Fungal Genetics Reports

Volume 47 Article 32

Neurospora 2000

Neurospora Conference

Recommended Citation
Neurospora Conference. (2000) "Neurospora 2000," *Fungal Genetics Reports*: Vol. 47, Article 32.
https://doi.org/10.4148/1941-4765.1228

Neurospora 2000

Abstract

Abstracts from the Neurospora 2000 Conference, March 9 - 12

Information	
The Neurospora 2000 Conference was held at the Asilomar Conference Grounds, Pacific Grove, California (near Monterey, California) from the afternoon of March 9 to noon, March 12, 2000 (Thursday-Sunday). The meeting consisted of five plenary sessions and two poster sessions.	**Schedule** **Invited talk abstracts** **Poster abstracts** **SCIENTIFIC PROGRAM CHAIRS** **Dan Ebbole** **Eric Selker**

Neurospora 2000

Schedule of Activities

Thursday, March 9

3:00-6:00 P.M.
Registration: Administration

6:00-7:00 P.M.
Dinner: Crocker

7:30-10:30 P.M.
Mixer: Fred Farr Forum (formerly Firelight Forum)

Friday, March 10

7:30-8:30 A.M. **Breakfast: Crocker**

Session 1: CELL BIOLOGY AND MORPHOGENESIS
8:30-12:00 Noon **Chapel**

8:40 Welcome/Introduction (session leader: Michael Plamann)

8:45 David Perkins: **Celebrating 75 years of Neurospora genetics**

9:00 Nick Read: **Imaging the vesicle trafficking network in living Neurospora hyphae**

9:25 Louise Glass: **Programmed hyphal compartmentation and death in *Neurospora crassa***

9:50 Frank Nargang: **Structure and function of the Neurospora TOM complex**

10:05-10:25 Break

10:30 Michael Plamann: **Analysis of dynactin and its role in regulating cytoplasmic dynein activity**

10:55 Alan Lambowitz: **Mitochondrial introns and mitochondrial retroplasmids of Neurospora**

11:20 Oded Yarden: **Expression and localization of two COT1 kinase isoforms in *N. crassa***

11:35 Tony Griffiths: **Interaction of colonial mutations of *Neurospora crassa***

11:50 P.M. End of Session

12:00-1:00 P.M. **Lunch: Crocker**

1:00-2:30 P.M. **Free Time**

Session 2: BIOCHEMISTRY AND METABOLISM
2:30-6:00 P.M. **Chapel**

2:30 Introduction (session leader: Matthew Sachs)

2:35 Barry Bowman: **The vacuolar ATPase is a surprisingly complex enxyme with multiple roles to play in growth and differentiation in fungi**

3:00 John Paietta: **Regulation of sulfur metabolism in *Neurospora crassa***

3:15 Bo Feng: **A new GATA factor, ASD, regulates sexual development in*Neurospora crassa***

3:30 Richard Weiss: **Regulation of arginine metabolism**

3:45-4:05 Break

4:10 Matthew Sachs: **Translational control of *Neurospora crassa arg-2* by arginine**

4:35 Manju Kapoor: **Properties and interactions of HSP70 and Hsp80: Heat- inducible, cytosolic molecular chaperones of Neurospora**

5:00 Gloria E. Turner: **Metabolite regulation of multiple arginases in conidia and early germination of *Neurospora crassa***"

5:15 Piotr Bobrowicz: **Characterization of *Neurospora crassa mak-2* gene encoding mitogen-activated protein kinase similar to yeast Fus3 and Kss1**"

5:30 Javier Avalos: **Search for carotenogenesis mutants, new phenotypes and cloning of the *ovc* and *cut* loci**

5:45 Robert Metzenberg: ***hpp*, a third phosphate permease gene**

5:50 P.M. End of Session

6:00-7:00 P.M. **Dinner: Crocker**

7:00-10:00 P.M. **Poster Session: Fred Farr Forum**

Saturday, March 11

7:30-8:30 A.M. **Breakfast: Crocker**

Session 3: GENETICS AND GENE REGULATION
8:30-12:00 Noon **Chapel**

8:30 Introduction (session leader: Eric Selker)

8:35 Carlo Cogoni: **Neurospora as model system to study homology dependent gene silencing**

9:00 Eric Selker: **Neurospora as a model system to investigate DNA methylation**

9:25 Jak Kinsey: **Tad transcription and transposition**

9:40 Alberta Rosa: **S-adenosylmethionine, DNA methylation and DNA mutation in *N. crassa***

9:55 Hirokazu Inoue: **DNA damage, repair and aging in *Neurospora crassa***

10:10 Break

10:35 David Catcheside: **Events at recombination hotspots in Neurospora and their control**

11:00 Robert Metzenberg: **Suppression of certain ascus-dominant mutations**

11:25 Rodolfo Aramayo: **The Transvection Control Region (TCR) of *Neurospora crassa***

11:40 Edward G. Barry: **Detached nuclear genes of Neurospora transmitted cytoplasmically through infectious contact**

11:55 A.M. End of Session

12:00-1:00 P.M. **Lunch: Crocker**

1:00-2:30 P.M. **Free Time**

Session 4: SIGNALING AND DEVELOPMENT
2:30-6:00 P.M. **Chapel**

2:30 Introduction (session leader: Dan Ebbole)

2:35 Jennifer Loros: **Feedback loops in the Neurospora circadian system**

2:55 Deanna L. Denault: **Biochemical interactions between FRQ and WC-2: critical clock proteins required for the normal operation of the Neurospora circadian oscillator**

3:10 Christian Heintzen: ***vvd* encodes a novel PAS protein involved in light perception for the Neurospora circadian clock**

3:25 Deborah Bell-Pedersen: **Control of conidial development by the circadian clock in *Neurospora***

3:50 Break

4:15 Dan Ebbole: **Regulation of macroconidiation by fluffy**

4:40 Kathy Borkovich: **Signal transduction through heterotrimeric G proteins and opsins in *Neurospora crassa***

5:05 Chuck Staben: **Interaction between and transactivation by mating type polypeptides of *Neurospora crassa***

5:20 Jin-Rong Xu: **The hyper-osmotic stress response pathway of *Neurospora crassa* is the target of phenylpyrrole fungicides**

5:35 Hartmut Linden: **Localization and light-dependent phosphorylation of White Collar 1 and 2, the two central components of blue light signaling in *Neurospora crassa***

5:50 End of Session

6:00-7:00 P.M. **Dinner: Crocker**

7:00-9:00 P.M. **Poster Session: Fred Farr Forum**

9:00-12:00 P.M. **Party: Fred Farr Forum**

Sunday, March 12

7:30-8:30 A.M. **Breakfast: Crocker**

Session 5: GENOMICS/EVOLUTION/TECHNOLOGY
8:30-12:00 Noon **Chapel**

8:30 Introduction (session leader: Mary Anne Nelson)

8:35 John Taylor: **Phylogenetics of Neurospora and closely related genera**

8:50 Mary Anne Nelson: **The Neurospora genome project at UNM: Gold from the mold**

9:05 Jonathan Arnold: **Physical map of *Neurospora crassa***

9:20 Ulrich Schulte: **The German Neurospora sequencing project: Achievements and perspectives**"

9:35 Alan Radford **The Neurospora gene compendium - lessons from the past and plans for the future**

9:45 Genomics Roundtable Discussion led by the Neurospora Policy Committee, the Neurospora Genomics Policy Committee and other members of this session

10:35 Break

11:00 Ad hoc workshops on future genomics projects and other topics.

11:55 End of Session

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

1:00-2:30 **Check-out**

Neurospora 2000 Invited Talks

Session 1: CELL BIOLOGY AND MORPHOGENESIS

Celebrating 75 years of Neurospora genetics.

David D. Perkins. Biological Sciences Stanford University, Stanford CA, USA.

Genetic work with Neurospora had its beginning not in 1941 with Beadle and Tatum, but in 1925 with experiments of B. O. Dodge, who described life cycles and ascus development in heterothallic and pseudohomothallic species, described heterokaryons and complementation, demonstrated Mendelian segregation of mating type, and performed the first tetrad analysis. Dodge's work and his enthusiasm for the organism were largely responsible for Neurospora being chosen to produce the first biochemical mutants, leading to its use by many workers and its development as a model organism. Mating type was the only marker available to Dodge when he began. In contrast, over 1000 loci have now been mapped to linkage group; 25% of these have been cloned and sequenced.

Imaging the vesicle trafficking network in living *Neurospora* hyphae.
Nick D. Read, Patrick R. Hickey, Sabine Fischer-Parton, Richard M. Parton. Fungal Cell Biology Group, Institute of Cell and Molecular Biology, University of Edinburgh, Rutherford Building, Edinburgh, EH9 3JH, UK.

The amphiphilic styryl dye FM4-64 has been used to track vesicle trafficking in growing *Neurospora* hyphae using confocal microscopy. FM4-64 is internalised by endocytosis, and stains putative endosomes prior to staining vacuole membranes and the apical vesicle cluster within the Spitzenkörper. Our data indicate that (1) the endocytic pathway integrates with the secretory pathways involved in tip growth and (2) wall-building secretory vesicles within the main Spitzenkörper are derived from two or possibly three sources (Golgi, satellite

Spitzenkörper and possibly endosomes). Time-lapse movies illustrating the dynamic behaviour of the Spitzenkörper in wild type and mutant strains of *Neurospora* will be shown and, finally, a model of the vesicle trafficking network within growing hyphae will be presented.

Programmed hyphal compartmentation and death in *Neurospora crassa*.

N. Louise Glass, Jennifer Wu, Qijun Xiang. Plant and Microbial Biology Department, University of California, Berkeley, CA 94720.

Vegetative incompatibility is a phenomenon whereby when two strains that differ in *het* genotype undergo hyphal fusion, the fusion cell is compartmentalized and dies. Vegetative incompatibility is usually assessed in *N. crassa* by forcing heterokaryons or partial diploids that differ in allelic specificities at *het* loci. Such incompatible strains exhibit slow growth rates, hyphal compartmentation and death and suppression of conidiation. The *het-c* locus encodes a glycine-rich polypeptide; a 24-48 amino acid variable domain mediates the three allelic specificities at het-c. Phylogenetic analysis of *het-c* in different species and genera in the Sordariaceae indicated that *het-c* is under balancing selection, presumably because of its nonself recognition function. To determine the requirements of specificity, we examined the role of natural polymorphisms on *het-c* specificity and thereby identified and subsequently generated new *het-c* allelic specificities. To determine the mechanism of recognition between strains containing alternative *het-c* polypeptides, we tagged HET-c and performed co-immunoprecipitation experiments. Heterocomplexes of alternative HET-c polypeptides were detected, but homocomplexes were not. We have undertaken a genetic approach to determine how HET-c heterocomplex formation triggers vegetative incompatibility and have identified three suppressor mutants. Characterization of *vib* (vegetative incompatibility blocked) loci will identify downstream effectors of *het-c* mediated vegetative incompatibility.

Structure and function of the Neurospora TOM complex.

Frank E. Nargang and Rebecca D. Taylor. Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.

The majority of mitochondrial proteins are encoded by nuclear genes, synthesized as preproteins in the cytosol, and imported into the organelle. Preproteins destined for the matrix contain cleavable targeting signals at their N-termini, while the targeting information for other mitochondrial proteins lies within the mature protein coding sequence. Recognition and import of mitochondrial preproteins through the outer membrane is achieved by the TOM complex (translocase of the outer mitochondrial membrane). Further movement into the matrix or mitochondrial inner membrane requires the action of the TIM complexes (translocase of the inner mitochondrial membrane). The *N. crassa* TOM complex contains at least six subunits: TOM70, TOM40, TOM22, TOM20, TOM7, TOM6. Our investigations have concentrated on the function of the receptor domain of the TOM22 protein and on TOM40, the major component of the translocation pore. We have constructed null mutants for the genes encoding each of these essential proteins in sheltered heterokaryons. The existence of the mutant strains has allowed us to introduce various *in vitro* generated mutant alleles of the genes so that the phenotypic effects of the altered gene products can be studied. For TOM22 we have shown that mutations affecting

the negatively charged region of the cytosolic domain have little affect on the growth rate of cells or their ability to import mitochondrial precursors. Mutations affecting various conserved regions of the TOM40 protein result in only a mild growth phenotype. In at least some of these strains, native blue gel electrophoresis and chemical cross-linking studies suggest that the conformation of the TOM complex may be altered in the mutants.

Analysis of dynactin and its role in regulating cytoplasmic dynein activity.

In Hyung Lee, Santosh Kumar, and Mike Plamann. School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO, USA.

Dynactin is a multisubunit complex that mediates the interaction of the microtubule-associated motor cytoplasmic dynein with membranous cargo. We have isolated hundreds of mutants in *Neurospora crassa*, that are defective in cytoplasmic dynein/dynactin function. We report the identification of three subunits of the Arp1 pointed-end capping complex of Neurospora dynactin. RO2, RO7 and RO12 are apparent homologs of mammalian dynactin subunits p62, ARP11, and p25, respectively. Mutations in *ro-2* and *ro-7* disrupted nuclear and vacuole distribution; however the degree of defects was less severe in *ro-2* mutants than in *ro-7* mutants. In contrast, the *ro-12* mutation disrupted vacuole distribution, but nuclear distribution was normal. The results suggest that RO2, RO7, and RO12 have specific functions within the Arp1 filament pointed-end capping complex. We also examined the effects of dynactin mutations on dynein ATPase activity. Our results indicate that dynein ATPase activity is regulated by dynactin-dependent phosphorylation.

Expression and localization of two COT1 kinase isoforms in *Neurospora crassa*.

Oded Yarden,[1] Rena Gorovits[1], Klaas A. Sjollema[2] and J.Hans Sietsma[2], [1]Department of Plant Pathology and Microbiology and The Otto Warburg Center for Agricultural Biotechnology, The Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, 76100 Israel. [2]Department of Plant Biology and Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Biological Center, Nl-9715 NN Haren, The Netherlands.

Reversible protein phosphorylation is one of the mechanisms by which hyphal elongation and fungal morphogenesis are regulated. Complementation of the colonial temperature sensitive 1 (*cot-1*) mutant of *Neurospora crassa* has indicated that *cot-1* encodes a Ser/Thr protein kinase which is structurally related to the mammalian myotonic dystrophy kinase and other members of the Rho-kinase subfamily. Antibodies raised against COT1 detected and immunoprecipitated a predominant 73-kDa polypeptide in *N. crassa* extracts, whose abundance was constant under all growth conditions tested. An additional, lower MW, COT1 isoform (67-kDa) present in the wild-type, was not detected in *cot-1* grown at the restrictive temperature. Immunolocalization analysis provided evidence that COT1 is present in both the cytosol and in association with the cytoplasmic membrane. Immunoblotting experiments performed on extracts obtained from the wild type indicated that the lower MW isoform was present in all cellular components, yet was more abundant in the membrane fraction. However, the membrane-associated form was almost undetectable by immunolocalization or western blotting procedures in the *cot-1* mutant grown at

the restrictive temperature. Differential detection and immunolocalization of COT1 in various sub-cellular fractions indicate that COT1 is post-translationally processed and trafficked within the fungal cell. We propose that a lower MW COT1 isoform that, is associated with the plasma membrane, is involved in the regulation of hyphal elongation. Transcription profiling is currently being used in order to identify genes that are differentially expressed in *cot-1* grown at restrictive conditions.

Interaction of colonial mutations of *Neurospora crassa*.

Tony Griffiths, Florian Muller, Jennifer Haynes, and Cindy Yen. Botany, University of BC, Vancouver, BC, Canada.

In Neurospora there are numerous loci at which mutation gives rise to a compact "colonial" morphology, generally a manifestation of increased branching and slow growth. As part of a programme to dissect the genetics of branching, we have started to examine the functional relationships between these loci. The procedure has simply been to obtain double mutants and compare their phenotypes to the single mutants. We have used two general groups of mutants, the true colonials (*col*) and the partially colonials (*smco* and *spco*). From fertile crosses, 41 double mutants were obtained via NPD and T octads. In most cases, the double mutant phenotype is more defective than either single, and the mutants can be regarded as additive in their action. Assuming the mutants are nulls, the simplest interpretation is that they involve separate developmental pathways. However, there were several cases of epistasis in which the double resembles one of the singles in growth rate and branch pattern; for example *col-8* is epistatic to (>) *col-17*, *smco-6* > *smco-8*, *smco-1* > *spco-4*, *spco-5* > *smco-8*, and *spco-11* > *spco-10*. Such interaction might indicate a common pathway: for example *smco-6*, *spco-5* and *smco-8* might be grouped. In one case (*smco-9*; *spco-6*) the double is more vigorous than either single, indicating a different type of interaction of gene products.

Session 2: BIOCHEMISTRY AND METABOLISM

Translational control of *Neurospora crassa arg-2* by arginine.

Matthew S. Sachs. Department of Chemistry, Biochemistry & Molecular Biology, Oregon Graduate Institute, Portland, OR USA.

The *arg-2* gene encodes the small subunit of Arg-specific carbamoyl phosphate synthetase, the first enzyme in the Arg biosynthetic pathway. *arg-2* is unique among Arg biosynthetic genes because it is subject to negative regulation in response to Arg. An evolutionarily conserved peptide encoded by an upstream open reading frame (uORF) in the *arg-2* transcript mediates Arg-specific regulation at the level of translation. Using *N. crassa* cell-free extracts, we observe that ribosomes stall after synthesizing this uORF-encoded peptide when the level of Arg is high. The stalled ribosomes block the movement of trailing ribosomes, reducing translation initiation at the downstream ARG-2 start codon. The uORFs in the corresponding genes of *Saccharomyces cerevisiae*, *Magnaporthe grisea* and *Aspergillus nidulans* function similarly to stall ribosomes in *N. crassa* extracts. The sequence requirements for Arg-regulation by the uORF-encoded peptide have been investigated by systematic mutational analysis.

Regulation of sulfur metabolism in *Neurospora crassa*.

John V. Paietta. Biochemistry & Molecular Biology, Wright State University, Dayton, OH, USA.

The sulfur regulatory system of *Neurospora crassa* consists of a group of sulfur-regulated structural genes (e.g., arylsulfatase, *ars-1*) which are under coordinate control of the *cys-3* and sulfur controller (scon) regulatory genes. The CYS3 regulator is a bZIP DNA-binding protein and transcriptional activator, while the SCON2 negative regulator is a F-box/WD-40 protein. The importance of the SCON2 F-box in sulfur gene regulation has been demonstrated by site directed mutagenesis experiments. F-box proteins are thought to serve as adaptors that specfically link a targeted protein to the ubiquination/proteolysis system. The F-box of an adaptor protein (e.g., SCON2) binds to the core complex involved in the regulated proteolysis (referred to as the SCF) by a protein-protein interaction with SKP1 (suppressor of kinetochore protein 1). We have cloned and are studying a component of the Neurospora sulfur regulatory system that is homologous to SKP1. The current model of the control mechanism will be discussed.

A new GATA factor, ASD, regulates sexual development in *Neurospora crassa*.

Bo Feng[1], Hubertus Haas[2] and George A. Marzluf[1]. [1] Department of Biochemistry, The Ohio State University, Columbus, Ohio. [2]University of Innsbruck, Innsbruck, Germany.

A novel GATA factor, ASD, was cloned from *Neurospora crassa*. The sequence of Asd revealed an open reading frame of 427 amino acid residues containing a GATA type zinc finger and a putative coiled-coil domain. Unlike its homologs in yeast or Penicillium, however, Asd does not appear to be involved in nitrogen regulation. Disrupting Asd gene by Rip did not show any effect on nitrogen regulation, but resulted in severe defects in ascospore genesis. A strain bearing the disrupted Asd gene was unable to produce ascospores during genetic crossing with wild-type, though fruiting body development seemed normal macroscopically. Introduction of the Asd^+ gene into the Asd(Rip) strain corrected the failure of ascus and ascospore development. Mobility shift assay showed that ASD recognizes "GATA" DNA elements, thus is active in specific DNA-binding. The putative coiled-coil domain forms a dimer in solution, indicating that Asd binds DNA as a homodimer. Together, the results strongly suggest that Asd functions as a transcription regulator controlling sexual development in *Neurospora crassa*.

Regulation of arginine metabolism.

Richard L. Weiss. Department of Chemistry and Biochemistry, University of California, Los Angeles, CA.

Arginine metabolism in *Neurospora* involves a number of novel metabolic and regulatory features. The first seven biosynthetic enzymes are localized in the mitochondria, whereas the last two biosynthetic steps and the catabolic pathway are located in the cytosol. Feedback inhibition of acetylglutamate synthase (AGS) and acetylglutamate kinase (AGK), products of the unlinked *arg-6* and *arg-14* genes, involves an arginine carrier mediating rapid equilibration of arginine between the cytosol and mitochondrial matrix. AGK contains a C-terminal eukaryotic domain

not found in the bacterial enzyme. Mutations in the *arg-6* gene can affect the activity or feedback-sensitivity of both AGK and AGS. The proteins have been shown to interact using the yeast two-hybrid system. The eukaryotic domain appears to be responsible for this interaction. A mutation affecting the feedback sensitivity of AGK and AGS has been mapped to the N-terminal domain of AGK. AGK and acetylglutamyl-phosphate reductase (AGPR), the third enzyme of the biosynthetic pathway, are initially synthesized as a polyprotein precursor which is cleaved into separable proteins upon entry into the mitochondrion. Independently targeted proteins or an unprocessed polyprotein precursor appear to complement *arg-6* null mutants suggesting that the polyprotein precursor is not essential for protein targeting to the mitochondria or functional assembly into active enzymes. These organizational features may reflect the different regulatory requirements imposed upon eukaryotic (versus prokaryotic) organisms

The vacuolar ATPase is a surprisingly complex enzyme with multiple roles to play in growth and differentiation in fungi.

Barry Bowman. Department of Biology, University of California, Santa Cruz, CA, USA.

In most intracellular compartments the control of pH and the energy for active transport are provided by the vacuolar ATPase. This is a complex enzyme, which uses the energy in ATP to pump protons across membranes. The enzyme is composed of at least 13 different types of subunits, all of which have been identified in *Neurospora crassa*. The enzyme appears to function as a miniature mechanical pump. Some polypeptides provide the driving force and form the motor. Other polypeptides form a rotor, which spins at speeds of up to 10,000 rpm. Additional polypeptides attach the motor and rotor sectors to the membrane or are involved in regulation or cellular localization. To examine the role of the vacuolar ATPase we have generated strains in which genes encoding essential subunits have been mutated by RIPing. Several of these are true null strains that survive without a vacuolar ATPase. The most striking phenotypes of these strains are the inability to grow in alkaline medium (above pH7), and the dramatic alterations in mycelial morphology and differentiation. Mutations identified in other genes can suppress the pH sensitivity but not the morphological alterations. One group of suppressors is comprised of strains in which the kinetic properties of the plasma membrane ATPase have been changed. We have proposed a model to explain how the vacuolar and plasma membrane ATPases may regulate pH and control ion homeostasis.

Properties and interactions of HSP70 and Hsp80: Heat-inducible, cytosolic molecular chaperones of Neurospora.

Manju Kapoor. Biological Sciences, University of Calgary, Calgary, Alberta, Canada.

Formation of hetero-oligomeric complexes between purified HSP70 and HSP80, cytosolic molecular chaperones of Neurospora, is demonstrable by ELISA assays, inter-protein crosslinking using bifunctional reagents, gel filtration and Surface Plasmon Resonance measurements. The subunit structure of HSP80, in its native state, is consistent with a dimer-of-dimers. Inter-protomer crosslinking of HSP80 with dimethyl adipimidate and dimethyl suberimidate is suppressed by nucleotides. Crosslinking of HSP70 by glutaraldehyde yields dimers and higher order oligomers but binding of ATP, ADP and NAD reduces the yield of

oligomeric species. Interestingly, [HSP70:HSP80] complex formation- reflecting inter-protein interactions- appears to be stimulated by nucleotides. Although both HSP70 and HSP80 exhibit high intrinsic ATPase activity, that of HSP80 is subject to strong inhibition by the substrate, indicating the presence of two nucleotide binding sites. HSP70 and HSP80 act as molecular chaperones individually as well as in the form of a complex. The substrate specificity of the complex is distinct from that of HSP70 and HSP80 alone. An additional, ~40-kDa protein, interacting with HSP70, appears to compete for the binding site(s) for HSP80. These studies suggest the presence of a functionally flexible chaperone system in Neurospora.

Metabolite regulation of multiple arginases in conidia and early germination of *Neurospora crassa.*

Gloria E. Turner and Richard L. Weiss. Chem & Biochem, UCLA, Los Angeles, CA, USA.

The biological role of extrahepatic arginase in vertebrates is not well understood. It has been proposed that this arginase has the potential to regulate the availability of arginine for proline, polyamine, NO, and glutamate synthesis. Ornithine; a product of arginine hydrolysis by arginase would be used to preferentially synthesize any of these various metabolites. Unlike the major arginases of vertebrates that are encoded on separate genes, *N. crassa* arginases are differentially expressed from a single locus. Arginine, the substrate of arginase is required for production of the larger arginase transcript and protein. Previous work has shown that arginase is temporally expressed in germination and that the ratio of the two forms change in a dynamic fashion when the organism is grown on nitrogen sufficient minimal or arginine supplemented medium. We have examined the effects of arginine, ornithine and proline, on the ratio of the two major transcripts as well as their translation products in conidia and during germination. We have established that conidia under all conditions, minimal, minimal supplemented with arginine, ornithine or proline has both forms of arginase protein. This is also the case when arginine, ornithine or proline is used as a sole nitrogen source. Interestingly, only the large transcript is detected in our conidial samples supplemented with each amino acid. We were unable to detect the small transcript or any arginase activity in these conidial extracts. This suggests that both forms of arginase protein are stored in condia in an inactive form. We have shown that ornithine and proline can induce expression of the large form of arginase and these metabolites alter the ratio of the two forms during germination. This suggests that each form may preferentially be used for the synthesis of polyamines and proline. The ratio of the two forms is dramatically different when arginine, ornithine or proline are the sole nitrogen sources. This nitrogen stress condition induces the expression of both forms of arginase. The two protein forms are almost equal and remain at a constant level. This suggests the presence of a nitrogen regulatory factor that controls arginase expression during nitrogen stress.

Characterization of *Neurospora crassa mak-2* gene encoding mitogen-activated protein kinase similar to yeast Fus3 and Kss1.

Piotr Bobrowicz and Daniel Ebbole. Plant Pathology & Microbiology Texas A&M University College Station TX.

Using the *Magnaporthe grisea PMK1* cDNA as a heterologous probe we have isolated the *N. crasssa* gene that resembles the *Saccharomyces cerevisiae* MAP kinase genes, *FUS3* and *KSS1*, involved in pheromone signaling and invasive growth. This gene is identical to the *mak-2* gene previously isolated by a PCR approach (Margolis and Yanofsky). In *M. grisea*, *PMK1* is required for infection-related morphogenesis but its role in sexual development has only been partially characterized (Xu & Hamer, 1996. Genes Dev. 12:2874). The corresponding MAP kinase of *Cochliobolus heterostrophus* (Lev *et al*. 1999. PNAS 96:13547) is required for conidiation and mating as well as appressorium development. To determine the function of *mak-2* gene in *N. crassa* we constructed RIP and gene replacement mutants. The *mak-2* strains are morphologically abnormal producing strongly reduced aerial hyphae with uniformly distributed conidiophores. Mutants are fertile as a male partner but completely sterile as a female. Experiments are in progress to elucidate the role of the *mak-2* gene in sexual and asexual development.

Search for carotenogenesis mutants, new phenotypes and cloning of the *ovc* and *cut* loci.

Javier Avalos[1], Loubna Youssa[1], and Thomas J. Schmidhauser[2]. [1]Genetica, Universidad de Sevilla, Sevilla, Spain. [2]U. of Louisiana, Lafayette, LA, USA.

Standard UV mutagenesis coupled with screening of survivors at 6C in continuos light lead to a number of distinct classes of mutants. A reddish mutant is complemented by the *al-2+* gene and may be defective in the cyclase function recently assigned to the *al-2* gene product, the Neurospora phytoene synthase. Another mutant designated carotenoid producer in the dark, *cpd,* was isolated in a *ovc* background and accumulates carotenoid pigments in dark grown mycelia. No mutants of this class could be isolated by visual screening after mutagenesis of the wild type strain. Screening for the *ovc* locus identified a cosmid clone that also complements the *cut* locus. Preliminary analysis suggests that the two loci are independent, but in close proximity as both mutants are complemented by transformation with a 6 kb cloned DNA fragment.

Session 3: GENETICS AND GENE REGULATION

***Neurospora crassa* as a model system to study homology dependent gene silencing.**

Carlo Cogoni, Gianluca Azzalin, Caterina Catalanotto, and Giuseppe Macino. Se. Genetica Molecolare, Universita di Roma, Rome, Italy.

The introduction into cells of foreign nucleic acid molecules, either RNA or DNA, can induce sequence-specific gene silencing in a number of organisms. In *Neurospora crassa* transgenes can induce a Homology-Dependent Gene Silencing (HDGS) phenomenon called 'quelling'. Three classes of mutants impaired in quelling have been isolated by using random insertional mutagenesis and plasmid rescue procedure, identifying three qde (quelling deficient) genes involved in the gene silencing machinery. The first gene cloned was qde-1 which product shares significant identity with an RNA-dependent RNA polymerase (RdRP) characterized in tomato. This identity supports the idea of the involvement of an RdRP in the post-trascriptional gene silencing mechanism that could be responsible for the identification of aberrant RNA and its amplification in a cRNA form. Successively the cRNA could be responsible for targeting and

degradation of specific mRNA. More recently we have isolated the qde-3 gene. qde-3 belong to the RecQ DNA helicase family suggesting a role for this gene in DNA-DNA interaction between repeats and/or induction of chromatin modification required for aberrant transcription. QDE-3 may be essential in unwinding double stranded DNA, thus producing single stranded DNA that could function as an intermediate in a DNA-DNA interaction between transgenes and/or endogenous gene required for gene silencing activation. A more refined model of the quelling mechanism and analogies with HDGS phenomena in other organisms will be discused.

Neurospora as a model to investigate DNA methylation.

Eric U. Selker, Hisashi Tamaru, Brian Margolin, Elena Kuzminova, Michael Freitag, Vivian Miao, Greg Kothe, Joe Dobosy, and Shan Hays. Institute of Molecular Biology, University of Oregon, Eugene, Oregon, USA.

Although absent from some model organisms such as *S. cerevisiae*, *S. pombe*, *D. melanogaster* and *C. elegans*, DNA methylation is found in Neurospora, humans and many other eukaryotes. Methylation can serve to mark particular sequences for special treatment by the cell and typically plays roles in gene silencing. Neurospora seems to use methylation to prevent expression of foreign DNA and DNA mutated by RIP. Methylation is clearly not essential for life of this organism; methylation can be abolished by mutation or replacement of the DNA methyltransferase gene without noticeable effects. The dispensability of methylation in Neurospora contrasts the situation in higher eukaryotes and facilitates genetic studies on its control and function. One feature of DNA methylation is that once established, it is typically propagated through DNA replication, perpetuating its effect(s). The mechanism of such "maintenance methylation" is not well understood. In Neurospora, we have found evidence that maintenance methylation is sequence-dependent. We have also used Neurospora to carry out the most detailed analysis to date of de novo methylation signals. We found that both A:T-richness and high densities of TpA dinucleotides, typical attributes of methylated sequences in Neurospora, promote methylation, but neither are essential for methylation. We also found that methylated sequences contain multiple, positive signals that trigger methylation. To better understand the nature of these signals, various simple, synthetic sequences were tested for their ability to trigger de novo methylation. Certain patterns of alternating As and Ts, but not long runs of Ts or As or G:C-rich sequences, efficiently trigger methylation (see poster by Tamaru and Selker). We are also investigating the relationship between DNA methylation and modifications of chromatin. We showed that DNA methylation associated with products of RIP can prevent transcription elongation without affecting initiation and that trichostatin A (TSA), an inhibitor of histone deacetylases, can relieve this repression and cause loss of DNA methylation. TSA does not cause reduction of methylation in most of the genome, however, perhaps because not all regions have access to histone acetyltransferases. Analysis of the acetylation state of histones associated with specific DNA regions has revealed that not all regions become hyperacetylated by TSA treatment, consistent with this possibility. We also demonstrated that changes in DNA methylation can affect histone acetylation, consistent with evidence from animal systems that histone deacetylases are recruited to methylated DNA by methyl-DNA binding proteins. In addition, we found unmethylated hypoacetylated regions, indicating that hypoacetylation, per se, does not lead to DNA methylation. We are currently exploring the

possibility that TSA-induced loss of methylation is due to inhibition of methylation by transcription.

Tad transcription and transposition.

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Using *in-vitro* mutagenesis and targeted transformation we have determined a number of elements within the Tad retrotransposon that are essential for transcription and/or transposition. One of these elements is an internal promoter that is located between 131 and 179 bp internal to the consensus 5' end of Tad. The promoter was initially located by deletion mapping. Subsequently the promoter has been further defined by the use of reporter gene constructs. This promoter appears to have several unique features in addition to its internal location. For example it appears to trigger the initiation of transcription in a manner that is neither sequence nor distance specific. It also appears to act as a bi-directional promoter. Preliminary gel mobility shift analysis with this promoter suggests a complex gel shift pattern which might indicate interaction directly or indirectly with a number of proteins.

S-adenosylmethionine, DNA methylation and DNA mutation in *Neurospora crassa*.

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Cytosine methylation is associated with the occurrence of C-T transition mutations. About 30% of point mutations related to human diseases are C-T mutations that take place in CpG dinucleotides, which are the preferred substrate for the mammalian DNA-(5-cytosine) methyltransferases. *Neurospora crassa* is a particularly well suited organism to study the relationship between the processes of cytosine methylation and mutation. This fungus exhibits a particular mutagenic process called Repeat-Induced Point mutation (RIP) that detects and irreversibly alters repeated sequences through the introduction of C-T transition mutations. Although sequences altered by RIP often show cytosine DNA methylation, it is not yet clear whether cytosine methylation is the cause or the consequence of this mutagenic process. We have theoretically modeled the different enzymatic mechanisms proposed for the occurrence of C-T transition mutations in RIP. We suggested that the various models might be distinguished experimentally by studying the behaviour of the average frequency of transition mutation in populations of mutant alleles obtained by RIP at different levels of S-adenosylmethionine (SAM), the universal methyl-group donor for DNA methylation. RIP was investigated on a set of strains displaying a wide spectrum of both cellular SAM levels and DNA methylation. RIPing frequencies of unlinked duplicated copies of the *am* (NADP-glutamate dehydrogenase) gene decrease at high levels of SAM. The results suggest that the spontaneous deamination of the unstable cytosine-methylation intermediate (5,6-dihydrocytosine), is one of the main causes of the introduction of C-T transitions during RIP.

DNA damage, repair and aging in *Neurospora crassa*.

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To investigate DNA repair mechanism in *N. crassa*, we have isolated and characterized more than 40 different mutagen-sensitive mutants. *N. crassa* has 3 different repair systems as other orgnisms have. In addition to those repair systems, there are some repair groups which have not been identified yet. We are currently analyzing those mutants in molecular level. I will summerize DNA repair study in *N. crassa* and talk about DNA repair mutants showing relationship between DNA repair and aging. Also, I will give an example that Neurospora is usable as a model organism in DNA repair study.

Events at recombination hotspots in Neurospora and their control.

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Recombination in Neurospora is initiated from hotspots usually 5' of genes. The hotspots are regulated by transacting genes (*rec*-genes) that block recombination from specific subsets of hotspots. *rec* genes are polymorphic in the natural population with active and inactive alleles. This system has the potential to provide differential control of the rate of evolution of specific sets of genes. We have cloned and sequenced *rec-2*, which regulates the very active hotspot *cog* that we have also cloned. *rec-2* is transcribed in the sexual phase and does not show significant homology to known genes. In *rec-2* regulated recombination initiated at *cog*, conversion events frequently have an apparently associated crossover. In events initiated at a weaker hotspot 5' of the *am* locus, conversion is rarely associated with a crossover, although the occurrence of a conversion event substantially increases the probability of crossovers in an interval 5 to 80 kb proximal to *am*. This is the first demonstration of negative interference and does not fit well with current molecular models for recombination. Many conversion tracts in Neurospora are interrupted. This offers a means of diversifying heterologous genes. We have adapted *cog* for this purpose.

Suppression of certain ascus-dominant mutations.

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A number of mutations have been reported that affect steps in meiosis proper, the cutting out of ascospores, the shape of asci, the shape of the ascospores themselves, or the pigmentation and maturation of ascospores. Some of these are ascus-dominant, in that a mutant allele in one of the parents confers its characteristic phenotype equally on those that carry the allele and those that are genetically wild type. One such mutant, *Asm-1* (Ascus maturation) causes about 99% of all ascospores to fail to develop pigment and to become viable, and certain derivatives of it are even more detrimental in a cross than the original mutant. Closely-linked suppressors can be isolated that allow a considerably larger fraction of the ascospores to mature. A few examples of a second, much rarer class have recently been identified that likewise give an increased fraction of survival in crosses involving *Asm-1*, and also give considerable suppression of the dominance of another ascus-dominant mutation, *Roundspore*. One of these, which gives about 50%

suppression of Roundspore, also suppresses a third mutation, *Banana* which normally fails to cut out individual ascospores in about 99% of asci. In the suppression of *Roundspore*, an individual rosette in a perithecium will contain both asci with round spores and asci with normal spindle-shaped spores, but no ascus contains a mixture. Some implications will be discussed.

Detached nuclear genes of Neurospora transmitted cytoplasmically through infectious contact.

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This research demonstrates that a plasmid can evolve from a set of nuclear genes. The chromosome aberration *T(II;VI)R2459* produces acentric chromosome fragments through meiotic crossovers in linkage group IIR when paired with normal chromosome sets. The acentric fragments do not move to the division poles with the centric chromosomes but remain between poles in the cell cytoplasm. Acentric chromosomes from *R2459* organize a surrounding membrane which protects them from nuclease digestion. (Acentric fragments produced from other Neurospora chromosome aberrations do not generate membranes.) As a mitotic division in the ascus continues after meiosis, the *R2459* acentric replicates in synchrony with adjacent nuclei. The replicas may separate into distinct micronuclei. When ascospores form, the separate acentric fragments in micronuclei can be included passively in a spore along with either a translocation chromosome set or a normal sequence chromosome set. As the following mitotic divisions within developing ascospores proceed, the acentric chromosomes in micronuclei also replicate and may separate. After ascospore germination, selecting for prototrophic markers on the acentric fragment which complement reciprocal auxotrophic markers in the main nuclues has successfully shown that the fragment can be maintained in vegetative growth. A slower growth occurs when the culture is dependent on the acentric fragment for enzymatic activity determined by a gene carried by the fragment. The fragment may be transferred to a different cell line by construction of a heterokaryon and subsequent selection of both genetic activity of the fragment and a diagnostic marker of the infected second line. The acentric fragment is retained in high number only by selective pressure. When the fragment is required for continued growth, surveys of conidia in one typical serial set of isolations of colonies dependent on activity of the fragment show a range of 1:1,000, 1:100, and 1:20 conidia with the acentric fragment. The increasing frequency of recovery of the fragment in successive selective steps may indicate an adaptive evolution.

Session 4: SIGNALING AND DEVELOPMENT

Feedback loops in the Neurospora circadian system.

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Circadian rhythms are daily rhythms in physiology and behavior that are found among many living things and that are nearly ubiquitous among higher eukaryotes. The fundamental molecular biology underlying these cycles is based at the level of the single cell, and there are

similarities in mechanism running through all known circadian systems. An attractive model for elucidating these processes has been Neurospora; research on this fungus and on Drosophila has formed the basis for existing models of clock function. Models for the Neurospora circadian clock posit a negative feedback loop wherein the *frq* gene encodes two FRQ proteins which travel to the nucleus to block the activity of the heterodimeric activator of *frq* comprised of the WC-1 and WC-2 proteins (Dunlap, Cell 96:271-290, 1999). Given appropriate delays in the synthesis, action, and turnover of FRQ, this negative feedback loop yields oscillations in *frq* transcript and FRQ protein levels; this cycling of expression in clock components is characteristic of all circadian oscillators. The general layout of the feedback loop and the identity molecular identity of some of the components, particularly the heterodimeric positive elements WC-1 and WC-2 appear to be conserved among the Crown Eukaryotes (Dunlap, Science 280:1548-49, 1998; Cell 96:271-290, 1999). Light delivered at any point within the cycle acts through two regions within the *frq* promoter to rapidly increase the level of *frq* transcript thereby resetting the clock (Crosthwaite *et al*. Cell 81:1003 - 1012, 1995); mammalian circadian rhythms are reset in part in a similar manner (Shigeyoshi *et al* Cell 91:1043 - 1053, 1997). The WC proteins bind to these sites. Temperature acts posttranscriptionally to determine the absolute level of FRQ in the cell and the site of initiation of translation within the *frq* transcript, thereby dictating the ratio of long FRQ versus short FRQ (Liu *et al*, Cell 89:477 - 486, 1997). Resetting of the clock by changes in temperature can be understood in terms of changes in these set points (Liu *et al*, Science 281: 825 - 29, 1998). Dozens of circadianly expressed genes are known to act downstream of the clock. These "clock-controlled genes" include a hydrophobin (*eas=ccg-2*), trehalose synthase (*ccg-9*), and glyceraldehyde 3-P dehydrogenase (*ccg-7=gpd*) and play roles in clock regulation of development, stress responses, and intermediary metabolism (Loros, Curr. Opin. Microbiol. 1:698 - 706).

Biochemical interactions between FRQ and WC-2: critical clock proteins required for the normal operation of the Neurospora circadian oscillator.

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The White Collar proteins (WC) are PAS domain containing proteins required for blue-light responses and essential for normal operation of the circadian clock in *Neurospora crassa*. Specifically, both WC-1 and WC-2 are necessary for rhythmic expression of a central clock component, *frequency* (*frq*). Although it is well established that FRQ negatively regulates it's own expression and that the expression of *frq* requires WC-2, the molecular mechanisms governing both the activation and the feedback loop of *frq* are unknown. Previously, we have shown that WC-2 is nuclear, consistent with its anticipated role as a transcriptional activator, but does not display a robust rhythm in either the nuclear or cytoplasmic fraction. Current experiments set out to begin to determine the nature of the mechanism of WC-2 regulation of *frq* transcription. In independent experiments, it was found that WC-2 interacts directly with FRQ. *In vitro,* GST-tagged WC-2 specifically interacts with radiolabelled FRQ proteins. More importantly, *in vivo,* FRQ specifically co-immunoprecipitates with both WC-1 and WC-2 from Neurospora extracts. This demonstrates direct or indirect association between FRQ and the WC proteins. Thus, WC-2 may regulate rhythmic transcription of *frq* through this protein-protein

interaction. Further characterization of this interaction may provide insight to the molecular events underlying the basis of the circadian clock in Neurospora.

Control of conidial development by the circadian clock in *Neurospora crassa*.

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Organisms have a clock to temporally organize biological activities to appropriate time of the day. In *Neurospora crassa* the clock regulates the timing of asexual spore development (conidiation). In constant darkness, conidiation occurs once every 22 h during the subjective morning. Several environmental signals can trigger conidiation in Neurospora, including light, air, carbon and nitrogen starvation; however the biological clock provides the only known endogenous signal to initiate conidiation. Three key regulators of the developmental pathway are known and include *acon-2*, *fl* and *acon-3*. Different combinations of the regulators are thought to control the expression of downstream conidiation-specific genes, including *con-10, con-6* and *eas* (*ccg-2*). To begin to understand how the clock regulates development we are addressing the following questions: 1) what genes within the developmental pathway are regulated by the circadian oscillator? and, 2) How does initiation of development by the clock and by environmental signals differ? Answers to these questions will help us understand the mechanisms by which the clock regulates this critical cellular event.

Regulation of macroconidiation by *fluffy*.

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Fluffy (*fl*) encodes a member of the Gal4 class of transcription factors. Null mutations of *fl* block the budding growth characteristic of proconidial chain formation. These mutants are also blocked in expression of many of the known conidiation-specific genes. The pattern of *fl* mRNA expression is consistent with its role as a regulator of morphogenesis. A basal level of *fl* expression is observed in undifferentiated mycelia. *fl* mRNA levels are induced during development at approximately the time when budding growth initiates and *fl* mRNA levels declines at later stages of development. *acon-2* and *acon-3* also are regulators of conidial morphogenesis and *acon-2* is required for induction of *fl* mRNA while *acon-3* is not. Elevated expression of *fl* from a the *cpc-1* or *ars-1* promoter was sufficient to induce conidiophore morphogenesis. One current effort is to isolate the FL protein for DNA binding studies. The location of consensus FL DNA binding sites in the genome may provide clues as to which *N. crassa* genes may be subject to developmental regulation.

Signal transduction through heterotrimeric G proteins and opsins in *Neurospora crassa*.

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Heterotrimeric G proteins are components of major signal transduction pathways in eukaryotic organisms. We have characterized three G-alpha (GNA-1, GNA-2 and GNA-3), one G-beta (GNB-1) and one G-gamma (GNG-1) subunit from the filamentous fungus *Neurospora crassa*. Taken together, our results demonstrate that G proteins are crucial for regulation of cell proliferation, asexual and sexual differentiation and stress tolerance in *N. crassa*, through both cAMP-dependent and independent pathways. Of evolutionary interest, GNA-1 was the first microbial *G-alpha* demonstrated to belong to a family found in higher organisms (G-alpha$_i$). In contrast to mammalian systems, this fungal G-alpha$_i$ is a positive regulator of adenylyl cyclase activity. Homologues of GNA-1 and GNA-3 have now been implicated in virulence in several animal and plant fungal pathogens. We have recently initiated a second project in the laboratory, study of opsins and opsin-related proteins (ORPs) in *N. crassa*. We have cloned and mutated the first opsin gene identified in a eukaryotic microbe, *N. crassa nop-1*. The predicted amino acid sequence of *nop-1* is most similar to archaeal opsins. NOP-1 binds all-trans retinal using a Schiff base linkage to form a green-light absorbing pigment with an archaeal rhodopsin-like photocycle. Expression of *nop-1* is restricted to conditions that favor conidiation and *nop-1* null strains exhibit light-dependent conidiation defects under certain conditions. We are continuing our analysis of *nop-1*, and are also characterizing an ORP gene (*orp-1*) from *N. crassa*, in order to determine the functions that opsins and ORPs play during light-sensing in filamentous fungi.

Interaction between and transactivation by mating type polypeptides of *Neurospora crassa.*

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The polypeptides encoded by the mating type idiomorphs of *Neurospora crassa* control diverse aspects of the fungal life cycle. Biochemical characterization of MAT a-1, MAT A-1, and MAT A-3 reveal new activities that correlate with important biological activities of the polypeptides. All three polypeptides have domains capable of activating transcription in a yeast reporter system. The transcriptional activation domains of both MAT a-1 and MAT A-1 are not critical for either mating or vegetative incompatibility activities in Neurospora. Two hybrid assays establish the ability of MAT a-1 to interact with MAT A-1. Mutations that interfere with this interaction correlate with mutations that eliminate vegetative incompatibility, but not mating, in Neurospora. The MAT A-2 polypeptide did not transactivate nor did it appear to interact with any other mating type polypeptide. These results suggest the hypothesis that interaction of MAT a-1 with MAT A-1 stimulates vegetative incompatibility. Two-hybrid interaction screens of Neurospora cDNA libraries with MAT a-1 have identified other polypeptides potentiallyimportant in the activities associated with the mating type locus.

The Hyper-osmotic stress response pathway of *Neurospora crassa* is the target of phenylpyrrole fungicides.

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Neurospora crassa osmotic sensitive (*os*) mutants are sensitive to high osmolarity therefore unable to grow on medium containing 4% NaCl. In this study, we found that *os-2* and *os*-5

mutants were resistant to phenylpyrrole fungicides fludioxonil and fenpiclonil. To further understand the mode of action of phenylpyrroles, we cloned the *OS-2* gene by sib-selection. *OS-2* encodes a MAP kinase homologous to *HOG1* and can complement the osmosensitivity of a yeast *hog1* mutant. We sequenced three *os-2* alleles and found all of them were null alleles with either frameshift or nonsense point mutations. An delta-*os-2* gene replacement mutant was also generated and was found to be osmotic sensitive and resistant to phenylpyrrole fungicides. Nonetheless, *os-2* mutants transformed with the wild type *OS-2* gene could grow on media containing 4% NaCl and became sensitive to phenylpyrrole fungicides. Interestingly, fludioxonil could stimulate intracellular glycerol accumulation in wild type strains but not in *os-2* mutants. We also observed that fludioxonil could cause wild type conidia and hyphal cells to swell and burst. These results suggest that the hyper-osmotic stress response pathway of *N. crassa* is the target of phenylpyrrole fungicides and fungicidal effects may be resulted from a hyperactive *OS-2* MAP kinase pathway. Because phenylpyrrole fungicides have no effect on fission and budding yeast, this fungicidal effect may be specific for filamentous fungi.

Localization and light-dependent phosphorylation of White Collar 1 and 2, the two central components of blue light signaling in *Neurospora crassa*.

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In *N. crassa* only two *white collar (wc)* mutants, *wc*-1 and *wc*-2, have been described which seem to be blind to light. The pleiotropic phenotypes of these mutants suggest that they represent two central components of blue light signal transduction. The WC proteins have several characteristics of transcription factors consistent with an involvement in transcriptional control of light-regulated genes. Here, we present a biochemical analysis of WC1 and WC2 polypeptides in *Neurospora crassa*. Using specific antisera against WC1 and WC2 respectively, the subcellular localization of the WC polypeptides was investigated. The WC1 protein was localized exclusively in the nucleus, whereas WC2 was detected in both the nuclear and cytoplasmic fractions. The nuclear localization of WC1 and WC2 was shown to be independent of light and dimerization between the two proteins. In addition, WC1 and WC2 are phosphorylated in response to light. The phosphorylation of WC1 and WC2 was dependent on functional WC1 and WC2 proteins, respectively which clearly indicated a correlation between the light-dependent phosphorylation and the function of WC1 and WC2 in blue light signaling. However, the light-specific phosphorylation of the WC proteins revealed different kinetics. The phosphorylation of WC1 was transient whereas the WC2 phosphorylation was shown to be stable under constant light conditions. The analysis of the light-dependent phosphorylation of WC1 and WC2 in *wc*-2 and *wc*-1 mutants revealed an epistatic relationship for WC1 and WC2 with WC2 acting downstream of WC1 in the signal transduction pathway of blue light.

Session 5: GENOMICS/EVOLUTION/TECHNOLOGY

The Neurospora Genome Project at UNM: Gold from the mold.

Mary Anne Nelson and Donald O. Natvig. Biology, University of New Mexico, Albuquerque, NM.

In the Neurospora Genome Project (NGP) at the University of New Mexico, expressed sequence tags (ESTs) corresponding to four stages of the life cycle (germinating conidial, advanced-growth mycelial, perithecial and unfertilized sexual tissues) of the filamentous fungus *Neurospora crassa* are being analyzed. Our strategies have included both random clone selection (to generate information on relative levels of gene expression) and subtraction of clones representing highly-expressed genes. We have identified over 2,000 different genes and have also developed a system for classifying encoded proteins based on their predicted functions. The results demonstrate a high percentage of novel genes with no known homologues in any organism (greater than 50%), and many genes that were not predicted for filamentous fungi. Database searches reveal that only 33% of the EST sequences have homologues in *Saccharomyces cerevisiae*, and 30% have homologues in non-fungal organisms. Several of the most highly expressed genes in mycelial and perithecial tissues have no known homologues in other organisms. Analyses of genomic sequences support estimates of greater than 10,000 genes for N. crassa. The preliminary results of an analysis of alternative splicing in Neurospora will also be presented. The results of EST and genome sequencing efforts with N. crassa and other filamentous fungi strongly support the need for additional study. Filamentous fungal genomes are rich in novel genes specific to fungal biology, and also in genes shared with very diverse organisms. The latter class of genes will prove valuable toward understanding fundamental biological processes. Supported by grants from the National Science Foundation.

Physical map of *Neurospora crassa*.

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A physical map at 29 kb resolution covering 58% of the genome has been generated using a 13.3 genome equivalent cosmid library sorted by chromosome. The physical map is being used to test whether or not *N. crassa* has a chimeric genome organization like that of *Aspergillus nidulans*. Associated with the physical map is a chromosome walk across each chromosome with a scale in kilobases. Physical maps of each linkage group can be found at http://fungus.genetics.uga.edu:5080. Of the 100 genes with known assignments to the 13.3 genome equivalent cosmid library, 59 of these cloned genes have been assigned to date to the physical map as expected. There is slightly less than 1 EST per cosmid along the physical map so that most cosmids contain an identified *N. crassa* gene sequence. Our best estimate of gene density is currently 1 gene per 3.3-3.8 kb or between 11,000-13,000 genes in the genome. About 10% of the *N. crassa* genome is repetitive with 4% being rDNA. Work is supported by NSF MCB-9630910.

The German Neurospora sequencing project: Achievements and perspectives.

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The genome of *N. crassa* comprises 43 Megabases in 7 chromosomes. As part of an international effort to sequence the entire genome of the fungus, a German project has been set up to analyse two chromosomes with a total of 14 Mb. Based on the initiative of 8 institutes at 6 German universities with financial support from the Deutsche Forschungsgemeinschaft the project involves the cooperation of academic and industrial partners. BAC- and cosmid libraries are

being mapped in the labs of Jörg Hoheisel (DKFZ, Heidelberg) and Jonathan Arnold (Athens, Georgia). Selected cosmid and BAC- clones giving a minimal tiling path covering the chromosomes II and V are being sequenced by MWG-Biotech, Ebersberg. The sequence data are assembled and analyzed by Werner Mewes and coworkers at MIPS, Munich. Open reading frames are identified and annotated and all the data are provided in a data base accessible through WWW. The data base is supposed to finally hold the entire genome as the analysis of the genomic sequence procedes in the US. Currently the database holds some 6 Mb of analyzed sequence. In my contribution to this meeting I will report on the progress of the project.

http://www.uni-duesseldorf.de/WWW/MathNat/biochem/genome.html

http://www.mips.biochem.mpg.de/proj/neurospora/

The Neurospora gene compendium - lessons from the past and plans for the future.

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Working for the past three years on the update of the 1982 compendium, we find that new genes have been described at the rate of approximately two per month, and the rate is rising. Actually, more new genes have been described than that, but some later have been shown to be allelic with known genes. Such multiple namings arise when two different aspects of the phenotype are observed, when a sequence is determined which subsequently is found to correspond to a gene known from a mutant phenotype, etc.. These situations are relatively easy to resolve once the allelism becomes apparent. Using the same name for different genes is a more difficult problem. The commonest cause is when a sequence is found to be the homolog of a known gene from another species, and the name is transferred without checking if it is already used in Neurospora. Another problem arises when an apparently novel gene justifying inclusion had been described, probably based on a sequence, but the originator had not named it. We propose to put on the web a list of all symbols and names that have been used in the organism in order to make checking easier and naming according to the conventions for Neurospora more creative. With new genes at two per month and rising, the millennium compendium will need updating, and we cannot wait fifteen years this time before starting. We will endeavour to supplement the published compendium with a WWW database of new genes and major revisions of existing gene entries. To facilitate this, we propose to put an electronic submission form on the web, and request the support of the community in maintaining an up to date compendium.

Neurospora 2000 Poster Abstracts

1. Mutants of Neurospora complex I as models of human mitochondrial disease.

Arnaldo Videira[1], Margarida Duarte[1], Helena Populo[1], and Ulrich Schulte[2]. [1]Instituto de Biologia Molecular e Celular, Porto, Portugal. [2] Duesseldorf Institute of Biochemistry, Duesseldorf, Germany.

Mitochondrial complex I transfers electrons from NADH to ubiquinone, through protein-bound prosthetic groups, coupled with proton translocation through the inner membrane of the organelle. It contains about 40 proteins of dual genetic origin distributed in two major domains, the peripheral and membrane arms. Specific mutations in iron-sulphur subunits of the enzyme were found associated with human mitochondrial diseases. Namely, the P79L and R102H mutations in the TYKY protein and the V135M mutation in the PSST protein were found. Disruption of either of the N. crassa homologues of these proteins prevents assembly of the peripheral arm of complex I. We generated the equivalent human mutations in the fungal cDNAs encoding the TYKY and PSST homologues, by site-directed mutagenesis. The cDNAs harbouring the mutations were then expressed in the relevant fungal mutant. The correspondent mutant proteins were able to restore the assembly of a complex I enzyme. Further characterisation of the strains will be presented.

2. Characterization of NAD(P)H dehydrogenases from the inner membrane of Neurospora mitochondria.

Arnaldo Videira[1], Ana M.P. Melo[1] and Ian M. Moller[2]. [1]Instituto de Biologia Molecular e Celular, Porto, Portugal. [2]University of Lund, Lund, Sweden.

Disruption of an NAD(P)H dehydrogenase from the inner mitochondrial membrane of *Neurospora crassa*, NDE1, was achieved by repeat induced point mutations. A physiological characterization of the NDE1 mutant was performed by measuring the rates of respiration of intact mitochondria and inside-out submitochondrial particles with either NADH or NADPH as substrates. The results indicate that the mutant strain lacks an NADPH dehydrogenase, which contains a catalytic site facing the mitochondrial intermembrane space and works at pH 7. The observation of NADH oxidation at pH 7 and of NADPH oxidation at pH 6 by intact mitochondria from the NDE1 mutant evidences the presence of a second external NAD(P)H dehydrogenase activity. The cross of an NDE1 mutant with several mutants in subunits of complex I yielded double mutants, indicating that further NAD(P)H dehydrogenases exist in *N. crassa* mitochondria.

3. Initial characterization of the TIM17-23 translocase of the mitochondrial inner membrane in *Neurospora crassa*.

Dejana Mokranjac and Holger Prokisch. Physiological Chemistry, University of Munich, Munich, Germany.

The majority of mitochondrial proteins are encoded in the nucleus and are synthesized in the cytosol. In order to reach their functional destination, they must be transported into or across the mitochondrial membranes. Import into mitochondria is a multi-step process mediated by translocation systems in the mitochondrial outer and inner membranes. In yeast, the outer membrane contains one translocase, the TOM complex, which is probably used by all nuclear-

encoded preproteins. The inner membrane contains two different translocases, TIM17-23 and TIM22-54 complexes. TIM17-23 complex is used by the preproteins which carry the classical mitochondrial presequence. TIM22-54 complex mediates the import of hydrophobic membrane proteins with internal targeting signals. In *Neurospora crassa*, TOM complex has been purified and characterized in certain details. However, neither of the Neurospora TIM complexes has been known so far. Here, we have cloned and characterized *Neurospora crassa tim23*. The encoded protein shares 40% sequence identity with its yeast homologue TIM23p. We have constructed a Neurospora strain which expresses the TIM23 protein with an octahistidine tag on its C-terminus and only negligible amounts of the wt protein. The tagged protein is fully functional. Construction of the strain and its use in the purification of the TIM17-23 complex will be presented.

4. Effect of mutation in vacuolar ATPase on basic amino acids pools in *Neurospora crassa*.

Kelly A. Keenan, Keith Mindish, Kristi L. Muldoon, and Dawn M. Moore. NAMS, Richard Stockton College, Pomona, NJ, USA.

Three strains with decreased vacuolar ATPase activity have been isolated using a filtration enrichment procedure. All three strains show an increase sensitivity to the three basic amino acids(arginine, lysine and ornithine) stored in the vacuole. The levels of the basic amino acids in the cytosol was measured using a cupric ion permeabilization procedure and compared to wild type. All three strains had increased levels of basic amino acids. The vacuolar pools for the three basic amino acids was measured and shown to be decreased. Uptake of the basic amino acids into the vacuole was measured and shown to be signficantly reduced. This was further characterized kinetically by measuring the Michaelis constant and maximum velocity for uptake for the basic amino acids. For all amino acids studied so far, there have been changes in these values. The results suggest that the vacuolar ATPase plays an integral role in controlling the levels of vacuolar amino acids.

5. Mitochondrial morphology and inheritance in *Neurospora crassa*: Cloning and characterisation of *mmm-1*.

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Mitochondria are essential organelles which are often located at sites of high energy consumption in the cell. Since they cannot be synthesized de novo, they have to be inherited from the mother to the daughter cell during cell division. There is mounting evidence that positioning and transport of mitochondria are controlled by the cytoskeleton. All three major cytoskeleton classes have been implicated in mitochondrial movement in fungi. In the budding yeast *S. cerevisiae*, the actin cytoskeleton appears to play a major role in inheritance of mitochondria. In filamentous fungi, different data exist as to whether microtubules or microfilaments mediate inheritance of mitochondria. Disruption of microtubules does not affect mitochondrial movement in *Aspergillus nidulans*, whereas it has been suggested that cytoplasmic microtubules are required for transport of mitochondria in *Neurospora crassa*. Here, we describe the cloning and characterization of *Neurospora crassa mmm-1*. The encoded protein shares 30% sequence identity with its yeast homologue, Mmm1p, that was proposed to act as a mitochondrial

receptor for actin binding proteins. An *mmm-1RIP* mutant strain of Neurospora exhibits a temperature-sensitive growth phenotype and harbours mitochondria with an abnormal giant morphology. The MMM-1 protein is imported into the mitochondrial outer membrane in a receptor-dependent manner. We provide evidence that the MMM-1 protein has a single transmembranesegment in the mitochondrial outer membrane with a large C-terminal domain exposed to the cytoplasm. Implications of the mutant phenotype and the toplogy of the protein on its role in mitochondrial inheritance will be discussed.

6. Structure of the vacuolar H+ ATPase from *Neurospora crassa*.

Jack C. Reidling, Emma Jean Bowman, Emilio Margolles-Clark, Karen Tenney, June Pounder, and Barry J. Bowman. Biology, UCSC, Santa Cruz, CA, USA.

The filamentous fungus *Neurospora crassa* contains many small vacuoles. These organelles contain hydrolytic enzymes, high concentrations of polyphosphates, and basic amino acids such as arginine and ornithine. To generate an acidic interior and to drive the transport of small molecules, the vacuolar membranes are densely studded with a proton-pumping ATPase. The ATP-driven proton pump in the vacuolar membrane is a typical V-type ATPase. It is a large and complex enzyme distantly related to the F-type ATPase of bacteria and mitochondria. We have found that about half of the 13 subunits in the vacuolar ATPase are homologs of subunits of F-type ATPases. Several of the subunits appear to be unique to the V-ATPase. An interesting question is whether all V-ATPases have 13 subunits. We have characterized the genes that encode 11 of the Neurospora V-ATPase subunits, and have recently obtained the 12th and 13th. Ten of the Neurospora V-ATPase genes have been mapped and the locations are not clustered. A major goal of our laboratory has been to understand the function and structure of the vacuolar ATPase. One approach we are using is to purify the peripheral V1 complex for structural studies.

7. Neurospora mating pheromone precursor genes.

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The mating type loci of *N. crassa* encode regulators that control expression of genes involved in sexual fertility and development. We have begun to analyze the genes encoding the sex pheromones of *N. crassa*. One gene, expressed in Mat-A strains, encodes a polypeptide containing multiple repeats of a putative pheromone sequence bordered by KEX2 processing sites. The predicted sequence of the pheromone is remarkably similar to those encoded by the rice blast fungus, *Magnaporthe grisea*, and the chestnut blight fungus, *Cryphonectria parasitica*. Mat-a strains express a pheromone precursor gene, whose polypeptide contains a C-terminal CAAX motif predicted to produce a mature pheromone with a C-terminal carboxy-methyl isoprenylated cysteine. The pheromone precursor genes are regulated by nutrients, macroconidiation, and the circadian clock, but display strict mating type-specificity. They are repressed by the RCO1 repressor, but repression by RCO1 is not required to maintain mating type-specificity.

8. Protein synthesis in germinating ascospores of *Neurospora crassa*.

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Dormant ascospores of *Neurospora crassa* exhibit a high level of thermotolerance, and this protection is gradually lost during germination. The goal of this study is to determine at what point during germination the ascospores become capable of synthesizing both normal and heat shock proteins. We determined survival rates of activated ascospores germinated at normal temperature (30 C) and then exposed to a heat shock treatment (45 C) to induce heat shock protein synthesis, a lethal heat treatment (50 C), or a heat shock treatment followed by a lethal heat treatment. These survival experiments showed that 240 min. after activation ascospores become sensitive to lethal heat treatments; they also benefit from receiving a heat shock treatment prior to exposure to the lethal heat treatment. We measured accumulation of the small heat shock protein, Hsp30, a protein that is heat shock-inducible and not constitutively synthesized, by Western analysis. Results showed that cells cannot be induced by heat shock to synthesize Hsp30 until 135 min. after ascospore activation. Further, radiolabelling experiments showed that synthesis of normal proteins as well as heat shock-induced proteins are initially detectable 120 min. after activation. The results of this study show that there is a period of approximately two hours after activation before the ascospores initiate new protein synthesis.

9. Analysis of two novel genes that are highly expressed in starved and sexual tissues of *Neurospora crassa*.

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Two novel and highly expressed genes were identified by the Neurospora Genome Project at UNM (Nelson *et al*., Fungal Genetics and Biology 21:348-363, 1997). These genes are tentatively named *poi-1* and *poi-2* (for plenty of it), since they are the most highly expressed genes in the starved mycelial and perithecial tissues of *Neurospora crassa*. The most abundantly expressed of the two genes, *poi-1*, has no easily identifiable ORF. The mRNA is mostly non-coding with multiple stop codons and no region of good codon bias. *poi-1* mRNA also has an unusual predicted secondary structure. The gene might encode a 10 kDa putative protein as determined by in vitro transcription and translation, and it appears to be essential. An ORF with good codon bias has been identified in the second most abundantly expressed gene, *poi-2*. It encodes a 27 kDa putative protein that contains a possible transmembrane helix and a possible signal peptide. The putative *poi-2* protein also contains a novel 16 tandem repeat of 13-14 amino acid residues. We are currently using repeat-induced point mutation (RIP) to analyze the functions of these two genes and their products.

10. A novel bZip transcription factor expressed during sexual development in *Neurospora crassa*.

Harriett J Bowannie Platero, and Mary Anne Nelson, Department of Biology, University of New Mexico, Albuquerque, NM, USA.

The research goal is to characterize a highly expressed gene, tentatively named *zip-1*, found predominantly in the perithecial stage of *Neurospora crassa*. This gene is of particular interest for two reasons. First, it exhibits a higher level of expression during the perithecial stage than during the vegetative growth stages. Second, similarity search algorithms have identified a region of homology to the transcription factor, c-jun. c-jun plays an important role in activating gene transcription, it is also a proto-oncogene and was the first transcription factor shown to induce cancer. Our hypothesis is that the zip-1 gene of *Neurospora crassa* is a novel transcription factor belonging to the same family of transcription factors as c-jun, the bZip subfamily.

11. Regulation of macroconidiation by fluffy.

Panan Rerngsamran, Lori Bailey-Shrode and Daniel Ebbole. Plant Pathology & Microbiology, Texas A&M University, College Station, TX, USA.

Fluffy (*fl*) encodes a member of the Gal4 class of transcription factors. Null mutations of *fl* block the budding growth characteristic of proconidial chain formation. These mutants are also blocked in expression of many of the known conidiation-specific genes. The pattern of *fl* mRNA expression is consistent with its role as a regulator of morphogenesis. A basal level of *fl* expression is observed in undifferentiated mycelia. *fl* mRNA levels are induced during development at approximately the time when budding growth initiates and *fl* mRNA levels declines at later stages of development. *acon-2* and *acon-3* also are regulators of conidial morphogenesis and *acon-2* is required for induction of *fl* mRNA while *acon-3* is not. Elevated expression of *fl* from a the *cpc-1* or *ars-1* promoter was sufficient to induce conidiophore morphogenesis. One current effort is to isolate the FL protein for DNA binding studies. The location of consensus FL DNA binding sites in the genome may provide clues as to which *N. crassa* genes are subject to developmental regulation.

12. The role of the dgr class of mutants in conidiation.

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The *rco-3* mutant is resistant to growth inhibition by 2-deoxyglucose and L-sorbose. These are the phenotypes of *dgr-1*, *-2*, and *-3* and *rco-3* is allelic to *dgr-3*. The *rco-3* gene was found to negatively regulate conidiation. *rco-3* mutants conidiate in submerged aerated liquid culture without the usual requirement for carbon or nitrogen starvation. The *rco-3* mutant was also found to be required for proper regulation of glucose transport activity and carbon catabolite repression. *rco-3* encodes a member of the sugar transporter superfamily and our characterization of *rco-3* suggests that it functions as a sugar sensor rather than a sugar transporter. As one approach to identifying genes that genetically interact with *rco-3* we have isolated mutants that suppress the sorbose resistance of *rco-3* mutants.

13. Kinesin as a motor for organelle movement in mammalian and fungal cells .

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Kinesin is a molecular motor protein which moves cargo along microtubules in cells. Although kinesin has been implicated in the transport of various membrane-bound organelles and their positioning within the cell, its in vivo function is still unclear. Previous experiments in mammalian cells have shown that the majority of the kinesin protein in cells is inactive and not bound to either membranes or microtubules. This is due to the folded conformation of the protein which allows the C-terminal tail of the protein to directly inhibit the N-terminal motor. Current experiments are aimed at addressing whether fungal kinesins are also auto-inhibited. In Drospohila and *C. elegans*, kinesin is an essential protein whose deletion leads to embyonic lethality. In contrast, deletion of the motor in Neurospora is not lethal but results in severe alterations in cell morphogenesis, notably hyphal extension, morphology and branching (Seiler *et al*., 1997 EMBO J 16:3025-3034). We have begun further analysis of the phenotype of the kinesin null strain, with the aim of identifying conditions which can later be used in a screen for mutants with a similar defect in organelle transport.

14. *htl*, a gene unique to filamentous fungi, encodes a protein involved in hyphal tip growth.

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We have identified a gene, *htl* (hyphal tip lysis), that encodes an abundant 19 kDa protein in mycelia of *Neurospora crassa*. In the current genome databases, homologs to the htl gene are unique to filamentous fungi such as *Aspergillus nidulans*, *Magnaporthe grisea*, *Fusarium veneratum*, and *Botrytis cinerea*. The morphology of RIP strains (in which the gene is inactivated and the HTL protein is deficient) suggests a role in hyphal tip growth. Phenotypic characteristics of htl- strains include swelling and bursting of hyphal tips as well as the gross pigment accumulation ("bleeding") similar to that seen in *N. crassa* osmotic strains. Although the HTL protein is present at sufficiently high levels to detect on Western blots of whole cell extracts probed with polyclonal anti-htl antibody, it is not present in detectable levels in conidia. When isolated by cell fractionation the protein behaves like a particle of high density, but is not associated with the cell wall. We present evidence that HTL forms oligomers that are not dissociated by SDS and mercaptoethanol but can be dissociated by treatment with phosphatase. HTL proteins may form oligomeric complexes that are associated with cytoskeletal components involved in the unique hyphal tip growth of filamentous fungi.

15. Aspects of the membrane skeleton in *Neurospora crassa*.

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Filamentous fungi produce tubular forms called hyphae by means of tip growth. Tip growth involves several coordinated processes that include vesicle transport and fusion, and synthesis and expansion of cell wall and plasma membrane. It has been proposed that the cytoskeleton,

chiefly F-actin and its associated proteins, is important as an integrative scaffold to localize, support, and regulate these tip-growth processes which produce the hyphal form (membrane skeleton model). Our recent work with the ascomycete *Neurospora crassa* supports various aspects of this model. Notably, we have identified a spectrin-like protein in *Neurospora* that is concentrated prominently in hyphal apices and can bind actin in a blot overlay assay. Its distribution and properties indicate the presence of a membrane skeleton in hyphae. A Western blot survey of several fungal species with antibodies to animal spectrin suggests that spectrin-like proteins that may form membrane skeletons may be widespread in fungi. A complementary aspect of this model is the regulated exocytosis of wall vesicles in tip-growing regions. Our work, together with information from the *Neurospora* sequencing projects, has identified several t-SNAREs (highly conserved proteins that are required for intracellular membrane fusion) in *Neurospora* . At least one of these t-SNAREs has been partially characterized. This t-SNARE seems to be delivered to the plasma membrane by the wall vesicles. It occurs in a tip-high gradient on the plasma membrane, but the slope of this gradient does not match the mathematical exocytotic gradient required to produce the hyphal shape. This suggests that other factors, including those that regulate SNARE activity, are involved in the regulation of exocytosis in hyphal tips.

16. Phenotypic and genetic analysis of *ham* (hyphal anastomosis mutant) in *Neurospora crassa*.

Sergio Daniel Haedo and N. Louise Glass. Plant & Microbial Biology, University of California, Berkeley, CA.

Hyphal anastomosis is a fundamental process during both vegetative growth and sexual reproduction in filamentous fungi. Although several genes are probably involved in regulating this complex process, very little is understood about the mechanistic and genetic aspects controlling cell fusion events. A *Neurospora crassa* hyphal anastomosis mutant (*ham*) has been recently isolated that is not able to form heterokayon in the standard period of 24 h at 30C. This mutant shows a slight growth defect and is female-sterile (Wilson and Dempsey 1999. Fungal Genet. Newsl. 46:31). The study of *ham* can help us to unravel the genetic basis underlying in the hyphal anastomosis process in *N. crassa*. We selected ham OR-compatible strains (the original *ham* strain is incompatible with Oak Ridge strains) using a modified heterokayon test. Phenotypic analysis of these strains shown that the frequency of hyphal fusion events is severely reduced in *ham* strains. By plating different numbers of *ham* conidia along with conidia from an OR tester strain in sorbose-containing plates, we observed a reduction of several magnitudes in the ability to form heterokaryon in *ham* compared to several wild type strains. By genetic analysis we mapped *ham* in the right arm of LGI, linked to *arg-13* (2 to 9% recombination). The *ham* locus maps close to a known morphological mutant named *so* (soft) whose phenotype resembles *ham* strains (fuzzy short aerial hyphae and conidia formed more uniformly than the wild type. Moreover *so*, like *ham*, is female sterile). No wild-type progeny was recovered from crosses *so* x *ham* (0/220) and heterokayon tests shown reduced hyphal fusion frequency in *so* strains. We have initiated molecular experiments to clone *ham*. Two approaches will be used: a) chromosome walking from cosmid-libraries around *arg-13* and, b) phenotypic complementation of *ham* morphological phenotype using cosmids from a LGI-specific library.

17. Mutations at *vib-1* reduce hyphal fusion frequency and block *het-c* mediated vegetative incompatibility in *Neurospora crassa*.

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Vegetative incompatibility triggered by allelic differences in *het-c* locus of *Neurospora crassa* is characterized by growth inhibition and arrest, suppression of conidiation, hyphal compartmentation and death. To genetically dissect the pathway of *het-c* vegetative incompatibility, we allowed incompatible transformants to escape from growth inhibition and suppression of conidiation. Several mutations that blocked *het-c* mediated vegetative incompatibility were identified. A mutation at the *vib-1* (*v*egetative *i*ncompatibility *b*locked) locus greatly reduces heterokaryon formation efficiency. High conidial concentrations are needed to force heterokaryons between a *vib-1* mutant and a wild-type strain. Heterokaryons were not obtained between two *vib-1* strains when conidia were used to force the heterokaryons. However, heterokaryons between two *vib-1* strains could be formed by either of spheroplast fusion or forcing mycelial heterokaryons. Reduced frequency of hyphal fusion contributes to the low heterokaryon formation efficiency. Hyphal fusion events have not been detected in leading hyphae of a homokaryotic *vib-1* colony. The effect of *vib-1* mutation on *het-c* vegetative incompatibility has been tested by four ways: spheroplast fusion, mycelial heterokaryons, transformation and partial diploids. All data indicated that *vib-1* fully blocks *het-c* vegetative incompatibility. The *vib-1* mutation is recessive and causes multiple developmental defects as well. A *vib-1* mutant has a reduced mycelial growth rate, reduced aerial hyphae differentiation and is fully sterile as female, but partially sterile as male. We also have identified two other independent mutants that suppress *het-c* vegetative incompatibility. We are testing whether these mutants contain mutations that are allelic to *vib-1*.

18. Characterization of a G-beta Subunit from *Neurospora crassa*.

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Heterotrimeric G protein-mediated signal transduction is one of many pathways used by cells to respond to environmental stimuli. Experimental evidence has demonstrated that G protein beta-gamma dimers play pivotal roles in transduction of extracellular signals. Low-stringency hybridization using degenerate oligonucleotides based on conserved regions of mammalian G-beta proteins as probes resulted in identification of the *Neurospora crassa* G-beta gene, *gnb-1*. The predicted amino acid sequence of *gnb-1* is most identical to *C. parasitica* CPGB-1 (91%), followed by human G-beta subunits (~49-67% identity) and *D. discoideum* GPB-1 (66% identical). Results from restriction fragment-length polymorphism (RFLP) mapping demonstrate that the *gnb-1* gene is located on the right arm of chromosome III near the *con-7* and *trp-1* genes. Northern analysis revealed two *gnb-1* specific transcripts of 2.7 and 1.4 kb. *gnb-1* null strains have numerous defects, including shorter germ tubes, aberrant vacuole number and size during growth in submerged culture, reduced mass accumulation and female sterility. Loss of *gnb-1* also impacts levels of the G-alpha subunits GNA-1 and GNA-2 in *N. crassa*.

19. Isolation of components involved in mating-type associated heterokaryon incompatibility using the yeast 2-hybrid system.

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The mating-type locus (mat) in *Neurospora crassa* controls mating and sexual development. The fusion of reproductive structures of opposite mating-type, A and a, is required to initiate sexual reproduction. During vegetative phase, the mat locus also functions as a one of the 11 heterokaryon incompatibility (*het*) loci. Heterokaryon incompatibility is a mechanism that prevents the formation of vigorous heterokaryons between genetically dissimilar individuals and is believed to be a ubiquitous phenomenon among ascomycetes and basidiomycetes. The fusion of *mat A* and *mat a* hyphae during vegetative growth results in growth inhibition, hyphal compartmentation and death. Vegetative incompatibility between opposite mating-types is due to the molecular actions of MAT A-1 and MAT a-1. The incompatibility reaction between MAT A-1 and MAT a-1 is mediated by TOL - mutations in tol are recessive and suppress mating-type associated heterokaryon incompatibility. The MAT A-1 and MAT a-1 are transcriptional regulators while the molecular function of TOL is not apparent although it contains protein-protein interaction domains. To understand the role of TOL in mating-type heterokaryon incompatibility and to identify other proteins involved in the process, we have isolated several TOL-interacting proteins (tip) using the yeast 2-hybrid system. The six potential tip genes have been identified and encode a regulator for acriflavine drug-resistance, a *Schizosaccharomyces pombe* VIP-1 homolog (a p53 related protein) and other novel proteins. RIP mutational analyses of the tip genes are undergoing and the phenotype of the mutants will reveal their functions, as well as their roles in mediating mating-type associated heterokaryon incompatibility.

20. G gamma subunit identified in *Neurospora crassa*.

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Heterotrimeric guanine nucleotide-binding proteins (G proteins) consisting of alpha, beta and gamma subunits mediate signalling between cell surface receptors and intracellular effectors in eucaryotic cells. Upon agonist binding to the receptor, the G protein alpha subunit releases GDP, binds GTP, and dissociates from the G protein beta gamma subunit dimer. Depending on the system, either G alpha or G beta gamma go on to activate downstream effectors. Three G alpha subunit genes, *gna-1*, *gna-2* and *gna-3*, and one G beta subunit gene, *gnb-1*, have been identified in *Neurospora crassa*. A G gamma subunit gene, *gng-1*, has been identified during searches of the University of Oklahoma EST database. The full length of *gng-1* cDNA (273 bp) has been found in the cDNA clone. The predicted amino acid sequence of GNG-1 shares 35% identity with the *Saccharomyces cerevisiae* STE18 and 30-33% identity with other G gamma subunits identified in mammalian cells. A *gng-1* gene clone was isolated from lambda-BARGEM7 genomic library using the *gng-1* cDNA clone as a probe. Our current focus is making *gng-1* mutants, and to analyze the phenotypes of them.

21. GNA-3, a G protein alpha subunit, regulates the expression of adenylyl cyclase in *Neuropora crassa*.

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In eukaryotic systems, cellular responses to signals, such as odorants and light, are mediated by heterotrimeric G proteins coupled to seven transmembrane receptors. Our lab identified two G alpha subunits, *gna-1* and *gna-2*, and demonstrated GNA-1 directly regulates adenylyl cyclase. A third G alpha, *gna-3*, was cloned in *Neurospora crassa* and shown to be a positive and negative regulator of aerial hyphae formation and conidiation, respectively. Addition of cAMP suppresses both the aerial hyphae and conidiation defects in standing liquid cultures, but only suppresses the conidiation defect on plates. *N. crassa* conidiaiton requires an air/water interface; however, delta *gna-3* strains conidiate abundantly in submerged culture. Analysis of the glucose-repressible gene, *qa-2*, suggests delta *gna-3* submerged culture conidiation is due to glucose starvation. Deletion of *gna-3* results in significantly decreased intracellular cAMP and adenylyl cyclase activity. Western analysis indicates the reduced delta *gna-3* cAMP is due to decreased levels of *N. crassa* adenylyl cyclase enzyme, NAC. To explore the role of GNA-1 and GNA-3 in regulating adenylyl cyclase, a double *gna-1* and *gna-3* mutant was constructed by a sexual cross. The resulting delta *gna-1* delta *gna-3* strain displays phenotypic morphology similar to an adenylyl cyclase mutant, *cr-1*. Biochemical results suggest that GNA-3 modulates intracellular cAMP levels by regulating the expression of adenylyl cyclase to facilitate *N. crassa* development.

22. pH sensing in *Neurospora crassa*.

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Although pH regulation in filamentous fungi has been implicated in the selective de-repression of many genes as a function of ambient pH, which is mediated by the transcriptional activation protein PacC, gene *pho*-2 is expressed irrespective of the growth pH when *N. crassa* is grown in low-Pi. This is because the same amount of *pho*-2 encoded alkaline phosphatase is secreted irrespective of ambient pH. Also, the loss of activity observed for the alkaline phosphatase secreted at pH 5.4 is an effect probably due to its low glycosylation as compared to the glycosylation observed for the enzyme secreted at alkaline pH. Thus, we could argue the functioning (or even the existence) of an *A. nidulans pac*C homologue in *N. crassa*. However, we cloned an *N. crassa pac*C homologue by using degenerate oligonucleotides to amplify genomic fragments by PCR (~93% identity to the *A. nidulans* PacC protein) and to generate probes to screen a genomic library. One of the recovered sub-clones of 6 Kb (~40% sequenced) complemented the *pac*C14 strain of *A. nidulans*, including the remediation of both the Pi-repressible acid and alkaline phosphatases secreted at pH 5.0. Thus, *pac*C14 behaved as a loss-of-function mutation in a clear indication that *N. crassa* and *A. nidulans* have similar adaptive responses to ambient pH.

Financial support: FAPESP, CNPq, CAPES

23. Neurospora in nature: Exploring the natural history of a traditional laboratory model.

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Although thousands of Neurospora isolates exist from worldwide collections, fundamental questions remain about the natural history of Neurospora. Clearly, species of Neurospora have evolved to respond to cyclical fires, because they appear profusely on vegetation that has been killed by recent fire, and ascospores are induced to germinate by either heat or chemical byproducts of burnt vegetation. However, important questions remain only partially answered: To what extent are colonization and dispersal dependent on conidia versus ascospores? Do different conidial eruptions on a single stem represent clonal propagation of a single genetic individual or multiple ascospore-derived individuals? How do sympatric species partition the environment? We are addressing these questions with isolates acquired after Florida Everglades fires in the spring, 1999. This collection includes multiple isolates from single plants and plants across transects. Our results confirm that individual stems of sugar cane and woody shrubs can be colonized by multiple species of Neurospora. Of 35 isolates examined from three stems, three (9%) were *Neurospora crassa*, and 24 (69%) were self-fertile *Neurospora tetrasperma* individuals. The remaining eight (22%) are still being identified. The conidial tufts representing different species were indistinguishable in the field and in some cases were only centimeters apart. Within- and between-species variation among isolates is currently being assessed by sequence analysis of specific genes.

24. Mutants of Neurospora altered in the adaptation to blue light.

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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: the mycelium adapts to light and a period of darkness is required before *con-10* can be photoactivated again. To investigate the molecular nature of light adaptation in Neurospora, we have designed a protocol that should allow us to isolate mutants altered in the adaptation of *con-10* photoactivation. A strain of Neurospora with a fusion of the *con-10* promoter to the gene conferring resistance to hygromycin is available. 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 introduced into the genome of this strain a fusion of the *con-10* promoter to the lacZ gene. The presence of this reporter fusion allows us to screen hygromycin resistant mutants for lacZ expression, which should reveal whether a mutation conferring resistance acts in cis or in trans. Using this strain we have isolated three mutants that grow in the presence of hygromycin under continuous light but not in the dark. Presumably this is due to a defect in the mechanism controlling light adaptation. One of these mutants has been investigated in some detail. This

strain exhibits high expression of the con-10::lacZ fusion, suggesting that the mutation affects a trans-acting protein that is required for the photoadaptation of *con-10*.

25. Light sensitivity of the circadian clock and regulation of a clock controlled gene.

Minou Nowrousian, Jay C. Dunlap, and Jennifer J. Loros. Genetics and Biochemistry, Dartmouth Medical School, Hanover, NH, USA.

The *Neurospora crassa* circadian clock controls many aspects of growth and development, including the timing of the initiation of conidiogenesis. A core feedback loop of the clock consists of an autoregulatory cycle, central components of which are the *frq*, *wc-1*, and *wc-2* gene products. The clock is able to react to environmental factors such as light and temperature. Continuous light represses clock function by supressing the cycling of *frq* mRNA and FRQ protein. The *lis* (*light insensitive*) mutants express the circadian rhythm of conidiation under dim light conidtions where wild type strains conidiate continuously. FRQ protein levels are low in the *lis-2* mutant in dim light whereas they are induced to high levels in the wild type in response to constant light. Thus, it may be that the circadian oscillator is altered in some manner in the mutant or, alternatively, perception of light levels may be changed. The molecular clock acts by controlling the expression of many genes (*ccgs*, clock controlled genes, Loros *et al*., Science 243: 385-388) involved in different aspects of the physiology of the fungus. Several genes have been shown to be controlled by the clock at the level of transcription, among them *ccg-1* (Loros and Dunlap, Mol Cell Biol 11: 558-563). Besides clock control, *ccg-1* displays other levels of regulation, including light, development, and heat shock. Deletion analysis of the *ccg-1* promoter sequences suggests that the clock regulatory elements lie near the start site of transcription and are distinct from sequences conferring developmental regulation and glucose repression.

26. Biochemical interactions between FRQ and WC-2: critical clock proteins required for the normal operation of the Neurospora circadian oscillator.

Deanna L Denault, Jay L. Dunlap, and Jennifer J. Loros. Genetics and Biochemistry, Dartmouth Medical School, Hanover, NH,USA.

The White Collar proteins (WC) are PAS domain containing proteins required for blue-light perception and essential for normal operation of the circadian clock in *Neurospora crassa*. Specifically, both WC-1 and WC-2 are necessary for rhythmic expression of a central clock component, *frequency (frq)*. Although it is well established that the protein product FRQ negatively regulates it's own expression and that the expression of the *frq* gene requires WC-2, the molecular mechanisms governing both the activation and the feedback loop of *frq* are not well understood. We have shown that WC-2 is nuclear, consistent with its anticipated role as a transcriptional activator, and does not display a robust rhythm in either nuclear or cytoplasmic content. Current experiments set out to begin to determine the nature of the mechanism of WC-2 regulation of *frq* transcription. In independent experiments, it was found that WC-2 interacts directly with FRQ. *In vitro*, GST-tagged WC-2 specifically interacts with radiolabeled FRQ proteins. More importantly, *in vivo*, FRQ specifically co-immunoprecipitates with both WC-1 and WC-2 from Neurospora extracts. This demonstrates direct or indirect association between FRQ and the WC proteins. Thus, WC-2 may regulate rhythmic transcription of *frq* through

protein-protein associations that affect transcriptional activation. Further characterization of this interaction may provide insight to the molecular events underlying the basis of the circadian clock in Neurospora.

27. Mutant screens to identify circadian clock components in *Neurospora crassa*.

Irene J. March, Victor V. Keasler, Kathyrn J. Wessels, and Deborah Bell-Pedersen. Biology, Texas A&M University, College Station, Texas, USA.

Circadian rhythms are intrinsic daily fluctuations in physiological, behavioral and biochemical activities that have been identified in organisms as diverse as cyanobacteria, plants, and mammals. In *Neurospora crassa* , conidiation is under control of the circadian clock. Previous genetic screens using mutant strains on race tubes have identified molecular components of the circadian clock. However, these screens were not saturating. Furthermore, no screens for mutations involved in the resetting of the clock have been carried out. In order to identify additional molecular components required for circadian rhythmicity and for resetting of the circadian clock, three genetic screens are being employed. In the first screen, UV mutagenized cultures of *N. crassa* are grown on race tubes and arrhythmic cultures are tested further. In the second screen, UV mutagenized cultures are grown on race tubes and given a light pulse 48 hours after they have been in the dark. In wild type strains, this results in a delay of the next cycle. Mutants that are arrhythmic, have altered periods, or that do not respond to the light pulse are candidates for further study. In the third screen, an amino acid permease is placed under control of the clock-controlled gene *ccg-2*, and mutants are selected based on their growth property, which is dependent on the circadian clock. Currently, 10,000 mutagenized strains have been tested in the first screen. From this, 17 arrhythmic mutants have been identified. In the second screen, 1,500 mutant strains have been tested, 4 arrhythmic strains and 1 possible light resetting mutant has been identified.

28. Identification of factors which regulate circadian rhythmicity of the clock-controlled *eas*(*ccg-2*) gene in *Neurospora crassa*.

Louis W. Morgan, Zachary Lewis, and Deborah Bell-Pedersen. Biology, Texas A&M, College Station, TX, USA.

Circadian rhythms are biochemical or physiological rhythms that persist in constant environmental conditions with a period close to 24 h. Viewed simply, the circadian clock is comprised of at least three components; a central oscillator, input pathways to and output pathways from the oscillator. The central oscillator regulates the expression of genes within the output pathways in a time-of-day-specific manner. However, the mechanisms and components responsible for the regulation of clock-controlled genes is poorly understood. We have identified a 68 bp promoter sequence, termed the ACE element, that is both necessary and sufficient for circadian expression of the clock-controlled *eas* (*ccg-2*) gene. Using gel mobility shift assays, we have identified protein factors that specifically bind to this sequence. Consistent with the factors being involved in clock-regulation of *eas*(*ccg-2*), binding to ACE occurs with a circadian period. Currently, we are purifying the factors and, once isolated, we will determine their role in circadian output pathways.

29. *vvd* encodes a novel PAS protein involved in light perception for the *Neurospora* circadian clock.

Christian Heintzen, Jay C. Dunlap and Jennifer J. Loros. Biochemistry, Dartmouth Medical School, Hanover, NH, USA.

The *vivid* (*vvd*) locus has been characterized by mutations affecting light-induced pigmentation in *Neurospora* . We have cloned *vvd* and find that it is a new member of the PAS protein superfamily, the sequence of the VVD protein having strong homologies to the LOV domain of the *Neurospora* clock gene *white collar-1* (*wc-1*). This gene is mutated in two *Neurospora* mutant alleles of *vvd* confirming that it encodes for the VVD protein. Physiological and molecular data indicate that VVD is involved in the light adaptation process of *Neurospora crassa* , which ensures a transient but not constitutive response of light regulated processes. The *vvd* gene itself is strongly and rapidly light induced. Moreover, it negatively autoregulates its own steady state levels. This indicates that VVD is an early repressor of light regulated processes. *vvd , band* (*bd*) double mutants have a slightly longer free running period (23.5 h) than the clock wt, *bd* strains, and show differences in their phase response to light suggesting that - among other functions - *vvd* is important for the resetting mechanism of the *Neurospora* circadian clock.

30. Genetic mapping of the *un-25* locus and ordering of the *aro-6* and *cpl-1* loci on linkage group VI.

Thomas J. Schmidhauser. Biology, U. of Louisiana, Lafayette, LA USA.

Complementation analysis has identified the locus *un(T51M54)* as a new un locus designated *un-25*. This temperature sensitive mutation has been shown to be tightly linked to the *lys-5* locus; 0/44 recombination (Perkins *et al*., (1973, Neurospora Newsl. 20:45-49). Our results place *un-25* between the *cpl-1* and *ylo-1* loci consistent with the tight linkage to the *lys-5* locus. In addition we establish the order of the *aro-6* and *cpl-1* loci on the left arm of linkage group VI.

31. A chromosme walk on chromosome IV encompassing *arg-2* and *trp-4*.

Sabine Mohr, John M. Stryker, and Alan M. Lambowitz. ICMB, University of Texas, Austin, Texas, USA.

The Neurospora mutant *cyt-19-1* is deficient in mitochondrial splicing. The nuclear gene maps to chromosome IV. Three-factor crosses and crosses with translocation strains determined the order *T(S4342)*L,*arg-14, cyt-19, T(NM152)*L, *pyr-3,his-5*. We are presenting a chromosome walk between *arg-2* and *trp-4* with the position and partial sequence of several novel genes. A *cyt-19* candidate gene has been found close to the *arg-14* gene and is currently under investigation.

32. *De novo* cytosine methylation associated with recognition of A:T base pairs in vegetative cells of *Neurospora crassa*.

Hisashi Tamaru and Eric U. Selker. Molecular Biology, University of Oregon, Eugene, OR, USA.

Most cytosine residues in the genome of *N. crassa* that are subject to *de novo* methylation are in sequences that have undergone RIP and are therefore relatively A:T-rich and enriched for TpA, the most common dinucleotide resulting from RIP. To understand how DNA sequences signal *de novo* methylation we conducted systematic tests of the capacity of various synthetic oligonucleotides to trigger methylation in controlled sequence contexts at the *his-3* locus. We show that various sequences consisting only of A and T can efficiently trigger methylation of nearby Cs, but to different extents. Both A and T are required on the same strand to induce significant methylation. Interestingly, both TpA and ApT can promote *de novo* methylation, but neither is essential. Moreover, the number of TpAs and/or ApTs in test fragments did not perfectly correlate with the levels of methylation induced. G:C pairs inhibit *de novo* methylation but to different extents depending on their position relative to TpAs and ApTs. Methylation is promoted by increases in the length of signal sequences. Using gel mobility shift assays we have identified DNA binding activities in cell extracts of both wild-type and methylation-defective strains that bind methylation signals. These results support the idea that a protein other than the DNA methyltransferase recognizes a feature of methylation signals and initiates the methylation process.

33. Purification of *Neurospora crassa* proteins that bind methylated DNA and DNA mutated by RIP.

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DNA methylation has been implicated in a diverse array of phenomena in eukaryotes such as genomic imprinting, X chromosome inactivation, and genome surveillance but a detailed understanding of the mechanism and function of methylation remains elusive. As one approach to better understand the mechanics and biological function of methylation we have chosen to isolate and characterize factors that bind to methylated DNA. Using gel-mobility-shift assays we have detected two factors in *Neurospora crassa* that bind to methylated DNA. A high-mobility factor was identified that is specific for methylated DNA. We refer to this factor as M-BP1 (Methyl Binding Protein 1). A low-mobility factor was identified that binds methylated DNA or DNA mutated by RIP. This factor binds most efficiently to DNA that is both methylated and contains mutations from RIP. We refer to this factor as M/R-BP1 (Methyl/RIP Binding Protein 1). M/R-BP1 may be involved in establishing methylation patterns in Neurospora. Both M/R-BP1 and M-BP1 may be involved in maintenance of methylation patterns as well as in exerting the effects of methylation. To test these possibilities we are purifying M/R-BP1 and M-BP1, characterizing their properties, and cloning the genes that encode them. After six chromatographic steps three polypeptides can be identified in silver-stained SDS-PAGE gels that correlate with M/R-BP1 activity.

34. Disruption of the *Neurospora crassa* histone 1 gene, *hH1*.

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The linker histone, H1, has long been thought to be an essential component of condensed chromatin and a general negative regulator of gene expression. Recent experiments in Xenopus (1), Tetrahymena (2), *Saccharomyces cerevisiae* (3), *Aspergillus nidulans* (4) and *Ascobolus immersus* (5) suggest that H1 has more specific roles. H1 appears to be nonessential in most of these organisms. In *A. immersus*, however, silencing of the H1 gene resulted in reduced lifespan as well as increased DNA methylation and increased sensitivity of the chromatin to micrococcal nuclease (MNase). To test the generality of these results, we cloned and inactivated the H1 gene of *Neurospora crassa*. A sequence for a Neurospora H1 homologue in an EST database (6) was used to isolate the full gene, which was then sequenced. Conceptual translation revealed a protein with *bona fide* H1 structure, i.e. highly basic N- and C-terminal tails flanking a core globular domain. A genomic fragment that contained the H1 gene, designated *hH1*, was targeted to the *his-3* locus and this strain was used to disrupt the gene by RIP. Mutants with no detectable H1 were found, suggesting that there is only one H1 gene and that this histone is not essential in Neurospora. Mutants grew and developed normally at 10, 32 and 39C on minimal medium. No obvious changes in global DNA methylation or methylation at specific chromosomal sites was observed. Chromatin from the mutant showed normal sensitivity to digestion with MNase. The absence of an obvious phenotype in H1 mutants of Neurospora is consistent with findings with *A. nidulans* (4) and *S. cerevisiae* (3) but contrasts the findings with Ascobolus. (1) Bouvet, P. et al. (1994) Genes Dev., 8:1147-1159. (2) Shen, X. *et al*. (1995) Cell, 82:47-56. (3) Escher, D. and W. Schaffner (1997) Mol. Gen. Genet., 256:456-461. (4) Ramon, A. *et al*. (2000) Mol. Microbiol., 35:223-233. (5) Barra, J. *et al*. (2000) Mol. Cell. Biol., 20:61-69. (6) http://www.genome.ou.edu/fungal.html.

35. Processing of the *arg-6* polyprotein.

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The complex *arg-6* locus in *Neurospora crassa* encodes a polyprotein precursor for two early mitochondrial arginine biosynthetic enzymes, acetylglutamate kinase (AGK) and acetylglutamyl phosphate reductase (AGPR). This polyprotein is processed into two mature proteins as it is translocated into the mitochondria. Processing involves cleavage of the polyprotein at three different sites. Cleavage of the first site, upstream of the proximal AGK, removes the mitochondrial targeting sequence. The two other sites are upstream of the distal AGPR, and cleavage removes a 20 amino acid region connecting both enzymes. The presence of a multi-protein coding gene is rare in eukaryotes; however, *arg-6* homologues have persisted in the polyprotein form in *N. crassa* and two other fungi. This work investigates the reasons behind the presence of this polyprotein. This is done by introducing mutant constructs that either contain a separate AGK and AGPR genes, or an *arg-6* gene that lacks the processing signal into a strain that contains a null *arg-6* mutation. Each set of transformants is then evaluated for the production of the recombinant protein(s) and the ability to synthesize arginine.

36. Feedback inhibition of arginine biosynthesis.

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Arginine biosynthesis is regulated primarily by feedback inhibition of acetylglutamate synthase (AGS) and acetylglutamate kinase (AGK). Previous genetic studies suggested a coordinate mechanism of inhibition mediated by interaction between AGS and AGK. Mutations in the gene for AGK (*arg-6*) have been shown to affect not only the activity and feedback sensitivity of AGK, but also of AGS. Direct interaction between these two enzymes has been shown in a yeast two hybrid system. We have used yeast two hybrid analysis to define the regions of interaction, and the effects of different mutations. The interaction domain of AGK maps to a unique C-terminal region found only in eukaryotic organisms. A mutation rendering AGK and AGS feedback insensitive was mapped to the N-terminal portion of AGK. Fusion proteins have also been made for the purification of the two enzymes, in order to further investigate the mechanism of feedback inhibition.

37. Interacting domains of Hsp30 of *Neurospora crassa*.

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Hsp30 of *Neurospora crassa* belongs to the class of alpha-crystallin-related small hsps, which share 90 amino acids of conserved sequence. These proteins have similar hydropathy profiles, and they self-assemble into multimeric particles, typically of 24 subunits. Crystallography suggests that a dimer is the core structural unit. We are characterizing the self-interactions of Hsp30 that contribute to its higher order structure, employing the *S. cerevisiae* two-hybrid system to report protein interactions in vivo. We measured the reporter beta-galactosidase activity by a sensitive luminescence assay. Monomers of Hsp30, co-expressed in transformed yeast cells, clearly interacted with one another, as expected for proteins that dimerize. We assayed portions of Hsp30 for interaction, dividing Hsp30 into an N-terminal (residues 1-134) and a C-terminal region (residues 129-228), both containing conserved sequence. The N-terminal half of Hsp30 interacted very strongly with the C-terminal half; this interaction is consistent with dimerization contacts within the small hsp crystal structure. We are continuing to define interacting domains within the N- and C-terminal halves of Hsp30 and have found that the conserved part of the C-terminal half is responsible for interaction with the N-terminal half. A truncated derivative of the N-terminal half, which contains the dimerization contact site of the crystal structure, retained binding to the C-terminal half.

38. Glyoxylase I of Neurospora is a stress-responsive enzyme.

Sanjeev Kalia and Manju Kapoor. Biological Sciences, University of Calgary, Calgary, Alberta, Canada.

Glyoxalase I (EC 4.4.1.5) catalyses the transformation of methylglyoxal and glutathione into S-lactoylglutathione. It is a highly conserved, ubiquitous protein, belonging to the superfamily of Zn-dependent hydrolases. It is a marker enzyme for cell division in eukaryotes and a potential therapeutic target for cancer and diabetes. Due to its ability to eliminate methylglyoxal, a

cytotoxic byproduct of glycolysis, from the system, it is postulated to be a defense related enzyme. To determine whether glyoxalase I is a stress-responsive enzyme, 14-h-old mycelial cultures of wild-type *N. crassa* and an albino strain were exposed to various types of stress: heat shock, nutrient depletion, oxidative stress, toxic metal ions. These treatments increased the specific activity of this enzyme up to 3-fold, relative to untreated controls. In addition, elevated enzyme activity was also apparent during early stages of conidial germination and during formation/maturation of perithecia. These studies suggest that glyoxalase I of *Neurospora* is a stress-inducible and developmentally regulated enzyme.

39. Mapping and osmotic sensitivity of the mutants *os-9* (allele SS-788 and allele SS-462) and SS-18.

Wayne A. Krissinger and Sara Neville Bennett. Biology, Georgia Southern University, Statesboro, GA, USA.

The osmotic-sensitive (os) mutants of *Neurospora crassa* fail to grow on medium containing elevated concentrations of NaCl. The first of these mutants (*os-1* through *os-5*) were mapped to LG I and LG IV and could also be scored on the basis of their non-wild type morphology. In our laboratory, three osmotic-sensitive mutants, alleles SS-788, SS-462, and SS-18 were isolated following UV irradiation. The gross morphology of these mutants is like that of wild type. SS-788 has been designated *os-9* and was mapped to LG VI between ad-1 and *trp-2*. Additional crosses placed *os-9* distal to *del* SS-462 is an allele of *os-9* as shown by the failure to recover wild type recombinants from a cross between the two mutant strains. SS-18 is in LG III linked by 36% recombination to *vel* and by 19% recombination to *os-8* which was also isolated in our laboratory and is located between *ad-2* and *trp-1*. We suggest that SS-18 be designated *os-11*. SS-788, SS-462, and SS-18, all grew as wild type 74 on media containing elevated KCl or glucose and failed to grow only on medium containing elevated NaCl. This is in contrast to the osmotic-sensitive mutant, *os-1*, which failed to grow on any of the three tested osmotica. These mutants may be useful in studies of transport of ions.

40. Expression of heat shock protein 70 (Hsp70) of Neurospora in *E. coli*.

Carol A. Curle and Manju Kapoor. Biological Sciences, University of Calgary, Calgary, Alberta, Canada.

RT-PCR was used to generate two overlapping DNA fragments of the hsp70 gene, from total RNA, isolated from heat-shocked mycelium. These fragments (1.21 kb and 1.07 kb) were then spliced by overlap extension to yield a product ~2kb in size, representing the entire coding sequence of the hsp70 gene, without introns. The resulting fragment was inserted into the Pin Point Xa-3 (Promega) plasmid to produce a biotinylated fusion protein in *E. coli*. Following induction with IPTG, a biotinylated p

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with specific antibodies raised against *N. crassa* Hsp70, was produced. This protein was affinity purified using the Soft-Link Soft-Release avidin resin. The yield of soluble protein was low, as

most of the fusion protein accumulated in an insoluble form. The coding region of the hsp70 gene was also expressed as a poly-His-containing recombinant protein. A product of the correct size that cross-reacted with anti-Hsp70 IgG was obtained, but it was not over-expressed. A higher level of expression was achieved with the N- and C-terminal domains of the Hsp70 polypeptide.

41. Molecular cloning and regulatory analysis of the cystathionine beta- and gamma-lyase genes of *Neurospora crassa.*

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The sulfur regulatory system of *Neurospora crassa* is composed of a set of structural genes involved in sulfur metabolism controlled by the CYS3 transcriptional activator and SCON (sulfur controller) negative regulators. We report here on the cloning and regulation of two additional genes under control of the system. Cystathionine beta-lyase coverts cystathionine into homocysteine, while cystathionine gamma-lyase converts cystathionine into cysteine. Northern blot analysis using wild type cells showed that the lyase transcripts were abundant under low sulfur (derepressing) conditions and at a low level under high sulfur (repressing) conditions. Northern analysis with a *cys-3* deletion mutant showed low levels of the two lyase transcripts under either derepressing or repressing conditions. A negative regulatory mutant, *scon-2* displayed constitutive levels of the lyase mRNAs. Finally, homology comparisions to other lyases will be described.

42. Fungal cell wall production and utilization as a raw resource for textiles.

William J. Dschida. MAD BIO Research Institute, Blue Lake, CA, USA.

The present invention reveals a product from fungi and a method for making the product. The method involves producing spores from a filamentous fungus, producing mycelia from the spores, growing the mycelia into a flat sheet, and recovering the fungus product. The fungus product is used as a raw resource for the production of textiles. (United States Patent 5,854,056)

43. Characterization of the *paba-2* gene from *Neurospora crassa*.

Jason Edwards, Malcolm Robb and John P. Vierula. Biology, Carleton University, Ottawa, Ontario, CANADA.

Para-aminobenzoic acid (PABA) is a precursor to tetrahydrofolate, an essential vitamin for nucleic acid biosynthesis in microorganisms. Since humans ingest folates, this pathway has been an attractive antibiotic target in bacterial and more recently, some fungal infections. PABA biosynthesis is not well characterized in fungi, but it is believed to be similar to the scheme for bacteria. To aid in drug design, we have initiated a molecular genetic study of the PABA pathway in *Neurospora crassa*. The *pab-2*$^{+}$ gene was cloned by complementation and cDNA clones were obtained by hybridization screening of the NO_3^- induced cDNA library and PCR amplification from various cDNA pools. The mutant allele of *pab-2* was cloned via PCR.

Sequencing of several *pab-2*$^{+}$ cDNA clones revealed that the single intron is alternatively spliced. A common 5' splice junction and alternative 3' splice junctions give rise to C-terminal variant ORFs. The major transcript encodes a 276 amino acid ORF and the minor transcript, a 254 amino acid ORF. The *pab-2* mutation is a frameshift/transition which results in a truncated, 208 residue protein.

44. Analysis of the *Neurospora crassa* opsin, NOP-1 and the opsin-related protein, ORP-1.

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Opsins are seven-transmembrane helical apoproteins that form light absorbing pigments upon binding retinal. Genes encoding opsins had only been identified in animals and the archaea until the discovery of an opsin gene, *nop-1* in the filamentous fungus *Neurospora crassa*. The NOP-1 protein sequence is 81.1% identical to archaeal opsins in the retinal-binding pocket. Previously, we have shown that heterogously expressed NOP-1 in *P. pastoris* membranes could bind all-trans retinal ($lambda_{max}$ = 534nm), and undergo a photochemical reaction cycle similar to archaeal rhodopsins. Also, we found the *nop-1* gene expression during *N. crassa* development is highest under conditions that favor conidiation and is also positively influenced by the presence of light. A role for NOP-1 in conidiation was demonstrated by the light-dependent conidiation phenotype of delta *nop-1* strains in the presence of the mictochondrial H^{+}-ATPase inhibitor oligomycin. We have recently determined that delta *nop-1* strains exhibit temperature-sensitive defects in the presence of light. Together, the phenotypic results suggests NOP-1 may have a light-dependent function in response to stress. Evolutionary analysis reveals clear similarity between NOP-1, archaeal rhodopsins, and another group of fungal proteins we have termed Opsin-Related Proteins (ORPs). ORPs are also predicted seven-transmembrane proteins, but they lack the conserved lysine residue required for retinal binding in opsins. Recently, we identified an EST encoding a *N. crassa* ORP (*orp-1*) in the University of Oklahoma Genome database. The putative ORP-1 protein sequence is most similar to HSP30 from *Coriolus versicolor*. Therefore, opsin-related proteins and opsins may be important for stress tolerance in *N. crassa*.

45. The *Neurospora crassa mus-11* gene is a homologue of the *S. cerevisiae RAD52* gene.

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Mutagen-sensitive mutants with phenotypes resembling mutants defective in recombination repair fall into two epistasis groups in the filamentous fungus, *Neurospora crassa*. In the Uvs-6 group, the *mei-3* gene has been shown to be a homologue of the *E. coli Rec A* and *S. cerevisiae RAD51* genes, while the *uvs-6* gene is a homologue of the *S.cerevisiae RAD50* gene. Using complementation of MMS sensitivity, we have used sib selection with the Orbach/Sachs *N. crassa* cosmid library to clone *mus-11* from the other (Uvs-3) recombination repair-like *N. crassa* epistasis group. The *mus-11* gene is contained in a 3128 bp EcoRI fragment from cosmid X19:8E. Sequencing of the fragment shows that this DNA has a strong similarity to the coding regions of the *RAD52* gene of *S. cerevisiae* and its *Schizosaccharomyces pombe* homologue,

rad22. The gene has two introns: an 88bp intron 9bp after the apparent start codon and a second intron of 52bp at 663bp from the start codon. The area from -127 bp to -98bp preceeding the start codon has an AT rich sequence of AAATATTTTTTTGAAAAAAGAAAAAAAAA. The 3' end of the genomic clone ends at an EcoRI site 2077bp from the presumed start codon. It is within the transcribed region, as a cDNA isolated by degenerate PCR from a UV induced *N. crassa* cDNA library has the same end. Fine mapping of the *mus-11* gene places it on the right arm of LGV in the order: *ade-7* - 9.5mu - *pab-2* - 7.3mu - *mus-11* - 3.7 - *inv*. A second gene in this group, *uvs-3*, has now been located on cosmid X18:7E of the Orbach/Sachs library.

46. Cloning and characterization of a RecQ homologue in *Neurospora crassa*.

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We cloned a *Neurospora crassa* homologue of RecQ using PCR method. The amino acid sequence of the homologue showed homology to that of other RecQ members within the helicase domain. We characterized a RecQ mutant which was made by RIP. This mutant was sensitive to MMS, MNNG and TBHP in spot test. These are the same characteristics shown by *mei-3* and *mus-23*, which are mutant in genes affecting recombination repair. The mutant of *Neurospora* RecQ-homologue gene was not sensitive to UV, different from phenotypes of *S. cerevisiae* recQ-mutant, *sgs1*, and *S. pombe* recQ-mutant, *rqh1*. In homozygous crosses of the RecQ mutants, spore were not produced. This characteristic is also seen in *mei-3* and *mus-23*. We are studying about the epistatic relationship between the $RecQ^-$ and $mei\text{-}3^-$. It was shown by RFLP mapping that the RecQ homologue was located near the end of the right arm of LGI.