused with the green fluorescent protein (GFP) and was also inactivated by RIPing. We have characterized the respiratory activities of the resulting mutant strain. Our results suggest that this enzyme also resides in mitochondria. In parallel, we have disrupted the gene encoding a subunit of *N. crassa* complex I, which is similar to a cell-death regulator. Characterization of an isolated mutant strain is underway and will be presented.

9) Analysis of vegetative growth of *Neurospora crassa* during the circadian cycle. E. Castro-Longoria[1], S. Brody[2], and S. Bartnicki-García[1]. [1]División de Biología Experimental y Aplicada, CICESE, Ensenada, Baja California, México. [2]División of Biology/Molecular Biology, University of California, San Diego.

The circadian rhythm of *Neurospora crassa* is best observed in "clock" mutants. The rhythm produces spectacular concentric bands of conidiation separated by vegetative bands, or interbands, where aerial hyphae and conidia are scarce. By video-microscopy and image analysis, we evaluated the relationship between vegetative and reproductive development during the circadian cycle. The growing edge of a colony of *N. crassa (bd csp oli)* was recorded continuously at low magnification for 24 h (11h light-13h dark) at 22-24 C. Growth rate was measured for groups of 10-15 leading hyphae and found to decrease gradually at the start of the dark period. This coincided with the formation of the conidiation band. At the start of the light period, the growth rateof vegetative hyphae increased gradually. Growth rate reduction was correlated with increased formation of aerial hyphae and subsequent conidiation. During this period the cytoplasm of vegetative hyphae behind the edge of the colony was seen (magnification 2000 X) to flow backwards towards a branch, possibly an aerial hypha. Clearly, the circadian cycle comprises not only a periodic band of conidiation but also a corresponding decrease in vegetative growth rate. Presumably cytoplasmic resources that flow towards the tip to maintain apical growth are partly diverted to form aerial mycelium and conidia.

10) Heterokaryon incompatibility, prion formation and protein aggregation in *Neurospora crassa.* Karine Dementhon and N. Louise Glass. University of California, Plant and Microbial Biology Department, Berkeley, CA 94720.

Neurospora crassa can undergo hyphal fusion between different individuals to form vegetative heterokaryons. Hyphal fusion between two individuals with identical genotype at all *het* loci leads to stable heterokaryon formation. In contrast, if the two individuals differ in allelic specificity at a *het* locus, the fusion cell is rapidly destroyed by a programmed cell death (PCD) reaction termed heterokaryon incompatibility. In *Podospora anserina*, the *het-s* locus has two incompatible alleles, *het-s* and *het-S*. A *het-s* strain can have two alternative HET-s forms: a [Het-s] prion form, and a [Het-s*] non-prion form. Only the [Het-s] prion form triggers incompatibility when a *het-s* strain fuses with a *het-S* strain. The prion-free [het-s*] strain will form a viable heterokaryon with a *het-S* strain. The mechanism by which the prion protein triggers the cell death reaction in combination with HET-S is unknown.

N. crassa offers an excellent system to explore the relationship between protein aggregation and PCD. We found that over-expression of *het-s* in *N. crassa* results in HET-s aggregation, and, unlike in *P. anserina*, triggers growth inhibition. Our preliminary results suggest that co-expression of *het-s* and *het-S* in *N. crassa* triggers heterokaryon incompatibility. In *N. crassa*, genetic differences at *het-c* locus trigger incompatibility, which is associated with the formation of HET-C heterocomplex composed of HET-C proteins encoded by the alternative alleles. Incompatibility at *het-c* is suppressed by *vib-1*, which encodes nuclear-localized putative transcriptional factor. The relationship between incompatibility mediated by *het-s* and *het-c*, protein mis-folding, and *vib-1* is currently under investigation.

11) The *Neurospora crassa* immunophilin FKBP50 is located in the nucleus. Margarida Duarte, Patrícia Carneiro and Arnaldo Videira. Instituto de Biologia Molecular e Celular and Instituto de Ciências Biomédicas de Abel Salazar, Porto, Portugal

The FK506 binding-proteins (FKBPs) define a subfamily of peptidyl-prolyl cis/trans isomerases known to play roles in cellular processes such as protein folding, protein interactions and signal transduction. Four FKBP members (FKBP11, FKBP13, FKBP22 and FKBP50) were identified in the last annotation of the *N. crassa* genome (Neurospora sequencing project, Whitehead Institute/MIT Center for Genome Research). We have recently disrupted the gene for FKBP22, an immunophilin that resides in the endoplasmic reticulum. FKBP50 displays a high similarity to the FPR3 and FPR4 homologues of *Saccharomyces cerevisiae*, along with its homology to other Neurospora FKBPs. To characterise the cellular function of FKBP50 we are inactivating the fungus gene and used a green fluorescent protein (GFP) fusion to determine its subcellular localization. The cDNA encoding FKBP50 was cloned in frame with the GFP gene, under the control of an inducible promoter. The fusion construct and a control plasmid were targeted to the his-3 locus of *N. crassa*. The transformants were analysed for the correct targeting of the plasmids by southern blot and for the expression of the FKBP50-GFP fusion protein with specific antibodies against FKBP50 and by fluorescence microscopy. Our results indicate that the FKBP50 protein is located in the nucleus. Further work is undergoing to characterise its biological function.

12) Characterization of *soft*, a hyphal fusion mutant of *Neurospora crassa.* Andre Fleissner, David J. Jacobson, Sovan Sarkar and N. Louise Glass. University of California, Berkeley

The mycelial colony of filamentous fungi consists of a network of interconnected multinucleate hyphae. The colony grows by hyphal tip extension, branching andfusion (anastomosis). The ability to form hyphal fusions within one colony, butalso between different individuals enables fungi to establish complex functional units that show coordinated growth and exploration of their environment.To gain a better understanding of the basic, yet not well described, process of hyphal fusion, we are characterizing *soft*, a hyphal fusion mutant of *Neurospora crassa*.The phenotype of *soft* includes the lack of anastomosis within a colony or between conidial germlings, female sterility, reduced aerial hyphae and a slower growth rate. We were able to clone *soft* by complementation. Database analysis showed that this gene is highly conserved in filamentous ascomycetes, but not present in *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*. The encoded protein contains a conserved WW-domain involved in protein-protein interactions, but has unknown function. Genetic, complementation and sequence analysis revealed that *soft* is allelic to *ham-1*. *Ham-1* mutants show the same phenotype as *soft* strains. Both alleles show single point mutations resulting in stop codons in the first part of the gene. Using microscopy, genetics and biochemical analysis we are revealing the function of the *SOFT* protein in the process of hyphal fusion and, in a broader view, the role of anastomosis for the biology of filamentous fungi.

13) Sharp Peaks of Circadian Conidial Development for *Neurospora crassa* as Determined by Time Lapse Video. Van Gooch, University of Minnesota Morris, Division of Science and Math, Morris MN 56267

Using time lapse video, the growth of *Neurospora crassa bd* was analyzed. The *Neurospora* were grown in classic conditions that demonstrate circadian rhythms of conidial formation (agar in a race tube with 0.3% glucose, 0.5% arginine, and 1X Vogel's salts at 25°C with red safety lights). The conidial development largely occurs simultaneously over a period ofabout one hour in a band width of about 6 mm. The peak time of this activity is about 11.4 hours after the white lights are last turned off. The time lapse video also reveals an approximately two fold change in the growth front rate during a daily circadian cycle. Using a variety of conditions that alter the conidiation rhythm, the period and magnitude of the growth rate rhythm correlates to the period and magnitude of the conidiation rhythm. *Neurospora* circadian rhythms have been classically measured by linearly translating the position along a race tube into time. In fact there is a variation in growth rate by a factor of about two and

https://newprairiepress.org/fgr/vol51/iss1/16
DOI: 10.4148/1941-4765.1146
http://newprairiepress.org/fgr/vol51/iss1/16

DOI: 10.4148/1941-4765.1146

calculations show that the linear translation of position to time can cause errors in period and phase on the order of magnitude of one hour. Secondly, when one uses a linear translation of position to time, one would determine from the conidial band width that conidiation development occurs over several hours when in fact the time lapse video analysis shows that it occurs more on the time frame of one hour.

14) A Mauriceville derivative strain that escapes senescence is defective in the retrograde response pathway. John C. Kennell, Erica Larson and Maze Ndonwi. Department of Biology, Saint Louis University, St. Louis, MO

Wild-type *Neurospora* strains display indefinite growth potential and with few exceptions, senescence is restricted to strains harboring certain mitochondrial plasmids. The MS4416 strain has a variant form of the Mauriceville mitochondrial retroplasmid and has been shown to senesce at highly predictable frequencies. Growth cessation of MS4416 cultures was shown to be associated with the inhibition of mitochondrial gene expression due to plasmid over-replication. Here, we describe a mutant derivative of the MS4416 strain that escapes senescence. This so-called long-lived (LL) strain shows indefinite growth while still tolerating high levels of the variant plasmid. New forms of variant plasmids arise and replace existing plasmids, indicating that longevity is not related to particular changes in the Mauriceville plasmid. The LL strain appears to be defective in the retrograde response pathway as it is sensitive to electron transport chain (ETC) inhibitors and fails to induce specific nuclear genes, such as the structural gene for alternative oxidase (*aod-1*). Genetic studies show that the sensitivity to ETC inhibitors is controlled by a single nuclear gene, and the mutation is not allelic to *aod-1*. The inability of the LL mutant to induce retrograde response genes appears to preventthe accumulation of defective mitochondria and enables the strain to escape senescence.

15) A novel senescent phenotype appears to result from a dominant-lethal nuclear mutation that influences cytochrome *c* expression. John C. Kennell, Marci Tauzin, Stephanie Cohen and Janice Chyi. Department of Biology, Saint Louis University, St. Louis, MO

Senescence of filamentous fungal cultures invariably involves mitochondrial dysfunction, which in most cases is associated with the integration of mitochondrial plasmids into the mitochondrial genome. Here we describe a senescent mutant of *N. crassa* (M6) that derives from the wild-type Mauriceville strain (FGSC 2225). Surprisingly, M6 cultures that are close to senescence have no detectable changes in either the Mauriceville mitochondrial plasmid or mitochondrial DNA, features that are commonly associated with Mauriceville senescent strains. Tetrad analysis of crosses with M6 as a conidial parent indicate that the senescent phenotype is inherited as a single nuclear mutation, with mutant ascospores senescing shortly after germination. The cytochrome spectra of M6 cultures are highly unusual as they lack a peak corresponding to cytochrome *c* and Northern analysis indicates that *cyc-1* transcripts are not produced. Uninucleate microconidia isolated from M6 cultures are not viable, suggesting that the mutation is lethal as a homokaryon. As M6 arose spontaneously during vegetative transfer, it appears that the mutation is dominant; however, formal (i.e. forced) heterokaryon studies have not yet been conducted. Further studies of the M6 strain may reveal attractive targets to control fungal growth.

16) Pheromone receptor genes, *pre-1* and *pre-2*, are essential for mating type-specific directional growth and fusion of trichogynes and female fertility in *Neurospora crassa.* Hyojeong Kim and Katherine Borkovich. University of California, Riverside, U.S.A.

Pheromone receptor genes for the heterothallic filamentous fungus *Neurosporacrassa*, *pre-1* and *pre-2*, were identified during BLAST searches against the entire genome sequence (http://www-genome.wi.mit.edu/annotation/fungi/neurospora/). The encoded proteins belong to the G-protein-coupled receptors

containing seven-transmembrane helices and show significant sequence similarity to other fungal pheromone receptors. *pre-1* and *pre-2* deletion mutants are not greatly affected in vegetative growth, heterokaryon formation or male fertility in either mating type.They form normal protoperithecia with fully differentiated trichogynes as well. However, *pre-1 mat A* and *pre-2 mat a* strains are unable to undergo fertilization; their trichogynes are unable to recognize and fuse with *mat a* and *mat A* cells, respectively. Previous work has demonstrated that GNA-1 (Ga) and GNB-1 (Gb) are required for female fertility in*N. crassa*. Trichogynes of *gna-1* and *gnb-1* mutants displayed severe defects in growth towards and fusion with male cells, similar to that of *pre-1 mat A* and *pre-2 mat a* strains. Thus, PRE-1 and PRE-2 are pheromone receptors coupled to GNA-1 that is essential for the mating of *mat A* and *mat a* strains as females, respectively, consistent with a role in launching the pheromone response pathway in *N. crassa*.

17) A G-protein coupled receptor gene, *gpr-1* is involved in regulation of sexual development in Neurospora crassa. S. Krystofova and K. A. Borkovich, University of California Riverside, Plant Pathology, Riverside, CA

10 potential G-protein coupled recepetors (GPCR's) have been identified in the entire *Neurospora crassa* genome database. Based on the phylogenetic analysis, three of those genes *gpr-1*, *gpr-2* and *gpr-3* form a family with homology to the second class of GPCR's. *gpr-1* null mutants analyzed exhibit defects in sexual development. This corresponds with *gpr-1* expression pattern that shows the highest mRNA level in perithecial tissue. In comparison to wild type, *gpr-1* strains produce small and pale protoperithecia that are often buried in solid media. Perithecia are smaller than those from wild type crosses and defective in the light-induced polarity and ascospore ejection.

18) Blue light effects on ion transport in *Neurospora crassa* slime mutant. N.N.Levina[1], A.Y.Dunina-Barkovskaya[2], S.N.Shabala[3], and R.R. Lew[1]. [1]York University, Toronto, Ontario, Canada; [2]Moscow State University, Moscow, Russia; [3]University of Tasmania, Hobart, Australia

Blue light regulates a number of cellular functions in *N. crassa*, such as carotenogenesis, conidium formation, protoperithecia formation and phototropism. We studied the effects of blue light on ion transport processes across plasma membrane, since electrical changes are the earliest recorded responses to blue light in *N. crassa*. We used the slime mutant (FGSC#1118), it lacks a cell wall and grows as amoeboid-like spheroplasts. It exhibits normal photoinduced carotenogenesis and is suitable for the study of photoeffects on plasma membrane ion currents. Patch clamp experiments were complemented by measurements of ion fluxes across plasma membrane using the ion-selective vibration probe technique. Within 2-4 min of illumination, blue light caused a decline in whole-cell conductance attributed to the decrease in inward potassium current. Simultaneous hyperpolarization of the plasma membrane may develop as a result of the decrease in inward potassium current or/and activation of the plasma membrane proton pump, supported by a decrease in the proton influx. Blue light also causes an increase in chloride ions influx. Therefore, blue light regulates an ensemble of transport processes: H+, Cl-, and K+ transport. Since changes in the total conductance are detected within a few seconds after the onset of blue light, we suggest that these changes may be part ofthe initial signal transduction.

19) Expression of clock-associated genes in agar cultures of Neurospora. Sanshu Li and Patricia Lakin-Thomas. Dept. of Biology, York University, Toronto, ON, Canada.

The circadian clock of *N. crassa* drives a rhythm in spore formation that is assayed by observing bands of conidiospores when cultures are grown on the surface of agar medium. Rhythmic gene expression has been assayed in Neurospora by other laboratories using liquid culture systems in which conidiation is suppressed and the rhythm of spore formation cannot be assayed. We are using a culture system in which conidiation and biochemical rhythms can be assayed under identical conditions, by growing cultures on solid agar overlaid with cellophane. We are comparing biochemical rhythms in the newly-formed hyphae at the growth front with hyphae 24-48 hours old behind the growth front. We have found rhythmic expression (RNA and protein) of the frq (frequency) gene in old and new areas, and rhythmic levels of WC-1 (white-collar-1) protein. However, the phase relationship between the

http://newprairiepress.org/fgr/vol51/iss1/16

DOI: 10.4148/1941-4765.1146

FRQ and WC-1 protein peaks differs between old and new areas, and differs from the phase relationship reported by other laboratories using liquid culture systems. RNA levels for clock-controlled genes ccg-2 and ccg-7 are rhythmic in new areas but not rhythmic in old areas. We found similar results for the neutral lipid diacylglycerol (DAG): Levels are rhythmic in new areas but low and arrhythmic in old areas. Mycelial transfer experiments indicate that hyphae from both old and new areas carry similar clock phase information. We are also investigating gene expression in the long-period *chol-1* mutant strain, which has an elevated level of DAG. Funding from NSERC grant 504210 is acknowledged.

20) Analysis of a G-protein coupled receptor from *Neurospora crassa*. Liande Li and Katherine A. Borkovich, University of California Riverside, Plant Pathology, Riverside, CA

gpr-4 (G-protein coupled receptor-4) is a gene encoding a G-protein coupled receptor identified in *Neurospora* genome database (http://www-genome.wi.mit.edu/annotation/fungi/neurospora) that is most similar to putative carbon sensory receptors: Gpr1p from *Saccharomyces cerevisiae* and Git3from *Schizosaccharomyces pombe*. The *gpr-4* deletion mutants do not show any obvious differences with wild type in regards to 1) apical extension rate on minimal medium and medium with poor carbon or nitrogen sources, 2) aerial hyphae height in standing liquid cultures, 3) male and female fertility, 4) ascospore germination, or 5) dry mass of submerged cultures grown with different carbon sources. However, delta *gpr-4* strains accumulate much less biomass than the wild type when cultured on solid medium containing poor carbon sources, such as glycerol, mannitol, arabinose, or low concentrations of sucrose or glucose, suggesting a possible role as a carbon sensor. delta *gpr-4* forms less aerial hyphae and produces more conidia per aerial hyphae than wild type. In addition, delta *gpr-4* showed higher resistance to heat shock and H_2O_2 stress. delta *gpr-4* shares some characteristics with delta *cr-1*, delta *gna-1*, delta *gna-2* and delta *gna-3* mutants. Further experiments, including epistasis analysis of *gpr-4*, with *gna-1*, *gna-3*, *cr-1* and *pka-cat* are in progress, in order to determine the function of GPR-4.

21) Characterization of meiotic silencing by unpaired DNA (MSUD) in *Neurospora tetrasperma*. Namboori Raju and David Jacobson. Department of Biological Sciences, Stanford University, California.

Genes that are unpaired during meiosis are silenced in *N. crassa*. GFP-tagged *histone H1* (*hH1::GFP*, courtesy of M. Freitag, U. Oregon), when inserted at the *his-3* locus on linkage group I (LGI), allows visualization of MSUD in developing asci by fluorescence microscopy. When homozygous, *hH1::GFP* is paired during meiosis; it expresses normally and nuclei fluoresce throughout ascus development. However, when heterozygous, *hH1::GFP* is unpaired and silenced during meiosis until ascospore delimitation. MSUD does not extend into the ascospore maturation stage and *hH1::GFP* nuclei in four of the eight ascospores begin to fluoresce ~18-24 h after delimitation. *N. tetrasperma* packages two nuclei of opposite mating type into each of its four ascospores. This is accomplished by blocking recombination in a large region of LGI. LGI also shows a large unpaired region during pachytene. To test whether the genes in this unpaired region are silenced, we introgressed *hH1::GFP* at the *his-3* locusfrom *N. crassa* into *N. tetrasperma*, The initial hybrid cross produced almost all 8-spored asci with a high level of ascospore abortion, but showed no silencing of *hH1::GFP*. After four backcrosses to *N.tetrasperma*, all progeny are now phenotypically *N. tetrasperma*: asci are four-spored and ascospores are heterokarotic for *hH1::GFP*. All nuclei in the developing asci and in the heterokaryotic ascospores fluoresce brightly. Thus, unpaired *hH1::GFP* on LGI is not silenced in *N. tetrasperma*. Whether the absence of MSUD in *N. tetrasperma* is limited to the unpaired region in LGI or is global in the genome is under investigation.

22) Screening for Supressors of Hyperbranching Mutants in *N. crassa.* Beth Rapa & Michael Watters. Valaparaiso University, Valparaiso Indiana

Growth in filamentous fungi occurs at a tip which branches as it extends. *Neurospora crassa* is a filamentous fungus for which there are many known morphological mutants which affect the periodicity of branching. The *col-16* mutant of *N. crassa* has a much greater branching frequency than the wild type and therefore grows more densely. Ultraviolet light was used on a *col-16* mutant strain in order to induce mutations with the goal of finding suppressors that return growth to normal. Following mutagenesis, the samples were plated and the resulting colonies screened for those in which more wild type growth had been restored (i.e. those which had gained a suppressor mutation). The presence of a suppressor was confirmed using crosses, crossing the potential suppressor to the wild type. The reappearance of *col-16* mutants among the progeny of these crosses confirms that the suspect strains indeed are suppressed (i.e. *col-16*/suppressor double mutants). The next goal is to separate the suppressor from *col-16* to obtain strains which contain only the suppressor mutation. This work was supported by a grant from the National Science Foundation.

23) A Novel Rho-type GTPase Required for Septation in *Neurospora crassa* . Rasmussen, C., Chiang, E. and N. L. Glass, Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720

Rho-type GTPases are small GTPases primarily involved in polarization, control of cell division and reorganization of cytoskeletal elements. Phylogenetic analysis of fungal Rho family members suggests that while several *Saccharomyces cerevisiae* Rho-type GTPases have orthologs in *Neurospora*, others do not. One of the Rho-type GTPases, RHO-4, which has an ortholog in *Schizosaccharomyces pombe* but not in *S. cerevisiae* was mutated in *Neurospora* by RIPping. *rho-4* loss of function mutations lead to a loss of septation, loss of conidiation and heavy cytoplasmic bleeding. Steps required for septation include formin localization, septin and actin localization, and finally new cell wall synthesis. In order to determine where RHO-4 acted in the septation pathway, localization of f-actin was observed in wild type and *rho-4* strains. *rho-4* mutants are unable to form actin rings, suggesting that RHO-4 acts upstream of actin localization. Further, preliminary immunofluorescence experiments suggest that RHO-4 is localized to septa. Characterization of dominant active and dominant negative alleles of *rho-4* as well as cloning of suppressor mutations will help to elucidate the role of RHO-4 in septation and conidiation in *N. crassa*.

24) Key differences between lateral and apical branching ontogeny in hyphae of *Neurospora crassa.* Meritxell Riquelme and Salomon Bartnicki-García.Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), 22860 Baja California, México.

We examined in fine detail growth kinetics and intracellular events during lateral and apical branching in hyphae of *Neurospora crassa* by high-resolution video-enhanced light microscopy. We found remarkable differences in the events preceding lateral vs. apical branching. While apical branching encompassed a significant disturbance of apical growth, lateral branching occurredwithout any detectable alterations in the apical growth of the parental hypha. Lateral branch formation did not interfere with the elongation rate of the primary hypha, the shape of its apex or the behavior of its Spitzenkörper. In sharp contrast, apical branching was preceded by marked changes in physiology and morphology of the parental hypha and by a sharp drop in elongation rate. The sequence involved a cytoplasmic contraction, followed by a retraction, dislocation, and disappearance of the Spitzenkörper; hyphal elongation decreased sharply and a transient phase of non-polar growth caused the hyphal apex to round up. Growth resumed with the formation of two or more apical branches, each one with a Spitzenkörper formed by gradual condensation of phase-dark material around an invisible nucleation site. The observed dissimilarities between lateral and apical branching suggest that these morphogenetic pathways are triggered differently. Whereas the trigger of apical branching may be traced to a sudden discrete disruption in cytoplasmic organization, the trigger of lateral branching probably stems from the subapical accumulation of wall precursors reaching a critical concentration.

25) Conidial Anastomosis in *Neurospora crassa.* M. Gabriela Roca M., Jochen Arlt & Nick D. Read. Institute of Cell and Molecular Biology/COSMIC, University of Edinburgh, Edinburgh, UK.

http://newprairiepress.org/fgr/vol51/iss1/16

DOI: 10.4148/1941-4765.1146

Using live-cell imaging, we have analysed the process of hyphal homing and fusion (anastomosis) between conidial germlings of *Neurospora crassa* labelled with different vital dyes and GFP probes. Specialised, morphologically distinct hyphae (called conidial anastomoses tubes [CATs]) are produced by conidia >4h following hydration. In wild type strains, CATs are thinner (2.72 +0.6 um) than germ tubes (3.52 + 0.3um). Conidial anastomosis tubes grow towards each other, and reorientate themselves back towards each other if they are moved relative to each other with laser tweezers. This provides clear evidence for the existence of, as yet unknown, diffusible chemotropic signals being involved in the homing response of CATs. In contrast to ‘fusion hyphae' whichundergo fusion in the centre of a mature colony, CATs do not undergo branching. Fusion between CATs is independent of mating type, and occurs between conidial germlings of the same and different mating types. We have imaged nuclear movement and shown continuity of the microtubular cytoskeleton between fused germlings. Further morphological, physiological andgenetic markers are currently being used to further characterize CATs and the role that these structures play in the life cycle of *N. crassa*.

26) Biogenesis of glyoxysomes and Woronin bodies in *N. crassa* as a model of selective protein import.
Hanspeter Rottensteiner, Institut für Physiologische Chemie, Bochum, Germany.

The biogenesis of microbodies has been studied in various model organisms ranging from yeast to man and is specifically governed by the so-called *PEX* genes. Also the genome of the filamentous fungus *Neurospora crassa* contains a set of at least 18 peroxins. Interestingly, *N. crassa* harbors two compartments of the microbody family; glyoxysomes that house the fatty acid beta-oxidation enzymes and two key enzymes of the glyoxylate cycle, as well as the Woronin body, whose main function is to plug the septal pore after hyphal wounding. The glyoxysomal matrix proteins as well as the predominant matrix enzyme of the Woronin body, Hex1p, contain classical peroxisomal targeting signals. Since both organelles coexist in a single cell, the question arises as to why Hex1p is imported into Woronin bodies and not into glyoxysomes and vice versa. Strategies to unravel the molecular mechanism of this selective protein import will be presented.

27) Mechanism of Polarized Growth and Hyphal Morphogenesis in *Neurospora crassa*. Maho Uchida, Elizabeth Perry, and Robert W. Roberson. School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA

Fungal hyphae grow and maintain their characteristic shape through cell extension at their tips. Mechanisms of polarized growth are maintained by cytoskeletal function and directed exocytotic events. Previous observations have lead us and others to speculate that microtubules (MTs) are involved in long distance transport of secretory vesicles from Golgi-equivalents to the Spitzenkörper (Spk), followed by a switch at the Spk from MTs- to actin microfilament-based motility. To better understand vesicle flow from sub-apical sites to the apical plasma membrane and the role(s) of the cytoskeleton, we have evaluated Spk dynamics and organization, and have mapped the distributions of MTs and cytoplasmic components using optical and transmission electron microscope (TEM) methods. This work was done in mature, wild type hyphae of *Neurospora crassa* and will serve as a baseline for future studies to elucidate important mechanism underlying hyphal morphogenesis. Using phase contrast digital light microscopy, a unique organization of the Spk and novel details of internal dynamics have been identified. The Spk consisted of three discrete phase-dark layers subtended by a phase-bright core. Unidentified materials, at or below the level of resolution, traveled through the core towards the hyphal apex. Serial cross-section reconstructions and quantitative analysis ofTEM data from a 2.0 m thick sub-apical region approximately 30 m behind the apex have been analyzed. Within this region, 61 microtubule cross-sections, 17 apical (secretory) vesicles and 13 Golgi-equivalents were counted. Mitochondria, multi-vesicular bodies, and nuclei were also noted. 0 to 7 MTs were located within 25 to 274 nm of Golgi-equivalents while only 3 apical vesicles positioned within 30 nm of a MT or MT bundle. Analysis of such structural and dynamic data will be discussed relative to the vesicle flow and and cytoskeletal function.

Developmental Biology

28) Oxidative stress and conidiation in *Neurospora crassa* Wilhelm Hansberg, Shaday Michán, Leonardo Peraza, Adelaida Díaz, Fernando Lledías, Pablo Rangel, Mauricio Rios-Momberg. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México, D.F.

Morphogenetic transitions of *N. crassa* asexual life cycle are responses to a hyperoxidant state. Catalase activity induction and catalase oxidation by singlet oxygen are consequences of this hyperoxidant state. The two large monofunctional catalases (CAT–1 and CAT–3) and catalase-peroxidase (CAT–2) are resistant to molar concentrations of hydrogen peroxide. These enzymes are oxidized by singlet oxygen at the heme, without significantly affecting enzyme activity, but oxidation increases enzyme degradation. CAT–1 is expressed in non-growing cells, such as hyphae in stationary growth and conidia. CAT-1 is accumulated to high levels in conidia. Crystallographic structure of CAT-1 showed an oxidized heme and an unusual covalent bond at the active site. CAT–2 is associated with lysing cells, such as hyphae in late stationary growth,in conidiating substrate mycelium, and base of aerial hyphae. CAT–3 is associated with growing hyphae and is expressed during late exponential and pre-stationary growth. CAT–3 has a signal peptide and is secreted. Light and oxidative stress induces CAT-3. A CAT–3 null mutant strain showed increased protein oxidation, carotene levels in the dark, and hyphae aggregates, features that are indicative of oxidative stress. The mutant strain formed and produced six fold the amount of wild type strain aerial hyphae and conidia. CAT-2 null mutant strain is sensitive to oxidative stress and to a temperature of 42ºC. It forms higher number of arthroconidia. These results support our hypothesis of cell differentiation as response to oxidative stress. Grants: CONACyT C01-40697, DGAPA/UNAM IN225402.

29) Characterization of the *rcm-1* gene of *Neurospora crassa.* Bheong-Uk Lee*, Eun Jung Kim, Sang Rae Kim, Ilji Jeon and Byung-Kap Jeong Division of Biological Sciences, Kosin University, Busan 606-701, Korea

Analysis of the complete genome of *Neurospora crassa* reveals that at least 15 proteins contain tetratricopeptide repeat (TPR) motifs. One of them shows over 60% homology to Ssn6 of *Saccharomyces cerevisiae*, a universal repressor that mediates repression of genes involved in various cellular processes. Mutant strains generated by RIP (repeat induced point mutation) process showed five distinctive vegetative growth patterns and slow growth in various rates: A) densemycelial, csp, looks like ropy, yellow, B) dense mycelial, csp, melanin-overproduction on SC agar, C) slow growth, dense mycelial, csp, D) extremely slow growth, acon and E) flat, little aerial hyphae, acon, looks like rco-1. They are male-fertile, yet all female-sterile and produced little or no perithecium. Reduced levels of con-8, eas and grg-1 mRNA were detected in type A mutant strain while they were elevated in type E. These results indicate that this gene is pleiotrophic and involved in several cellular processes during vegetative growth, and asexual and sexual spore formation. Analysis of cDNA shows that it encodesa putative 102kDa protein. This gene is designated rcm-1 (regulation of conidiation and morphology).

30) A suppressor mutant which suppresses *cr-1* mutation in *Neurospora.* Tadako Murayama, Michiko kudo, and Sei-ichi Kanzaki, Kanto-GakuinUniversity, Yokohama, Japan

A morphological mutant of *Neurospora cr-1* grows to be colonial, whereas the wild type grows to be filamentous. The *cr-1* mutant has been described as having a defect in adenylyl cyclase gene. Suppressor mutations of *cr-1* mutation frequently occurred. One of suppressor mutants, *hah*, which suppresses colonial growth of *cr-1* formed high aerial hyphae without conidia. Genetic analysis of the *hah* mutant showed that it has a mutation in the gene

http://newprairiepress.org/fgr/vol51/iss1/16

DOI: 10.4148/1941-4765.1146

which is located 13.3 units from *inl* and 4 units from *am* on the linkage group V. It was suggested by the physical map reported in *Neurospora crassa* Genetic Maps (http://www-genome.wi.mit.edu/annotation/fungi/neurospora/maps.html) that *HAH* is *MCB* which encodes the regulatory subunit of cAMP dependent proteinkinase, though the *hah* mutant is morphologically different from the *mcb* mutant which has been reported to form a lot of conidia(Bruno et al.,1996). The results of cloning and sequencing of the *MCB* gene from the *hah* mutant will be presented and the relatioships between the *hah* mutation and the morphology will be discussed.

31) *Neurospora crassa* catalase-peroxidase is required for heat shock and oxidative stress tolerance. Leonardo Peraza, Wilhelm Hansberg. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México, D.F.

Catalase-peroxidases (CP) are bifunctional antioxidant enzymes that evolved in a prokaryotic progenitor cell by tandem duplication of an ancestral peroxidase gene. *N. crassa cat–2* encodes a typical CP. Based on phylogenetic analysis, we have suggested a bacterial origin for fungal CPs. The enzyme is a homodimer of 83.4 kDa subunits that has both, catalase and peroxidase activities. CAT–2 mRNA and activity are associated with late stationary-phase mycelia, when arthroconidia are formed and hyphae undergo autolysis. CAT–2 was induced during stress conditions such as carbon deprivation, H_2O_2-generated oxidative stress and heat shock. CAT–2 activity was found to be regulated during macroconidiation, *cat–2* mRNA accumulated rapidly after induction of conidiation by air exposure of a mycelial mat, but CAT–2 activity was detected until aerial hyphae are formed. Both, mRNA and CAT-2 increase further in aerial hyphae and conidia. CAT–2 null mutant strains are sensitive to oxidative stress exerted by hydrogen peroxide or organic peroxides. It is also sensitive to high temperatures.CAT–2 null mutant strain showed increased formation of arthroconidia during late stationary-phase. Grants: CONACyT C01-40697, DGAPA/UNAM IN225402.

32) The *Neurospora crassa*, SBR protein exhibits specific DNA binding activity. John Vierula, Yanhua Yan, Katrina Campsall and Bin Zhang. Department of Biology, Carleton University, Ottawa, Ontario. Canada K1S 5B6

The *sbr* mutant of *Neurospora crassa*, forms very small, dense colonies which fail to produce conidiophores or conidia. Instead of hyphae, *sbr* initially forms sausage-shaped, cell compartments which give rise to large, randomly positioned, spherical buds. The *sbr* gene encodes a 612 amino acid protein with two poly-glutamine domains, a cysteine-rich region and a putative helix-turn-helix motif reminiscent of transcriptional activator proteins.To test this hypothesis, deletion derivatives of the SBR ORF were fused to a 6XHIS tag and over-expressed in *E.coli*. The purified, 6XHis-tagged polypeptides were then used to capture putative binding targets 300 bp and 600 bp in length from total genomic DNA. Electrophoretic mobililty shift assays (EMSA)were employed to demonstrate specific binding to both of the target sequences. Deletion of the zinc finger resulted in a loss of specific DNA binding by the SBR protein. The results of EMSA experiments using 100 bp subclones of the DNA targets have been used to further localize the SBR binding sites within each target sequence. Supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada.

Gene Regulation

33) *Neurospora crassa* SET-2 selectively methylates lysine 36 of histoneH3. Keyur K. Adhvaryu and Eric U. Selker. Institute of Molecular Biology, University of Oregon, Eugene, OR, 97403

The SET (**S**u(var)3-9, **E**nhancer-of-zeste, **T**rithorax) domain is an evolutionarily conserved domain found in many chromatin modifying proteins. Some SET domain containing proteins are histone methyltransferases. In *Neurospora crassa*, studies with the *dim-5* (*d*efective *i*n *m*ethylation) mutant have demonstrated a link between histone modifications and DNA methylation. DIM-5 is a SET domain histone methyltransferase with specificity for the lysine 9 residue of the amino terminal tail of the histone H3. Histone H3 and H4 have additional lysine residues (K4, K27, K36 and K79 on H3 and K20 on H4) that are potential targets for methylation. We searched the *Neurospora crassa* genome database for genes with putative SET domains and found eight in addition to *dim-5* (*set-1* through *set-8*).

The *set-2* (NCU00269.1) gene is closely linked to *ro-2* (NCU00257.1) on the right arm of LG III. It putatively encodes a 963 amino acid protein containing AWS, SET, postSET and WW domains. We used RIP to obtain a mutant (*set-2^{RIP1}*) that has multiple nonsense mutations. *set-2^{RIP1}* grows slowly, shows poor conidiation and is female sterile. Using specific antibodies we analyzed histones isolated from wild type *Neurospora crassa* strain (OR23-1V*A*) and find that lysine residues at position 4, 27, 36 and 79 in histone H3 and 20 in histone H4 are methylated. Methylation of the lysine 36 of H3 is completely lost in the *set-2^{RIP1}* suggesting that SET-2 is a lysine 36 specific methyltransferase.

This work was supported by AHA Postdoctoral Fellowship 0225370Z to K.K.A. and NIH grant GM 35690 to E.U.S.

34) *Neurospora crassa* Mutants Altered in Blue Light Transcription. Laura Navarro-Sampedro and Luis M. Corrochano
Departamento de Genetica, Universidad de Sevilla, Spain

The gene *con-10* of Neurospora is expressed during conidiation and following illumination of mycelia with light. The photoactivation of *con-10* disappears after two hours of illumination (light adaptation). We have designed a method to isolate mutants altered in the adaptation of *con-10* photoactivation. We are using a strain of Neurospora with a fusion of the *con-10* promoter to the gene conferring resistance to hygromycin. This strain is sensitive to the drug when the promoter is inactive, i.e. during vegetative growth either in the dark or under continuous light. We have isolated four mutants (SN1 to SN5) that grow in the presence of hygromycin under continuous light but not in the dark. All the mutants showed and enhanced accumulation of the *con-10*/hygromycin fusion gene after five hours of light compared to the parental strain. All the strains carry two copies of the *con-10* promoter, the one in the naturally occurring *con-10* gene and an additional one fused to the hygromycin gene. The mutations in strains SN1 to SN3 are specific for the *con-10*/hygromycin fusion. The photoactivation in the original *con-10* gene is similar to that in the parental strain. These mutants are likely to carry mutations in the *con-10* promoter fused to the hygromycin gene and will identify sequence elements required for the appropriate regulation of *con-10* photoactivation. On the contrary, the mutations in strains SN4 and SN5 have also altered the photoactivation of the original *con-10*. They should carry mutations in genes responsible for proteins regulating *con-10* photoactivation.

35) DNA Segments Involved in Regulation by Blue Light and Development in the *con-10* Promoter of *Neurospora crassa*. Maria Olmedo and Luis M. Corrochano. Departamento de Genetica, Universidad de Sevilla, Spain

The gene *con-10* of *Neurospora crassa* is expressed during conidiation and following illumination of vegetative mycelia with light. Dark repression sites have been located at positions -1559 to -779 (from the transcription start site) and -353 to -265. A mycelial repression site has been located at position -778 to -353. Two conidiation activation sites have been located at positions -353 to -265 and -236 to -191. We are using a series of fusions between segments of the *con-10* promoter and the lacZ gene to investigate the DNA sequences involved inregulating the expression of *con-10.* The strains were grown in the dark for 48 h before applying light. Beta-galactosidase activity in cell extracts was a measure of the *con-10* promoter activity. The complete *con-10* promoter (-1559) fused

to the lacZ gene was induced about ten fold after 30 min illumination. Other *con-10* promoter fusions contained sequences to -913, to -839, and to -517. Their light-dependent activity will locate the position of the first dark repression site and themycelial repression site. To confirm the presence of the second dark repression site (-353 to -265) and the mycelial repression site (-517 to -354) we have fused each segment to a *con-10* minimal promoter (-191) that shows no response to light or conidiation. Additional fusions will confirm the location of the dark repression site: from -353 to -282, and from -337 to -265. These and other *con-10*/lacZ fusions will allow us to locate the sequences involved in the regulation

36) Catabolite repression of the quinic acid *(qa)* genes in *Neurospora crassa*. Diana R. Arnett[1] and David K. Asch[1,2]. [1]School of Biomedical Sciences, Kent State University, Kent, Ohio and [2]Department of Biological Sciences, Youngstown State University, Youngstown, Ohio.

The quinic acid *(qa)* gene cluster of *Neurospora crassa* provides an unusual example of gene controlin a eukaryotic organism. Previous studies focused on the primary control mechanism, which is dependent on the inducer quinic acid. However, the expression of the *qa* genes is also repressed in the presence of a preferred carbon source, even in the presence of the inducer. This important secondary level of control, termed carbon catabolite repression, has not been well studied. In order to focus on this system of control we are utilizing a constitutive mutant of the *qa* gene cluster which contains a deletion of the *qa-1S* repressor gene. Deletion of the *qa-1S* gene removes the primary means ofregulation of the *qa* cluster, ensuring that any observed effect on *qa* gene expression is due to catabolite repression. By Northern blot analysis, we have demonstrated that the *qa-y* gene seems to be repressed in the presence of a preferred carbon source such as dextrose even when the *qa-1S* gene product is absent, while the remainder of the *qa* genes are not directly repressed to a significant degree by the presence of dextrose.

37) RRG-1, a Response Regulator Signaling Protein in *Neurospora crassa.* Suzanne E. Greer-Phillips and Katherine A. Borkovich. University of California, Riverside.

In yeasts, sensor histidine kinases respond to various environmental stresses and signal through a two-component-like signal transduction pathway. This pathway is a multistep phosphorelay system where a histidine-containing phosphotransfer protein (Hpt) and a response regulator (Rrg) propagate the signal from sensor to effector. Eleven sensor histidine kinases have been identified in the genome of *Neurospora crassa*, but only one Hpt and two Rrggenes have been found. Here we report the identification of response regulator - 1 (*rrg-1*) from *N. crassa*. RRG-1 is similar to Mcs-4 from *Schizosaccharomyces pombe* and Ssk-1p from *Saccharomyces cerevisiae*. Similar to the *ssk-1* mutant of *S. cerevisiae*, a *rrg-1* deletion mutant of *N. crassa* is inhibited in growth under osmotic stress conditions such as increased concentrations of NaCl, KCl, or sorbitol. In addition, *rrg-1* mutants are female sterile and show increased carotenoid pigmentation. These results suggest that RRG-1 plays a more significant role in regulating cellular functions than is known for response regulators of other systems.

38) FWD1-mediated degradation of FREQUENCY in Neurospora establishes a conserved mechanism for circadian clock regulation. He Q, Cheng P, Yang Y, He Q, Yu H, Liu Y.Department of Physiology, The University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390, USA.

Phosphorylation of the Neurospora circadian clock protein FREQUENCY (FRQ)regulates its degradation and the proper function of the clock. The mechanism by which FRQ undergoes degradation has not been established. Here we show that FRQ is likely ubiquitylated in vivo, and its proper degradation requires FWD1, an F-box/WD-40 repeat-containing protein. In the fwd1 disruption strains, FRQ degradation is severely impaired, resulting in the accumulation of hyperphosphorylated FRQ. Furthermore, the circadian rhythms of gene expression and the

circadian conidiation rhythms are abolished in these fwd1 mutants. Finally, FRQ and FWD1 interact physically in vivo, suggesting that FWD1 is the substrate-recruiting subunit of an SCF-type ubiquitin ligase responsible for FRQ ubiquitylation and degradation. Together with the recent finding that Slimb (the Drosophila homolog of FWD1) is involved in the degradation of the Period protein in flies, our results indicate that FWD1 regulates the degradation of FRQ in Neurospora and is an evolutionarily conserved component of the eukaryotic circadian clock.

39) Comparative sequencing of the *qa-2* gene of *Neurospora crassa* and *Neurospora africana*. James A. Shevchuk[1], Diana R. Arnett[2] and David K. Asch[1,2]. [1]Department of Biological Sciences, Youngstown State University,Youngstown, Ohio and [2]School of Biomedical Sciences, Kent State University, Kent, Ohio.

Gene systems like the quinic acid *(qa)* gene cluster have been studied in *Neurospora crassa* for many years. However, we know very little about gene systems in the homothallic species of *Neurospora*. Earlier it had been observed that *N. crassa* probes containing the *qa* gene cluster would hybridize to sequences in various homothallic species. To learn more about the *qa* systems in the homothallic species of *Neurospora* we have cloned the *qa* gene cluster from *Neurospora africana*. From these clones we have isolated and sequenced the *qa-2* gene and compared it with the *qa-2* gene sequence of *N. crassa* and the sequence of the *qut-E* gene of *Aspergillus nidulans*.

40) Analysis of Neurospora sirtuins: evidence for control of telomeric silencing and homologous recombination. Gregory O. Kothe[1], Cindy Matsen[2], Michael Freitag[1], Kriistina Smith[1], Melissa Hemphill[1], Matthew Sachs[3], Mark Farman[4], and Eric U. Selker[1]. [1]Institute of Molecular Biology, University of Oregon, Eugene, [2]University of Chicago Medical School, Chicago, IL, [3]Department of Biochemistry and Molecular Biology OGI School of Science and Engineering, Beaverton, OR, [4]Department of Plant Pathology, University of Kentucky, Lexington, KY

The *Saccharomyces cerevisiae* SIR2 protein is involved in silencing at telomeres, rDNA, and silent-mating loci, and controlling rDNA recombination. The SIR2 family of proteins (sirtuins in eukaryotes other than yeast) function as NAD-dependent deacetylases that regulate the activities of other proteins. We have mutated three Neurospora genes encoding sirtuins, and have investigated the effects of these mutations on silencing and recombination. We refer to these sirtuins as NST-1, 2, and 3 (Neurospora Sir Two). Preliminary evidence suggests that NST-1 and NST-3 control telomeric silencing, but not methylation dependent silencing. We also noticed a dramatic decrease in recombination between the mating-type locus and *his-3* in a cross of an *nst-1* mutant strain with an *nst-3* mutant. Recombination between *inl* and *am* was normal in this cross, but a test for homologous mitotic recombination by transformation in an nst-1 mutant strain revealed a dramatic decrease in comparison to wild-type. *nst-3* mutant homozygous crosses are nearly barren. Our findings suggest that surtuins function in mitotic and meiotic homologous recombination as well as silencing in Neurospora.

41) Characterization of two Neurospora proteins with motifs characteristic of DNA glycosylases and putative DNA demethylase. Gregory O. Kothe[1], Jean-Pierre Jost[2], and Eric U. Selker[1]. [1]Institute of Molecular Biology, University of Oregon, Eugene, OR, [2]Friedrich Miescher-Institut, Maulbeerstrasse 66, CH-4058 Basel, Switzerland

In Arabidoposis, two proteins, ROS1 and Demeter, which contain a motif characteristic of DNA glycosylases (helix-hairpin-helix motif), have been shown to regulate expression of methylated DNA sequences, and are proposed to function as DNA demethylases. In vertebrates, the methyl-CpG binding protein MBD4 has an hhh domain and is a T:G mismatch glycosylase that functions in base excision repair, apoptosis, and cell-cycle regulation. Biochemical studies have suggested that MBD4 has demethylase activity as well. We mutated two Neurospora genes encoding proteins containing hhh domains, one most similar to MBD4, and the other most similar to ROS1 and Demeter. We

http://newprairiepress.org/fgr/vol51/iss1/16

DOI: 10.4148/1941-4765.1146

have shown that the Neurospora factor related to MBD4 binds methylated DNA preferentially in a Southwestern assay, and we refer to this factor as MBP-3 (Methyl-Binding Protein 3). The second protein is encoded by a gene immediately adjacent to the rid gene. We refer to this protein as HHH-1 (Helix-Hairpin-Helix 1). We have not detected T:G mismatch or methylcytosine glycosylase activity for either MBP-3 or HHH-1. We are currently characterizing the mutant phenotypes for the genes in the vegetative and sexual cycle, and we are analyzing the effect of over-expression of the proteins as well.

42) Circadian regulation of the evening-specific gene *ccg-16.* Zachary Lewis and Deborah Bell-Pedersen. Texas A &M University.

Circadian clocks coordinate daily changes in behavior, physiology, and gene expression in organisms. In Neurospora the clock regulates the production of conidia and the rhythmic expression of a number of genes and proteins. Previous studies identified several *clock controlled genes (ccg's)* all of which peaked in mRNA accumulation in the late-night to early-morning. These data implied that the circadian clock in Neurospora is simple, only regulating output pathways at one phase of the circadian cycle. Using microarrays however, we have identified several genes that peak in mRNA accumulation in the early-evening. These data support the idea that the circadian clock of Neurospora is complex, analogous to that of higher eukaryotes. We are currently investigating the regulation of one evening-specific gene, *ccg-16*, in greater detail. Although *ccg-16* encodes a gene of unknown function, similar sequences can be found in the genomes of closely related fungi. Efforts are underway to produce a *ccg-16* knockout strain. *ccg-16* is repressed by light and development, consistent with an evening-specific expression profile. Interestingly, *ccg-16* mRNA accumulates with a ~24 hour rhythm in a *frq*-null strain. This strain lacks the FRQ-oscillator required for conidiation rhythms in constant conditions. These results suggested that a second FRQ-independent oscillator regulates some aspects of output from the circadian clock. It is not known, however, if this oscillator is involved in the time-keeping mechanism or is a slave oscillator that is driven by the FRQ-based oscillator. These possibilities are being examined.

43) Regulation of sulfur metabolism in *Neurospora crassa*. John V. Paietta, Department of Biochemistry and Molecular Biology, Wright State University, Dayton OH.

The sulfur regulatory system of *Neurospora crassa* consists of a group of sulfur-regulated structural genes which are coordinately controlled by the *cys-3* and sulfur controller (*scon*) genes. We are examining the entire set of structural genes involved in sulfur metabolism for control by the CYS3/SCON regulators. We have cloned and sequenced the *ars-1* (arylsulfatase), *cys-4* (sulfite reductase), *cys-16* (cystathionine gamma-lyase), *met-1* (methylene tetrahydrofolate reductase), *met-2* (cystathionine beta-lyase), *met-5* (homoserine o-acetyltransferase), and *met-8* (methionine synthase) genes for past and on-going studies. In addition, our analysis of the genomic sequence data has identified most of the remaining genes involved in sulfur metabolism (e.g., sulfur transport, generation of sulfide and cysteine, homocysteine and methionine metabolism, and glutathione metabolism). A number of these genes are under control of the CYS3/SCON regulatory system. The overall pattern of regulation in the sulfur metabolic network as determined from expression studies of available genes will be presented.

44) The transcriptional modulators *nuc*-1 and *pac*C-1 from *N. crassa* are required for the expression of a heat shock-inducible gene of the *hsp*70 family. Carlos J. Ono, Sergio R. Nozawa, Monica S. Ferreira-Nozawa, Nilce M. Martinez-Rossi and Antonio Rossi. University de Sao Paulo, Ribeirão Preto, Brazil

The *nuc*-1 and *pac*C-1 genes are wide-domain transcriptional factors involved in the regulation of the adaptive response of fungi to the levels ofphosphate (Pi) and to the pH of the medium, respectively. The *nuc*-1 gene activates

the transcription of various Pi-repressible structural genes, allowing molds to utilize nucleic acids as the sole Pi source. The *pac*C-1 gene activates the transcription of various alkaline genes, i.e., it allows the survival of molds at alkaline pH. Thus, the two genes are regulators apparently functioning independently from each other, but both are functionally active genes at alkaline pH. Furthermore, the promoter region of the *nuc*-1 gene has the consensus 5'-GCCAAG-3' (DNA binding domain of PACC-1), whereas the promoter region of a gene of the *hsp*70 family (XP_327938) has the consensus 5'-CACGTG-3' (DNA binding domain of NUC-1). Thus, it is possible that both the *nuc*-1 and *pac*C-1 genes are required for the expression of this heat shock-inducible member of the HSP70 family, suggesting a cascade effect i.e., at alkaline pH the *pac*C-1 gene would activate the expression of the *nuc*-1 gene, which in turn would activate the expression of this *hsp*70 gene. As detected by DDRT-PCR and confirmed by Northern blot analysis, strains of *N. crassa* carrying the *nuc*-1 or *pac*C-1 gene silenced by RIP (repeat-induced point mutations) did not express this *hsp*70 gene, confirming the predictions stated above. Financial support: FAPESP, CNPq, CAPES, FAEPA.

45) Expression of *arg-13* and ARG13. Gloria E. Turner, Rey David, and Richard L. Weiss. Department of Chemistry, University of California, Los Angeles.

The *arg-13* gene encodes a mitochondrial carrier family (MFC) protein. Examples of MFC proteins include carriers for ADP/ATP, dicarboxylate, citrate, ornithine, glutamate and glutamine. These proteins participate in metabolic trafficking, exchanging metabolites for inorganic cations across the inner mitochondrial membrane, using dual transport mechanisms, uniport and exchange. Proteoliposome assays with purified recombinant ARG13 reveal that ornithine is the substrate for this protein. ARG13 antibodies were used to determine the level of expression and subcellular localization. Results indicate that ARG13 is expressed at low levels and is localized in the mitochondrial fractions. Protein extracts supplemented with ornithine had increased levels of the protein. RNA's isolated from conidia and germinating cultures were used to determine the expression profile of *arg-13*. This profile is consistent with biosynthetic genes, where expression is detected early in germination but not seen in conidia or late germination. Interestingly arginine supplementation did not alter this profile. A lower molecular weight transcript is observed at the same time but only when arginine is absent. We are investigating if this transcript encodes a tryptophan-rich sensory protein (TspO/MBR) homolog that overlaps with the 3' region of *arg-13* on the opposite strand. This protein is involved in the efflux of porphyrin intermediates.

46) A Genetic Selection for Circadian Clock Mutations in *Neurospora crassa.* Michael Vitalini, Louis Morgan and Deborah Bell-Pedersen. Department of Biology, Texas A&M University, College Station, TX 77843

To identify components of the circadian clock in *Neurospora crassa*, we have carried out a genetic selection to isolate mutations that alter the expression of *clock-controlled genes (ccgs)*. The selection is based on the differential expression of the ccgs in the absence of the clock gene *frequency* (*frq*); *ccg-1* expression is high and *ccg-2* expression is low in the frq^{10} (null) strain. The promoters of *ccg-1* and *ccg-2* were fused to the *mtr* gene to create plasmids pCCG1M and pCCG2M, respectively. The *mtr* gene encodes a neutral amino acid permease that allows for both positive and negative selection. Loss of MTR function can be selected for based on resistance to the amino acid analog p-flourophenylalanine (FPA). Gain of MTR function can be selected for based on growth of tryptophan auxotrophs on high arginine/low tryptophan (TA) media. The pCCG1M and pCCG2M plasmids were transformed into a *bd;frq*^{10};*mtr;trp-2* strain. The pCCG1M transformed strain, CCG1M, displayed the predicted Mtr+ phenotype: growth on TA media, but not on FPA. The pCCG2M transformed strain, CCG2M, displayed an Mtr- phenotype. Both strains were subjected to UV light mutagenesis and assayed for growth on selective media. Eighty mutant strains that grew on FPA medium were isolated from CCG1M and one hundred forty mutant strains that grew on TA medium were isolated from CCG2M. The circadian phenotypes of the mutant strains will be discussed.

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DOI: 10.4148/1941-4765.1146

47) Distinct roles for PP1 and PP2A in the *Neurospora* circadian clock. Yuhong Yang[1], Qun He[1], Ping Cheng[1], Philip Wrage[1], Oded Yarden[2], and Yi Liu [1]. [1]Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX75390, USA; [2]Department of Plant Pathology and Microbiology, Faculty of Agricultural Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 7610, Israel

Phosphorylation of the *Neurospora* circadian clock protein FREQUENCY by several kinases promotes its degradation and is important for the function of the circadian feedback loop. Here, we show that FRQ is less stable in a *ppp-1* (catalytic subunit of PP1) mutant, resulting in its advanced phase and short period. In contrast, FRQ stability is not altered in an *rgb-1* (regulatory subunit of PP2A) mutant, but levels of *frq* protein and mRNA are low, resulting in a low-amplitude and long-period oscillation of the clock. Furthermore, PP1 and PP2A expressed in *Neurospora* can dephosphorylate the endogenous FRQ in vitro, sugguesting that these two phosphotases may differentially regulate FRQ and, consequently, the behavior of the circadian clock.

48) Characterization of the gene responsible of the *ovc* and *cut* phenotypes of *Neurospora*. Loubna Youssar[1], Tom Schmidhauser[2] and Javier Avalos[1]. [1]Departamento de Genética, Universidad de Sevilla, Spain. [2]present address: California State University Channel Islands. U.S.A.

Light induction of carotenogenesis in *Neurospora* is mediated by the WC proteins. Few mutants have been described with an enhanced photocarotenogenesis. One of them is *ovc* (Harding et al, 1984, *Neurospora* newsl. 31:23), a strain sensitive to high osmotic conditions and allelic with the *cut* mutant (Banks et al. 1997. FGN 44:10), also osmosensitive but normal for carotenogenesis. A phenotypic characterization of both strains is presented. Light induction of mRNA levels of the regulatory gene *wc-1*, the carotenoid genes *al-1* and *al-2*, or the conidiation specific gene *con-10*, is not significantly changed in the *ovc* mutant when compared with the wild type.The gene responsible of the *ovc* and cut phenotypes was identified by complementation of osmosensitivity with a cosmid library. The gene, that we call *cut-1*, codes for an enzyme of the haloacid dehalogenase family, which groups different classes of phosphatases. The gene is not present in the *ovc* mutant, which has suffered a deletion, and is able to restore the wild type phenotype upon transformation. Transcription of *cut-1* is low in either light or dark-grown cultures, and is high under hyperosmotic conditions. A blast search with the *cut* gene against the *Neurospora* genome reveals two additional genes with sequence similarity. None of them is induced by high osmotic conditions. Further experiments on *cut-1* function and regulation are under way.

49) HDA-1 of *Neurospora crassa* targets Histone 3 Lysine 14 and is required for proper DNA methylation. Kristina M. Smith, Joseph R. Dobosy, Hisashi Tamaru, and Eric U. Selker. University of Oregon, Eugene.

Previous studies have shown that histone deacetylase (HDAC) inhibitors selectively inhibit DNA methylation in *Neurospora crassa*. To investigate this phenomenon, we identified a gene encoding a homolog of the *Schizosaccharomyces pombe* Clr3 HDAC in *N. crassa* and used RIP to disrupt its function. The *hda-1* mutant lost DNA methylation at some chromosomal loci but not others. Western blotting revealed that the deacetylase activity of *N. crassa* HDA-1, like Clr3, is specific for H3 K14. The increased H3 K14 acetylation in the *hda-1* mutant correlated with a significant loss of H3 K9 trimethylation. The relationship between H3 K9 and K14 modification and their effect on DNA methylation was explored with chromatin immunoprecipitation experiments.

This study was funded by American Cancer Society fellowship PF0404301GMC to K.M.S. and NIH grant GM35690 to E.U.S.

Genomics and Proteomics

50) Modeling and Analysis of the Biological Clock in *Neurospora crassa*. Cara M. Altimus, Dr. Jonathan Arnold, Dr. H. Bernt Schuttler. Department of Genetics, University of Georgia, Athens, GA 30602

A biological clock is a recurring set of reactions within a system that produces anoscillating pattern. Unlike a traditional "clock," a biological clock can run continuously so long as all reaction components are present. Reaction rates are the main variants. Genetic networks are used to understand the relationships between genes, RNA, and proteins. These models show which genes are active, how they become active, what their products do, and their relationships with other genes and their products in the circuit. Then an ensemble of genetic networks for the biological clock was identified, fitting available RNA and protein profiling data. The fitted ensemble was used to identify essential features of the genetic network needed to sustain oscillations. Two features that appear necessary for oscillations are: (1) cooperativity in the action of two clock components, the White Collar (WCC) protein and Frequency (FRQ), and (2) a closed feedback loop in clock components. Along side the ensemble experiments, local stability analysis was done to examine equilibrium properties of the genetic network. Oscillations will only occur if the system does not have a stable fix point. Analytical conditions for instability are derived, permitting oscillations. In short, the clock needs several interacting proteins, a negative feedback loop, some cooperativity and the absence of a stable fix point to which the system would otherwise equilibrate.

51) Evidence of extensive plasmid integration into fungal mitochondrial DNAs. Patrick Cahan and John C. Kennell. Department of Biology, Saint Louis University, St. Louis, MO

Mitochondrial (mt) plasmids are autonomously-replicating genetic elements that reside in many filamentous fungi. Although they lack specific integrase functions, certain mt plasmids have been shown to integrate into mitochondrial DNA (mtDNA). In addition, annotation of fungal and plant mtDNAs has revealed regions having varying degrees of sequence similarity to mt plasmids. To directly assess the degree to which plasmids have invaded fungal mitochondrial genomes, BLAST search parameters were modified to identify plasmid sequences within highly AT-rich mtDNAs. Plasmid sequences representing four well-characterized plasmid homology groups of *N. crassa* were used as queries with completely sequenced fungal mtDNAs as subjects. Outputs were compared to previously-reported integration events and to randomly generated sequences. To facilitate analysis, algorithms were developed to parse the output data by E value, score and sequence complexity. Additional criteria included the presence of shared repetitive elements. Our results reveal several regions within the *N. crassa* mt genome that have homology with mt plasmids, and analysis of other mtDNAs indicates that plasmid integration is widespread among filamentous fungi. Our studies also revealed an unequal distribution of *PstI* palindromes within intergenic regions of *N. crassa* mtDNA that could be indicative of a recent recombination event. Tools developed here will be useful in understanding the co-evolution of plasmids and their hosts.

52) A rapid and efficient knockout strategy for filamentous fungi. H.V. Colot, K.A. Borkovich*, C.W. Pitt, J.J. Loros and J.C. Dunlap. Genetics Department, Dartmouth Medical School, Hanover, NH; and *Department of Plant Pathology, University of California, Riverside, CA

Disruption of genes by targeted gene replacement is widely used for assaying gene function. In Neurospora, homologous recombination occurs at a low frequency; long regions of homology are required in the knockout constructs, making their creation cumbersome. Insertion of transforming DNA at ectopic locations leads to a significant background. We have developed a strategy that uses recombination-mediated plasmid construction in *S. cerevisiae* (Oldenburg etal. 1997, Nucleic Acids Res. 25:451), followed by the generation of split-markerfragments for transformation (Catlett et al. 2003, Fungal Genet. News. 50:9). This strategy allows rapid creation of the

http://newprairiepress.org/fgr/vol51/iss1/16

DOI: 10.4148/1941-4765.1146

knockout DNA: two 3 kb flank fragments and the selectable marker cassette are prepared by PCR with primers that confer 29 nt homologous overhangs. These three fragments, along with a gapped yeast shuttle vector, are cotransformed into yeast. Crude DNA is then prepared from the mixed yeast transformants and used as a template in PCR reactions to yield two overlapping split-marker fragments for transformation into Neurospora. Use of split-marker fragments decreases ectopic insertions several-fold compared to the use of a single full-length fragment. On average, 35% of the transformants obtained with this strategy have the proper gene replacement and, as determined by Southern analysis, 73% of those are free of ectopic insertions. The complete protocol, along with strains and plasmids, will be provided.

53) Comparative Genomics within the Genus *Neurospora.* Luz B. Gilbert, Takao Kasuga, Jeff Townsend, Louise Glass, and John W. Taylor. Dept. of Plant and Microbial Biology, U.C. Berkeley, Berkeley, Ca 94720 USA

Little is known about the process of microbial adaptation. In the future I will use microarray technology to help identify genes responsible for environmental adaptation in natural isolates of *Neurospora discreta*. *Neurospora* offers a unique opportunity to characterize the variability of global gene regulation within and between species. The genus *Neurospora* consists of eight closely related conidiating species (see Dettman et al. 2003, in press), indistinguishable by morphology, as well as several non-conidiating species. We have constructed a 70mer oligomer array for *Neurospora crassa* representing 3366 genes, approximately one third of the genome. To assess the effectiveness of our array for other members of the *Neurospora* genus I have analyzed comparative genomic hybridizations for all eight conidiating species of *Neurospora* as well as a few non-conidiating isolates. This technique uses genomic DNA as a substrate for labeling and hybridization to an oligo array, consequently avoiding the biases associated with transcription of mRNA. We can now determine gene coverage and estimate genome divergence among conidiating and non-conidiating *Neurospora* isolates by comparing the ratio of fluorescences between samples.

54) mRNA profiling of conidial germination and hyphal growth in *Neurospora crassa* using oligomer microarrays. Takao Kasuga, Betty Gilbert, Jeffery Townsend, John Taylor and Louise Glass. Plant and Microbial Biology Department, University of California, Berkeley, CA94720

We have constructed a *Neurospora crassa* 70 base oligomer microarray representing 3,366 out of ten thousand genes predicted by WICGR and MIPS. In order to validate the performance of the oligomer microarrays and to develop techniques for baseline profiling for *N. crassa*, we chose to assess the transcriptional profile of conidial germination (up to 24 hr of growth). A considerable body of literature is available on biochemical aspects and mRNA profile of conidial germination in filamentous fungi and in *N. crassa* in particular. Total RNA was obtained from a time course during conidial germination from a series of liquid cultures. Hierarchical clustering was used to group genes with respect to their transcriptional profiles. Three distinct clusters were identified; the Max Time 0 cluster showed maximum expression at time 0 (dormant conidia), the Min Time 0 cluster showed minimum gene expression at time 0 and reached a peak between 2 to 8 hrs and Max Time 24 cluster, which showed maximum gene expression at 24 hrs. Gene functions categorized by MIPS (Mannhaupt et al., 2003) were used to assess the global picture of gene expression during germination. In dormant conidia (Max Time 0), the percentage of genes categorized as ENERGY and METABOLISM were lower than that of the other clusters. In the Min Time 0 cluster, the proportion of genes for PROTEIN SYNTHESIS and TRANSCRIPTION were highest, reflecting active growth between 2-8 hr. In the Max Time 24 cluster, the ratio ofgenes for CELL CYCLE AND DNA PROCESSING was relatively higher than the other clusters. Although our observations are preliminary, they agree well with published studies of conidial germination in *N. crassa*.

55) Development of positional cloning tools for *Neurospora crassa* to characterize a suppressor of a temperature-sensitive mutation in the large subunit of ribonucleotide reductase. Moshi Kotierk and Myron L. Smith. Biology Department, Carleton University, Ottawa, Ontario K1S 5B6

un-24 encodes a temperature sensitive form of the large subunit of ribonucleotide reductase, an evolutionarily conserved enzyme that is essential for the reduction of RNA precursors into DNA precursors. We have identified a spontaneous mutation, *su(un-24)-1*, that suppresses the *un-24* temperature-sensitive phenotype. This suppressor may act by increasing intracellular osmotic pressure, since *un-24* is osmotically remediated, or by directly stabilizing the UN-24 protein. Unlike in *Schizosaccharomyces pombe*, the suppressor is not due to a mutation in the small subunit of ribonucleotide reductase. To identify *su(un-24)-1*, we are using a map-based approach that utilizes the complete genome sequence of *N. crassa*. We developed 16 PCR-based markers, located on each of the seven nuclear chromosomes and on the mitochondrial DNA, that are polymorphic between OakRidge and Mauriceville background *N. crassa* strains. We analyzed these markers in 34 progeny of a cross between our temperature-sensitive, suppressed OR-background strain and a wildtype Mauriceville strain. From this analysis, we have mapped *su(un-24)-1* within a region of Linkage Group II near *nmt-1*. This map-based approach can be used in positional cloning of any mutation generated in the OR background of *N. crassa*.

56) Gene amplification in some glycosyl hydrolase families. Alan Radford. University of Leeds.

Neurospora crassa hydrolyses cellulose and other polysaccharides as its major carbon source. High levels of such enzymes are achieved via gene amplification of several glycosyl hydrolase families, including GH family 3 (beta-glucosidases), families 5, 6, 7 and 61 (cellulases), family 10 (xylanases), and also the starch-degrading family 13 (alpha-amylases) and 15 (glucoamylases).Comparison of *Neurospora* sequences in the above families from the WICGR database with other ascomycete genomes at WICGR (*M. grisea, A. nidulans, F. graminareum*), identified by BLASTP and using Clustal X alignment and bootstrap tree calculation, shows amplification pre-dating the split into plectomycetes, discomycetes and pyrenomycetes, and hence prior to the evolution of RIP.Using the predicted *Neurospora* protein sequences in TBLASTN searches of the *C. cinereus* DNA database at WICGR shows that different members of the same family in *Neurospora* may find different "besthits" in *Coprinus*, suggesting that certain amplifications pre-date the Ascomycete-Basidiomycete split. Comparable searches of *U. maydis* and *C. neoformans* find no cellulases in the former and neither cellulases nor hemicellulases in the latter, consistent with the lifestyles of the two parasitic species. In extant species in which RIP occurs, gene duplication would probably delete a copy of the gene of that species from a clade with orthologs from other species. Examples of this are observed.

57) Developing the Neurospora Gene List. Alan Radford. University of Leeds

The Neurospora Compendium (Perkins, Radford and Sachs,2001) contains data up to 2000. It provides a snapshot of our knowledge four years ago. Since its publication, many new genes have been published, and the entire genome sequence has become available at WICGR (Galagan et al, 2003).The Neurospora gene list at http://www.bioinf.leeds.ac.uk/~gen6ar/Neurospora/gene_list.htm has been an attempt to keep the classical gene data up to date and cross-referenced to the genome database. For genes of known sequence, their NCU number and physical mapping data have been incorporated into gene entries, new genes have been added, and the mitochondrial genome has been compiled in standard format as a section of the gene list. With the MMBR magnum opus (Borkovich et al, 2004) in press, many new genes will require inclusion.The gene list is currently in XML format, with a front end using ActiveX and therefore requiring Internet Explorer, although this limitation could be circumvented, and a more flexible query front end provided..At this stage I seek the input of the Neurospora community on the current usefulness and future development of the gene list.

http://newprairiepress.org/fgr/vol51/iss1/16

DOI: 10.4148/1941-4765.1146

58) An Integrated Analysis of a Chemical Reaction Network for the Metabolism of Quinic Acid in Neurospora crassa. Cale D Whitworth and Dr. Jonathan Arnold. Department of Genetics, University of Georgia, Athens, Georgia 30602

A chemical reaction network for the metabolism of Quinic Acid in *Neurospora crassa* has been proposed. In this reaction network two regulatory genes and five structural genes are responsible for the metabolism of Quinic Acid. The protein product of *qa*-1*F* transcriptionally controls the expression of all seven *qa* genes, including those encoding enzymes which utilize Quinic Acid as a carbon source, and the protein product, QA-1S, represses the activator protein, QA-1F. An ensemble of possible chemical reaction networks is developed with rate constants consistent with RNA and protein profiling data. An alternative network, in which several molecules of QA-1F (i.e. Hill coefficient is greater than one) cooperatively activate qa genes, is also developed.

59) Cloning of telomeric regions from *Neurospora crassa* wild-type strains Oak Ridge and Mauriceville. Cheng Wu[1], Mark L. Farman[2] and Matthew S. Sachs[1]. [1]Oregon Health & Science University, Beaverton, OR 97006 [2]University of Kentucky, Lexington, KY 40546

Eukaryotic chromosomes are linear molecules that terminate in simple sequence repeats called telomeres. New telomere repeats, typically 5' TTAGGG 3', are added to the existing chromosome ends to guard against the loss of DNA during replication. The G-rich strand can form hairpin structures, which are believed to prevent the degradation of chromosome ends and the fusion of different chromosomes. In fungi and other eukaryotic microbes, regions near telomeres are highly variable and are rich in genes for ecological adaptation. Telomeres are poorly represented in the draft genome sequence database for *Neurospora crassa*. By characterizing clones for *N. crassa* telomeres and subtelomeric regions, we will be able to assess the functional and evolutionary significance of these regions and complete the genome sequences. Here we describe our approach to clone *N. crassa* telomeric regions. Isolated chromosomal DNA fragments with polished ends were ligated to linearized pMLF4 cosmid vector. The ligated DNA was then packaged with Lambda packaging extract and used to infect *Escherichia coli* XL-10 cells. Recombinant colonies containing telomeres were then identified using a ^{32}P-labeled telomere probe. Our goal is to isolate and characterize the 14 telomeric regions of two different wild-type *N. crassa* strains, Oak Ridge and Mauriceville. Comparison of subtelomeric regions between strains will improve understanding of the pathways of genome evolution.

Industrial Biology and Biotechnology

60) Diversification of barley beta-D-glucan endohydrolases in *Neurospora crassa.* Graham Eariss[1], Maria Hrmova[2], Geoffrey Fincher [2] & David E. A. Catcheside[1]. [1]School of Biological Sciences, Flinders University, Adelaide, South Australia.[2] Department of Plant Science, University of Adelaide, South Australia.

Similarities in the primary sequences and three dimensional structures of barley (1-3)-beta-D-glucan endohydrolases and (1-3,1-4)-beta-D-glucan endohydrolases suggest they are closely related in evolutionary terms, yet they perform completely different functions (Stewart *et al.,* 2001). While (1-3)-beta-D-glucanases capable of hydrolyzing the linear, substituted and branched (1-3)-beta-D-glucans often found in fungal cell walls appear to be involved in plant protection, the (1-3,1-4)-beta-D-glucanases are responsible for digestion of the starchy endosperm cell wall during germination of barley grains (Hrmova and Fincher, 2001). We are using an *in vivo* gene diversification technique developed in *Neurospora crassa* (Catcheside *et al.,* 2003) to investigate the molecular basis of their divergence and

to generate a more thermostable (1-3,1-4)-beta-D-glucanase intended for industrial use. Gene diversification in Neurospora takes advantage of the meiotic recombination hotspot *cog*^{L},shuffling exogenous genes inserted between *cog*^{L} and the nearby *his-3* locus during meiosis. Functional genes encoding the barley beta-D-glucanases will be inserted between *cog*^{L} and *his-3* by transplacement while non-functional duplicates will be incorporated at random. Variant alleles generated by pre-meiotic Repeat-Induced Point mutation (RIP) will be shuffled by recombination, increasing variation and providing a potential rescue for deleterious mutations. Progeny will be screened using a colorimetric plate assay to identify novel variants.

Catcheside, D. E. A., Rasmussen, J. P., Yeadon, P. J., Bowring, F. J., Cambereri, E. B., Kato, E., Gabe, J. & Stuart, W. D. 2003, 'Diversification of exogenous genes *in vivo* in *Neurospora*', *Appl. Microbiol. Biotechnol.,* 62: 544-549.

Hrmova, M. and Fincher, G.B. 2001, 'Structure-function relationships of beta-D-glucan endo- and exohydrolases from higher plants', *Plant Mol. Biol.,* 47: 73-91.

Stewart, R.J., Varghese, J.N., Garret, T.P.J., Hoj, P.B. & Fincher, G.B. 2001 'Mutant barley (1-3,1-4)-beta-glucan endohydrolases with enhanced thermostability', *Protein Eng.,* 14: 245-253.

61) Restoration of *Saccharomyces cerevisiae* coq mutants by *Neurospora crassa* genes. Eun Jung Kim, Sang Rae Kim, Na Young Eo and Bheong-Uk Lee*Kosin University, Division of Biological SciencesBusan 606-701, South Korea

Coenzyme Q (CoQ, ubiquinone) is a quinone derivative with a long isoprenoid side chain. CoQ is a lipid component that transports electrons in the respiratory chains located in the inner mitochondrial membrane of eukaryotes and the plasma membrane of prokaryotes, and also functions as an antioxidant. CoQ is essential in aerobic growth of *Saccharomyces cerevisiae*. coq mutants, deficient ubiquinone biosynthesis fail to grow on nonfermentable carbon sources. Two putative genes involved in ubiquinone biosynthesis of *Neurospora crassa* were cloned and used for complementation test of *S. cerevisiae* coq4 and *S. cerevisiae* coq7 strains, respectively. The predicted amino acid sequences of both *N. crassa* COQ4 and COQ7 showed about 52% and 58% homology with those of *S. cerevisiae,* respectively. When putative *coq-4* and *coq-7* genes of *N. crassa* were transformed to yeast strains, the growth rates were restored to the wild-type level and were able to synthesize coenzyme Q6. They also showed less sensitivities to polyunsaturated fatty acids such as linoleic acid or linolenic acid.

62) *Agrobacterium tumefaciens*-mediated genetic transformation of *Neurospora crassa.* Richard S. Feinberg and Matthew S. Sachs. Oregon Health & Science University, Beaverton, OR 97006

Agrobacterium tumefaciens-mediated transformation has been successfully applied to *Neurospora crassa*. The transformants were selected on the basis of their resistance to hygromycin B. Plasmids were tested in which the *hph* gene was driven by either the *N. crassa cpc-1* or *arg-2* promoters, or the *Aspergillus nidulans trpC* promoter. A variety of different conditions for transformation were analyzed. The number of *N. crassa* conidia and *A. tumefaciens* cells which were co-cultivated were varied, as was the length of the co-cultivation period prior to transfer to selection medium. Hygromycin-resistant transformants were stable through repeated rounds of conidial passaging and following homokaryosis by a microconidiation procedure.

63) Diversification of Human Growth Factors in *Neurospora crassa*. Steven Henderson[1], Briony Forbes[2], Leah Cosgrove[2] & David E. A. Catcheside[1]. [1]School of Biological Sciences, Flinders University, Adelaide, South Australia 5042, Australia.[2]Health Sciences and Nutrition, Commonwealth Scientific and Industrial Research Organisation, Adelaide, South Australia 5000, Australia.

https://newprairiepress.org/fgr/vol51/iss1/16
DOI: 10.4148/1941-4765.1146
http://newprairiepress.org/fgr/vol51/iss1/16

DOI: 10.4148/1941-4765.1146

We are utilising an *in vivo* gene diversification system developed in *Neurospora crassa* (Catcheside *et al.*, 2003) to generate novel human growth factor (hGF) variants. Diversification of heterologous genes in Neurospora exploits the high rate of meiotic recombination initiated at the recombination hotspot *cog*^{L} to shuffle exogenous sequences juxtaposed to *cog*^{L}. Transplacement vectors will be used to insert a functional hGF gene between *his-3* and *cog*^{L}. A non-functional hGF duplicate gene will be used to induce low frequencies of Repeat-Induced Point mutation (RIP) to generate hGF alleles *in vivo*. Subsequent meiotic recombination shuffles the growth factor alleles creating additional sequence variation and can also result in separation of deleterious mutations. Progeny from the cross will be screened to identify novel hGF variants.

Catcheside, D. E. A., Rasmussen, J. P., Yeadon, P. J., Bowring, F. J., Cambareri, E. B., Kato, E., Gabe, J. & Stuart, W. D. 2003, ‘Diversification of exogenous genes in vivo in Neurospora’, Appl. Microbiol. Biotechnol., vol. 62, pp 544-549.

64) Functional expression of *Neurospora crassa* L-carnitine biosynthetic genes in *Escherichia coli*. Sangrae Kim[1], Sunduk Hwang[2,3], Bum-Chang Kim[2], Hyoung-Sik Kim[2], Eun Jung Kim[1,3], Whankoo Kang[2,3,] and Bheong-Uk Lee[1,3]. [1]Division of Biological Sciences, Kosin University, Busan 606-701, Korea, [2]Dept. Biochemical Engineering, Hannam University, Daejeon 306-791, Korea [3]Gene To Protein Inc. Daejeon306-800, Korea

Five genes involved in biosynthesis of L-carnitine in *Neurospora crassa* were cloned and functionally expressed in *Escherichia coli.* These genes encode enzymes that sequentially converts from L-lysine to L-carnitine. The first enzyme produce trimethyllysine from L-lysine. The second enzyme is epsilon-N-trimethyllysine hydroxylase (TMLH), which converts epsilone-N-trimethyllysine to beta-hydroxy-epsilon-N-trimethyllysine. The third one is beta-hydroxy-epsilon-N-trimethyllysine aldolase (SHMT), which converts beta-hydroxy-epsilon-N-trimethyllysine to gamma-N-trimethylaminobutyraldehyde. The fourth is gamma-N-trimethylaminobutyraldehyde dehydrogenase (TMABADH). This converts gamma-N-trimethylaminobutyraldehyde to gamma-butyrobetaine that was finally coverted to L-carnitne by gamma-butyrobetaine hydroxylase. About 1.7 grams of L-carnitine per liter can be produced by the partially-optimized fed-batch fermentation.

Population and Evolutionary Biology

65) Phylogenetic relationships within the diverse *Neurospora discreta* species complex. Jeremy Dettman, David Jacobson, and John Taylor. Dept. of Plant and Microbial Biology, University of California, Berkeley.

Neurospora discreta is the most widely distributed species of *Neurospora* known, having been collected from Alaska, Europe, New Zealand and intervening tropical and subtropical regions. Our recent phylogenetic analyses showed that outbreeding (conidiating) *Neurospora* diverged into two sister clades (Dettman *et al*. Evolution 57:2703-2720). The first clade contains only *N. discreta*, which appears to be a complex of species nearly as diverse as the second clade, which contains the other seven outbreeding species. Cryptic species within *N. discreta* are being recognized using analyses of sequence data from three unlinked, anonymous, nuclear loci, each containing a microsatellite and ~450 bp of flanking sequence. Our sample totals 75 isolates from the worldwide range of *N. discreta*, including new collections from Europe and North America. Criteria developed to recognize phylogenetic species in *Neurospora* will be applied to the *N. discreta* clade: species are recognized using genealogical concordance of loci with the added the criteria that species level clades are monophyletic and well-supported in at least one single-locus genealogy, and not contradicted by any other. The results will set the foundation for future comparative biology and genetics within the *N. discreta* clade, and between *N. discreta* and other species of *Neurospora*.

66) The mysterious origin of the Oak Ridge wild types. Olivera Gavric and Anthony Griffiths. Botany Department, UBC, Vancouver, Canada V6T 1Z4.

In the course of routine sequencing of the phospholipase C (PLC) gene, we have discovered a striking sequence dimorphism among Neurospora laboratory wild types. The two morphs differ by 29 base pair substitutions (of which two are in the intron) plus one single codon addition/deletion. Eleven seemingly important missense mutations result. The significance of this genetic divergence is not known, but the results reveal two clear lines of ancestry that might have been subjected to different selection pressures. We have tracked the PLC dimorphism in several key strains alleged to be ancestors of the Oak Ridge wild types. Both Oak Ridge strains have the same allele, which can be arbitrarily designated morph 1. However, both Emerson 5297a and Emerson 5256A, which are the supposed direct predecessors of Oak Ridge (Newmeyer et al., FGN 34), have morph 2. Taken on face value, this would mean that Oak Ridge cannot be descended from the Emerson strains listed. Three Lindegren strains that we have analyzed also have the Emerson morph 2. However, preliminary data show that the Abbott strains 4A (definitely) and 12a (probably) have the Oak Ridge morph 1, hence the PLC allele of Oak Ridge seems to be of Abbott ancestry. The results fit the general pedigree well except for the Oak Ridge branch. The authenticity of several of the specific isolates has been questioned (summarized by Newmeyer et al.) and this might partially explain the paradox.

67) Studying the evolutionary genetics of reproductive isolation between *Neurospora* lineages. Elizabeth Turner and John W. Taylor, Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720

Recent work characterizing phylogenetic relationships and reproductive isolation among several outcrossing *Neurospora* species has laid the groundwork for studies that go beyond the description of evolutionary patterns to explore the biological mechanisms underlying interspecies sexual isolation and intraspecies cohesion. We are currently studying the genetic basis of reproductive isolation between two pairs of taxa: (1) *N. crassa* and *N. intermedia* and (2) the more recently diverged, partially isolated, subspecific clades *N. crassa* A and *N. crassa* C. Using AFLP markers, we have created linkage maps that will be the bases of quantitative trait locus (QTL) analyses of reproductive isolation and compatibility in matings with species- and clade-specific tester strains. Here we present the linkage maps and compare the linkage, intermarker map distances, and map orders of *N. crassa* markers common to both maps. In order to assign linkage groups recognized in our analysis to the known chromosomes of *Neurospora,* we took advantage of commercially available RFLP mapping kits. We found that many of our AFLP markers segregated in the RFLP mapping strains, and we discuss linkage group/chromosome assignments and marker ordering in light of these segregation data. Finally, we present the results of preliminary mating experiments on a small number of genotyped *N. crassa/N. intermedia* f_1 hybrids. We identify several markers that were significantly associated with the fertility of f_1 hybrids in crosses to *N. crassa* or *N. intermedia* tester strains.

68) Influence of Parental Genotype and Mating Type on Quantitative Traits in Field Isolates of *Neurospora crassa*. Heather H. Wilkinson, Thomas J. DeWitt and Daniel J. Ebbole. Texas A&M University, College Station, TX.

Despite the relatively well elucidated genetic basis for development in *Neurospora crassa*, little is known about morphological and life history variation in natural populations. To begin to address the heritability and ecological implications of this sort of variation, we bred genotypically distinct isolates from nature and evaluated phenotypic variation. Specifically, 8 previously characterized isolates from a Louisiana sugar cane field (four of each mating type) were crossed in all possible combinations such that each isolate served as both the maternal and paternal parent (in total, 32 combinations X 4 replicates). Perithecium size and number, and ascospore number, size and

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DOI: 10.4148/1941-4765.1146
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DOI: 10.4148/1941-4765.1146

shape were all measured for each replicate. There was substantial variability in all phenotypic characters. Maternal genotype significantly influenced all these characters, and accounted for the majority of variation associated with perithecium size and number. Ascospore size, shape and number were all also influenced by paternal genotype and the maternal-paternal genotype interaction. Finally, mating type of the maternal parent significantly influenced spore size, shape and perithecium number. Path analysis is being used to investigate phenotypic integration among traits and functional aspects of variation in these traits are being explored (e.g. germination rates of ascospores based on size). We conclude that Neurospora is an excellent candidate for use in manipulative evolutionary studies.

Other

69) Mutation of *spo-11* and its effect upon meiotic recombination in *Neurospora crassa*. F.J. Bowring, P.J. Yeadon, R.G. Stainer and D.E.A. Catcheside. School of Biological Sciences, The Flinders University of South Australia

SPO11 is thought to initiate meiotic recombination by generating chromosomal double-strand breaks. While first identified in *Saccharomyces cerevisiae*, *SPO11* homologues have been found in numerous species including humans, and we have generated three Neurospora *spo-11* RIP mutants.

The predicted polypeptide of the best characterised of these (*spo-11* ^{RIP1}) is truncated near the midpoint deleting three of five conserved motifs. While we predicted a reduction in the amount of meiotic recombination in *spo-11*$^{RIP1/RIP1}$ diploids, we observed elevated levels of allelic and non-allelic recombination in *rec-2* regulated regions. Neurospora *rec* genes modulate the recombination frequency in one or more distinct chromosomal regions. We are currently characterising the other two *spo-11* RIP alleles.

70) Characterization of *ku-70* and *ku-80* knockout mutants in *Neurospora crassa.* Ninomiya, Y., Suzuki, K., Ishii, C. and Inoue, H. Saitama University, Japan

We are studying DNA repair mechanism in *N. crassa*. In this report, we will present disruptants of *N. crassa*-homolog genes of Human *KU70* and *KU80* genes to investigate roles in recombination repair. These mutants showed slightly more sensitive to UV than the wild type and 3 times more sensitive to MMS than the wild type. Mutant genes were mapped by crossing with strains carrying proper makers. Double repair-deficient mutants were isolated to test epistatic relationship. For this purpose, other mutants belonging excision-repair or postreplication repair or recombination repair group were used. Also, homologous integration was measured in these strains. These results will be presented.

71) Isolation and characterization of the methyl/RIP DNA binding protein MRBP-1 and Potential Base Unpairing of DNA mutated by RIP. Gregory O. Kothe[1], Michael R. Rountree[2], Ashley McCormack[3], Larry David[3], Yuji Nakayama[4], Terumi Kohwi-Shigematsu[4], and Eric U. Selker[1]. [1]Institute of Molecular Biology, University of Oregon, Eugene, OR, [2]St. Jude Children's Research Hospital, Memphis, TN, [3]Department of Oral Molecular Biology, School of Dentistry, Oregon Health and Science University, Portland, OR, [4]Lawrence Berkeley National Laboratory, University of California, Berkeley, California

We have purified a putative protein complex that binds RIPed and methylated DNA, and refer to this activity as MRBP-1 (Methyl/RIP Binding Protein 1). The putative complex contains a protein belonging to the telobox class of DNA binding proteins; containing a single myb domain in its carboxyl terminus. We have also identified two proteins that show high affinity for single-stranded, but not double-stranded, DNA. We propose that certain sequences that have undergone RIP may have single-stranded character, similar to matrix attachment regions. We also show that a RIPed sequence is bound preferentially in a gel-shift assay by the vertebrate BUR (Base Unpairing Region) binding protein

72) Choline Depletion does not Affect the Central Circadian Oscillator in *Neurospora crassa.* Mi Shi, Jennifer Loros, Jay Dunlap. Department of Genetics, Dartmouth Medical School, Hanover, NH 03755

Neurospora *chol-1* mutants display an elongated and unstable period rhythm of spore formation on solid medium without choline supplementation. Since the rhythm shows few characteristics of a typical circadian rhythm, it is not clear whether the long period rhythm is an output reflecting an elongated central circadian oscillator or is instead a morphological rhythm reflecting defects in lipid metabolism. Such a rhythm could mask expression of a circadian clock. To evaluate this, a liquid to solid transfer assay was adapted to measure the rhythm period under choline depletion conditions in liquid culture. Conidia from *chol-1* mutants were cultured in liquid media without choline supplementation in darkness and the conidial suspension was transferred to race tubes with choline supplementation at different time points. Since the circadian clock should cycle through all phases of the day in liquid as well as on solid medium, and since the transfer from liquid to solid medium does not reset the phase of the clock, one can measure the period length of the rhythm under choline starvation. We found that the phases of the rhythm on solid medium were similar for all time points, suggesting that the free-running period of the circadian clock under choline starvation is the same as that seen with supplementation. Consistent with this, levels of the clock component FRQ oscillate with a ~22-hour period in a two-daytime course in darkness following two days of choline starvation.

73) Recombination hotspot alleles are co-dominant. P. J. Yeadon, F. J. Bowring and D. E. A. Catcheside[1].
[1]School of Biological Sciences, Flinders University, PO Box 2100, Adelaide, South Australia, Australia

Naturally occurring alleles of the recombination hotspot *cog* in *Neurospora crassa* have two phenotypes with respect to recombination in the *his-3* region. Presence of the cog^+ allele in a cross significantly increases both allelic and local intergenic recombination compared to a cross homozygous for the *cog* allele. Data obtained in the 1960s suggested that cog^+ is fully dominant to *cog*, which was always difficult to explain. We show that *cog* alleles are co-dominant in effect on both allelic recombination in *his-3* and crossing over between loci flanking *his-3*. In addition, we present evidence of a two-fold range in allelic recombination frequency due to genetic background variation, the most likely explanation for the previous conclusion that cog^+ is dominant to *cog*.

EXTRA ABSTRACTS

Biosensors in Neurospora. Emma Perfect and Pat Hickey, Lux Biotechnology Ltd.

Biosensors utilise biological materials to detect and monitor the presence of various molecules in a sample. The majority of biosensors are based on bacteria, mammalian cells or yeast. In 2001 LUX Biotechnology Ltd. was established to develop filamentous fungal biosensors. Filamentous fungi are more versatile, grow in more diverse conditions and are easier to store and transport than bacteria or yeast. These features make Lux biosensors more flexible and thus more powerful than existing alternatives.
Lux biosensors harness the power of bioluminescence and fluorescence by generating fungal

http://newprairiepress.org/fgr/vol51/iss1/16
DOI: 10.4148/1941-4765.1146

strains whose light output gives an indication of the presence of specific molecules in the environment or of fungal health. It is predicted that Lux biosensors will make important contributions to environmental testing, drug discovery and R+D. The decision to utilise Neurospora crassa for Lux's prototype biosensor was influenced by the quality research available, the recently published genome sequence and the N. crassa community's supportive reputation.
Contact: pat@luxbiotech.com or visit our web site at www.luxbiotech.com

Recent Activities at the FGSC. Kevin McCluskey. Fungal Genetics Stock Center, University of Kansas Medical Center, Kansas City, KS

In our continuing effort to serve the fungal genetics community, the FGSC has expanded to include new organisms, categories, and molecular materials. We have maintained our distribution efforts and encourage clients to use the resources at the FGSC. Recent additions include a collection of Schizophyllum commune strains, Magnaporthe grisea tagged knockout strains, Neurospora wild-type collections, library pools and clones from the sequencing programs

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Nick Read

David Jacobson
David Perkins
John Taylor
And many field
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Types of mutants examined