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Abstracts from the Neurospora 2002 conference

Abstract

Abstracts and Poster abstracts from the Neurospora 2002 conference

Neurospora 2002 **March 14-17, 2002** **Asilomar Conference Center** **Pacific Grove, CA.** **SCIENTIFIC PROGRAM** **Barry Bowman** **Gloria Turner**	**Schedule** **Invited Abstracts** **Poster Abstracts** **Index**

Schedule of Activities

Thursday, March 14

3:00 - 6:00 pm, Registration: Administration
6:00 - 7:00 pm, Dinner: Crocker
7:00 - 10:00 pm, Mixer: Kiln

Friday, March 15
7:30 - 8:30 am, Breakfast, Crocker
8:30 - 12:00 Noon, Session I, Chapel

Genomic Analysis : Mary Anne Nelson, Chair

8:35 - Bruce Birren, MIT, Whitehead Institute. "Genome sequencing for Neurospora crassa."
9:05 - Gertrud Mannhaupt, Heinrich-Heine-University. "The MIPS Neurospora crassa database-MNCDB."
9:30 - Chuck Staben, U. of Kentucky. "Gene finding and annotation for fungal genomes."
9:55 - Alan Radford, University of Leeds. "An on-line comprehensive gene list of Neurospora crassa."

10:10 - Break
10:35 - Don Natvig, U. of New Mexico. "The pdx-1 (snz-1/sno-1) region of the Neurospora genome."
10:50 - Jeremy R. Dettman, University of California at Berkeley. "Phylogentic species recognition in Neurospora."
11:15 - Nisha Sahay, QIAGEN Inc. "Gene expression analysis using oligo based microarrays."
11:40 - Craig Tomlinson, University of Cincinnati. "Microarrays for Neurospora crassa."

12:00 - 1:00 pm, Lunch, Crocker
Posters may be set up in Kiln beginning after lunch and will remain up throughout the meeting.

Friday 2:00 - 5:30 pm, Session II, Chapel

Signaling and Development : Dan Ebbole, Chair

2:05 - Jennifer Loros, Dartmouth Medical School. "The clock in Neurospora."
2:30 - Kwangwon Lee, Dartmouth Medical School. "Roles for WHITE COLLAR-1 in circadian and general photo perception in Neurospora crassa."
2:55 - Deborah Bell-Pederson, Texas A&M U. "Identification of novel components involved in controlling circadian rhythmicity using genetic and genomic approaches."
3:20 - Yi Liu, U. Texas Southwestern Medical Center at Dallas. "The clock kinases in the Neurospora circadian clock."

3:45 - Break
4:10 - Kathy Borkovich, U. of California, Riverside. "Life without heterotrimeric G alpha proteins."
4:35 - Carolyn Rasmussen, U. of California, Berkeley. "Hyphal fusion pathway of Neurospora crassa."
5:00 - David J. Jacobson, Stanford University. "Neurospora in western north America."
5:20 - Krishnapuram Rashmi, Osmania U. "Characterization of colbalt-sensitive mutant of Neurospora crassa."

6:00 - 7:00 pm, Dinner, Crocker
7:00 - 10:00 pm, Poster Session, Kiln

Saturday, March 16
7:30 - 8:30 am, Breakfast, Crocker
8:30 - 12:00 Noon, Session III, Chapel

Gene Regulation/Gene Silencing: Eric Selker, Chair

8:35 - Bob Metzenberg, Stanford U. "DNA unpaired in meiotic prophase triggers specific silencing of itself and its homologs."
9:00 - Patrick K.T. Shiu, Stanford U. "A quick and east way to determine gene function in meiosis using MSUD."
9:25 - Rodolfo Aramayo, Texas A&M U. "The presence of unpaired DNA is necessary but not sufficient to trigger meiotic silencing."
9:50 - Break

10:10 - Michael Freitag, U. of Oregon. "A DNA methyltransferase-like protein is essential for repeat induced point mutation in Neurospora crassa."
10:35 - Greg Kothe, U. of Oregon. "Methyl/RIP-DNA-binding proteins of Neurospora."
11:00- Hisashi Tamaru, U. Oregon. "Regulation of DNA methylation by a histone H3-K9 methyltransferase in Neurospora crassa."
11:25- David Catcheside, Flinders U. "New insights into recombination in Neurospora."
11:45- Edward G. Barry, U. of North Carolina. "Spore killer sensitive isolates become the killers in heterokaryons."

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

Saturday, 2:00 - 5:30, Session IV, Chapel
Organelle Biogenesis/Metabolic Regulation: Gloria Turner, Chair

2:05 - Frank Nargang, U. of Alberta. "Function and assembly of the preprotein translocase of the outer mitochondrial membrane (the TOM complex)."
2:30 - Holger Prokisch, U. Munich. "Protein translocation into mitochondria: Purification and characterization of the inner membrane protein translocases of Neurospora crassa."
2:55 - Robert Brambl, U. of Minnesota. "Transcripts and transcript-binding proteins in mitochondria of Neurospora crassa."
3:15 - Arnaldo Videira, U. do Porto. "Complex I, sex and alternative NADH dehydrogenase in Neurospora mitochondria."

3:35 - Break
4:00 - Matt Sachs, Oregon Health and Science U. "Translational control by upstream open reading frames in mRNA."
4:25 - Dan Ebbole, Texas A&M U. "Sugar Sensing in Neurospora."
4:50 - John Paietta, Wright State U. "Regulation of sulfur metabolism in Neurospora crassa."
5:10 - S. R. Nozawa, U. of Porto. "RIP of pacC-1 affected both the glycosylation of secreted alkaline phosphatase and conidiation in Neurospora crassa."

6:00 - 7:00 pm SATUDAY EVENING BANQUET, Crocker

David Perkins, Presentation of the Beadle and Tatum award
Rowland Davis, Neurospora: the making of a model organism

7:00 - 10:00 pm, Poster Session, Kiln

Sunday, March 17

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

8:30 - 12:00, Session V, Chapel
Cell Biology and Morphogenesis: Barry Bowman, Chair

The Beadle and Tatum Award Lecture
8:30 - Mike Plamann, U. of Missouri, Kansas City. "Analysis of dynactin-membrane interaction in Neurospora."
9:10 - Louise Glass, U. of California, Berkeley. "Hyphal fusion and vegetative incompatibility."
9:35 - Qijun Xiang, U. of California in Berkeley. "The Identification of vib-1, a gene involved in vegetative incompatibility mediated by het-c Locus in Neurospora crassa."

10:00 - Break
10:25 - Greg Jedd, Rockefeller U. "Hex1 crystal structure reveals the mechanism of self-assembly and evolutionary origin of Hex1."
10:50 - Nick Read, U. of Edinburgh. "Measuring the calcium message in living hyphae of Neurospora expressing codon-optimised aequorin."

11:15 - Tony Griffiths, U. of British Columbia. "Morphological miscellany."
11:40 - Barry Bowman, U. of California, Santa Cruz. "Antibiotic drugs and the vacuolar ATPase."

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

Invited Talks

Session I, Genomic Analysis

The MIPS *Neurospora crassa* Database - MNCDB.
Gertrud Mannhaupt and Werner Mewes. GSF-Forschungszentrum f. Umwelt und Gesundheit, Institut f. Bioinformatik, 85764 Neuherberg, Germany

The German project of sequencing *Neurospora crassa* linkage groups II and V started in 1998 and is close to completion. The two chromosomes are estimated to be 4.6 Mb and 9.2 Mb in length, respectively. The total genome has a length of about 43 Mb which is more than 3 times larger than the first completely sequenced eukaryotic genome of *Saccharomyces cerevisiae*. The MNCDB database at MIPS contains about 17 Mb of genomic sequences. 8 Mb could be definitely assigned to LG II and V and about 9 Mb could be partly assigned to different chromosomes. The entire dataset was automatically analysed using the PEDANT system. PEDANT is a fully automated system using a wide spectrum of sequence analysis and structure prediction tools. 4700 genes are predicted by the program FGENESH, recently trained for Neurospora (V. Solovyev). The results of the capacious analysis provided by PEDANT can be viewed on the project page.
Moreover detailed manual supervised gene modelling and annotation of the genemodels have been carried out for at least all sequences belonging to LG II and V. More than 2500 proteins were processed manually. Beside annotating different features they are classified and assigned to functional categories. The deduced protein sequences are concurrently analysed by the PEDANT system. The genome of *Neurospora crassa* will complement the information supplied by the ones of *S. cerevisiae*, *C. elegans* and *D. melanogaster* for several aspects. Comparisons of these genomes to the one of *N. crassa* - the filamentous fungal model organism - will aid the definition of entities required for multi-cellularity and tissue organization.
Summaries of data and related information, such as EST hits, BLAST hits, literature links, DNA and Protein sequences, or functional classification is available on each gene/protein entry and can be viewed on our project homepage.
http://mips.gsf.de/proj/neurospora

The *pdx-1* (*snz-1/sno-1*) region of the *Neurospora crassa* genome
Donald O. Natvig, Laura E. Bean, William H. Dvorachek, Jr., Edward L. Braun, Allison Errett, Gregory S. Saenz, Mara D. Giles, Margaret Werner-Washburne, and Mary Anne Nelson. Department of Biology, University of New Mexico, Albuquerque, NM

The *pdx-1* region of the *N. crassa* genome contains homologs of two closely-linked stationary phase genes, *SNZ1* and *SNO1*, from *Saccharomyces cerevisiae*. Homologs of *SNZ1* encode extremely highly conserved proteins that have been implicated in pyridoxine (vitamin B6) metabolism in the filamentous fungi *Cercospora nicotianae* and *Aspergillus nidulans*. In *N. crassa*, *SNZ* and *SNO* homologs map to the region occupied by *pdx-1* (pyridoxine requiring), a gene known in *N. crassa* since the earliest studies with biochemical mutants, but which was not sequenced previously. Combining genomic sequence data, existing high-resolution mapping data from the *pdx-1* region, and sequence data obtained from known mutants previously classified as *pdx-1*, we demonstrated that the *pdx-1* mutant phenotype can derive from mutations in either the *SNZ* or *SNO* homolog of *N. crassa*. This provided the first firm experimental link between *SNZ* and *SNO* functions. One *N. crassa* mutant with a disrupted *SNO* homolog was at one

time designated *pdx-2*. It now appears appropriate to reserve the *pdx-1* designation for the *N. crassa SNZ* homolog and *pdx-2* for the *SNO* homolog. Analysis of 36 kbp in the pdx region revealed at least 12 protein coding genes, supporting a previous conclusion of high gene densities (12,000-13,000 total genes) for *N. crassa*. With the exception of *pdx-1* and *pdx-2* there is no evidence of shared function among the genes in this region.

Gene expression analysis using oligo based microarrays.
Nisha Sahay. Product Manager, QIAGEN Inc., Valencia, CA 91355

Gene expression analysis is a powerful step in the diagnosis of various diseases, appraisal of disease progression, evaluation of drug therapy effectiveness and the estimation of the severity of trauma, as well as many other potential uses. To date the focus of this type of analysis is in screening large numbers of genes (sometimes whole genomes) by utilizing DNA microarrays or in the quantitative analysis of smaller numbers of genes using various quantitative PCR methods. Early generation DNA microarrays based on cDNAs or ORFs were not very specific and also not very quantitative. The lack of specificity of the ORF-based microarrays is mostly due to cross-hybridization of related or overlapping genes found in complex genomes. At QIAGEN Operon we have addressed many of these limitations of the ORF-based microarrays by designing oligos that are concentration normalized, hybridization temperature normalized as well as sequence optimized. In order to achieve good sensitivity while maintaining specificity, the sequences we design are relatively long oligonucleotides.

Microarrays for *Neurospora crassa*.
Craig R. Tomlinson and Mario Medvedovic. Hyacinth Genomics, LLC, Cincinnati, OH.

The primary goal of Hyacinth Genomics, LLC, is to provide oligonucleotides representing the expressed genome of model systems that have made seminal contributions to biology. Many research groups lack sufficient libraries for genomics studies and prominent among them are researchers who work with *Neurospora crassa*. To fulfill this need, we propose to generate an oligonucleotide library representing the genes of *N. crassa* to be available as arrayed DNAs in microtiter plates and on microscope slides. Using the recently completed *N. crassa* genome sequence database, the latest advances in bioinformatics will be applied to the sequence design of more than 12,000 oligonucleotides using an approach that will minimize cross-hybridization and maximize specificity and sensitivity. Studies will be carried out to demonstrate the efficacy of the *N. crassa* oligonucleotide sequences. Our purpose here is to assess the interest and support of our plan to supply affordable microarrays to the *N. crassa* community. We intend to keep costs to a minimum by including the production of the *N. crassa* oligonucleotide library as part of the budget of an NIH-sponsored Small Business Initiative Research grant. The providing of arrayed DNAs representing the *N. crassa* genome would greatly promote genomics studies in this significant model system.

Session II, Signaling and Development

The clock in Neurospora.
Jennifer Loros and Jay Dunlap. Departments of Genetics and Biochemistry, Dartmouth Medical School

Neurospora is a uniquely tractable model system for the analysis of the molecular basis of eukaryotic circadian oscillatory systems. Molecular bases for the period length and sustainability of the rhythm, light and temperature resetting of the circadian system, and for gating of light input and light effects are becoming understood, and Neurospora promises to be an amenable system for examining the role of coupled feedback loops in the clock. Many of these insights have been shown to have, or to foreshadow, direct parallels in mammalian systems, including specifically the mechanism of light entrainment, the involvement of PAS:PAS heterodimers as transcriptional activators in essential clock-associated feedback loops, and the dual role of FRQ in the loop as both an activator and a repressor; similarities extend to the primary sequence level in at least one case, that of WC-1 and BMAL1. Work on circadian output in Neurospora, using several approaches, has identified numerous regulated genes and has been at the forefront of studies aimed at understanding clock control of gene expression.
This work was supported by grants from the National Science Foundation MCB-0084509 to J.J.L., the National Institutes of Health MH44651 to J.C.D. and J.J.L., MH01186 and GM34985 to J.C.D. and the Norris Cotton Cancer Center core grant at Dartmouth Medical School.

Roles for WHITE COLLAR-1 in circadian and general photoperception in *Neurospora crassa*. (updated abstract follows)

Kwangwon Lee, Jennifer J. Loros, and Jay C. Dunlap. Department of Biochemistry and Department of Genetics, Dartmouth Medical School. Hanover NH. 03755.

The transcription factors WHITE COLLAR-1 (WC-1) and WHITE COLLAR-2 (WC-2) interact to form a heterodimeric complex (WWC) that is essential for all light- mediated processes in Neurospora. Based on the blind phenotypes of mutants and sequence comparisons with known photoreceptor proteins, WC-1 has been proposed to be the Neurospora blue light photoreceptor. WCC also plays a distinct non-light related role as the transcriptional activator in the FREQUENCY(FRQ)/WCC feedback loop that is central to the Neurospora circadian system. To further investigate the roles of WC-1, we analyzed the phenotypes of five different *wc-1* mutant alleles for 1) FRQ expression in constant darkness 2) light induction of FRQ, and WC-1, 3) post-transcriptional modification of FRQ, 4) light induction of developmentally and light inducible genes. Our data show that WC-1 is involved in FRQ expression at at least two different levels, and only a small N-terminal fraction of the full length of WC-1 is necessary for light induction of *frq* and other light-inducible genes. Functional WC-1 is necessary for the WCC formation. We propose that WC-1 is not a light- receptor for the circadian clock.
This work was supported by grants from the National Science Foundation MCB-0084509 to J.J.L., the National Institutes of Health MH01186 and GM34985 to J.C.D., GM20553 to K. L. and the Norris Cotton Cancer Center core grant at Dartmouth Medical School.

UPDATED ABSTRACT: ROLES FOR WHITE COLLAR-1 IN CIRCADIAN AND GENERAL PHOTOPERCEPTION IN *Neurospora crassa*.

Kwangwon Lee, Jennifer J. Loros, and Jay C. Dunlap. Department of Biochemistry and Department of Genetics, Dartmouth Medical School. Hanover NH. 03755.

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Identification of novel components involved in controlling circadian rhythmicity using genetic and genomic approaches.

Deborah Bell-Pedersen. Department of Biology, Texas A&M University, College Station, TX 77843

To identify signaling components of the circadian clock system in *Neurospora crassa*, we have carried out a genetic selection to isolate mutations that alter the expression of clock-controlled genes (ccgs). This selection is based on the differential expression of the ccgs in response to the presence or absence of the clock gene product FRQ. For example, *ccg-1* expression is repressed and *ccg-2* expression is activated when FRQ is present in the cell. The promoter region of the *ccg-1* gene was ligated to *mtr*. The *mtr* gene encodes a neutral amino acid permease that allows both positive and negative selection. The fusion constructs were transformed into both *bd; mtr; trp-2* and *bd;frq10;mtr;trp-2* strains. Reconstruction experiments using the *ccg-1:mtr* transformants showed FRQ-dependent production of the amino acid

permease. Both strains were subjected to UV light mutagenesis and assayed for growth under the opposite conditions. Out of 100,000 colonies screened for each transformant, we identified 15 mutant strains that yielded the desired growth on the selective media. The phenotypes of these mutations will be discussed. In a related study, we are using transcriptional profiling to help us understand the mechanisms by which the clock regulates development and other critical cellular events. *N. crassa* DNA microarrays were probed with cDNA produced from mRNA obtained from cultures harvested at different times of the day, during developmental induction and from a strain that overexpresses WC-1. These analyses are allowing us to identify genes under circadian control at the level of transcript accumulation, and to identify novel candidate clock components.

The clock kinases in the Neurospora circadian clock.
Yuhong Yang, Ping Cheng, and Yi Liu. UT Southwestern Medicla Center, Dallas, TX.

Phosphorylation of clock proteins represents an important mechanism regulating circadian clocks. In Neurospora, clock protein FREQUENCY is progressively phosphorylated over time, and its level decreases when it is extensively phosphorylated. To identify the kinase phosphorylating FRQ and to understand the function of FRQ phosphorylation, a FRQ phosphorylating kinase was purified and identified as casein kinase II. Disruption of the catalytic subunit gene of CKII in Neurospora resulted in hypophosphorylation and increased levels of FRQ protein. In addition, the circadian rhythms of frq RNA, FRQ protein, and clock-controlled genes are abolished in the CKII mutant. Our data suggest that the phosphorylation of FRQ by casein kinase II may have at least three functions: it decreases the stability of FRQ, reduces the protein complex formation between FRQ and the WHITE COLLAR proteins, and is important for the closing of the Neurospora circadian negative feedback loop. Taken together, our results suggest that CKII is an essential component of the Neurospora circadian clock.

Life without heterotrimeric G alpha proteins.
Katherine A. Borkovich. Department of Plant Pathology, University of California, Riverside.

Our laboratory investigates heterotrimeric G protein signal transduction pathways in the filamentous fungus *Neurospora crassa*. We have cloned, mutated and characterized three Galpha, one Gbeta and one Ggamma subunits from this species. Our results have demonstrated crucial roles for G proteins in regulation of cell proliferation, asexual and sexual differentiation, and stress tolerance, through both cAMP-dependent and independent pathways. Now we report the relative contributions of the three Galpha proteins, GNA-1, GNA-2 and GNA-3, to these processes, through the construction of strains containing multiple Galpha mutations. GNA-1 and GNA-3 are required for normal adenylyl cyclase activity, and these subunits contribute to hyphal elongation, conidiation and sexual fertility. GNA-2 appears to play a supporting role, in that functions for the *gna-2* gene are only revealed in a *gna-1* or *gna-3* mutant background. Epistatic relationships between *mcb* (a mutation in the regulatory subunit of cAMP-dependent protein kinase, or PKA) and the *gna-1* and *gna-3* genes suggest that GNA-1 and GNA-3 lie upstream of PKA during the regulation of several processes in *N. crassa*.

Session III, Gene Regulation/Gene Silencing

DNA unpaired in meiotic prophase triggers specific silencing of itself and its homologs.
Robert L. Metzenberg, Patrick K. T. Shiu, Namboori B. Raju, and Denise Zickler*. Stanford University, Stanford, CA. *Universite Paris-Sud, Orsay, France.

In the sexual phase of Neurospora, fusion of nuclei of opposite mating type results in a diploid zygote which quickly enters prophase of the first meiotic division. Homologs become intimately paired during pachytene of prophase1. Any DNA that is not so paired generates a signal in which a double-stranded RNA is apparently made as an intermediate. The result is destruction of mRNA encoded by the unpaired DNA or by any gene of the same sequence, paired or unpaired. Thus any gene present in an odd number of copies in the two parents taken together will be unexpressed, as will any gene present in an even number, but located differently in the two parents so that it cannot be paired. This system of meiotic silencing by unpaired DNA (MSUD) offers a unifying explanation of several seemingly unrelated

details of Neurospora biology. These include the ascus- dominance of some classical mutants, of some deletion mutants, and of gene insertion mutants; the barrenness of strains with segmental duplications; and (in part),the barrenness of crosses of *N. crassa* to related species like *N. tetrasperma* and *N. sitophila*. A mutant (Sad-1) in which the gene for RNA-directed RNA polymerase is deleted allows even unpaired genes to be expressed. Thus the mutation suppresses partially or completely the MSUD underlying these examples.

RID, a DNA methyltransferase-like protein that is essential for repeat-induced point mutation in *Neurospora crassa*.
Michael Freitag, Gregory O. Kothe, Rebecca Williams and E. U. Selker. Institute for Molecular Biology, University of Oregon, Eugene, OR.

All organisms have mechanisms to safeguard the integrity of their genomes. During sexual development *Neurospora crassa* inactivates duplicated DNA segments by "repeat-induced point mutation" (RIP). RIP operates on paired DNA repeats, introduces G:C to A:T transition mutations and creates targets for subsequent DNA methylation in vegetative tissue. The mechanism of RIP and its relationship to DNA methylation are poorly understood. We show that a cytosine methyltransferase-like protein is essential for RIP, the first implication of a eukaryotic DNA methyltransferases in mutagenesis under natural conditions. Disruption of the *rid* (*RI*P-*d*efective) gene did not affect normal development or vegetative DNA methylation. We isolated conserved *rid* genes from *N. intermedia* and *N. tetrasperma*, and portions of the gene from the homothallic *N. africana, N. terricola, N. pannonica* and *N. galapagosensis*. We propose that RID-like proteins constitute a new class of fungal DNA methyltransferases that are generally important during sexual development and for gene silencing processes.

Control of DNA methylation by a histone H3-K9 methyltransferase in *Neurospora crassa*.
Hisashi Tamaru & Eric U. Selker. Institute of Molecular Biology, University of Oregon, Eugene, OR97403, USA.

Cytosine methylation is the only known epigenetic mark on DNA in eukaryotes. In contrast histones, which wrap DNA around them, are marked by a variety of combinations of different modifications such as acetylation, phosphorylation and methylation at a variety of positions. DNA methylation is essential for the normal development of mammals and plants and is involved in epigenetic processes such as X-chromosome inactivation, genomic imprinting and silencing of transposons. However, little is know about how DNA methylation is controlled. In an attempt to tag a previously identified DNA methylation gene *dim-1* (defective in methylation) by insertional mutagenesis we unexpectedly generated a mutation in a novel gene, *dim-5*,which like the *dim-2*(DNA methyltransferase) mutation, eliminates all or nearly all DNA methylation. We mapped *dim-5*between *trp-4*and *leu-2*on LG IV. A contig containing both *trp-4*and *leu-2*was found in a *N. crassa*genomic sequence data base, based on their expected homology to *S. cerevisiae TRP4*and *LEU1*,respectively. We scrutinized the interval between the putative *trp-4*and *leu-2*for DIM-5 candidates using BLASTx. One striking candidate was found to encode a protein related to histone H3-K9 methyltransferases involved in heterochromatin silencing in fission yeast and fly. We confirmed that the candidate gene is *dim-5* by complementation and quelling tests using DNA fragments containing the gene. Sequencing of the *dim-5* gene in *dim-5* mutant identified a nonsense mutation in a SET domain that is known to be required for several previously identified histone H3 methyltransferases. Biochemical studies on recombinant DIM-5 demonstrated that this protein specifically methylates K9 of histone H3. Substitutions of K9 in histone H3 caused dominant loss of DNA methylation in *vivo.* We conclude that histone H3-K9 methylation signals DNA methylation in *Neurospora crassa.*

New insights into recombination in Neurospora.
David EA Catcheside, P Jane Yeadon, J Paul Rasmussen, Frederick J Bowring and Lin Koh. School of Biological Sciences, Flinders University, PO Box 2601, Adelaide, SA 5001, Australia.

Meiotic recombination in the *his-3* locus is predominantly initiated at or close to cog^L, a recombination hotspot within the promoter region of the nearby gene *lpl*. We have found that interposition of blocks of sequence heterology between cog^L and *his-3* scarcely affect the frequency of recombination within *his-3*, showing that propagation of recombination

events from this hotspot does not require contiguous tracts of sequence homology in the parental chromosomes. His+ progeny are readily obtained from crosses heterozygous for a *his-3* point mutation and a chromosomal translocation that disrupts *his-3* between the point mutation and cog^L. A substantial proportion of the recombinant progeny show evidence of sequence exchange both sides of the translocation breakpoint, suggesting that DNA repair synthesis can make multiple excursions between the parental chromosomes. This would resurrect a copy choice mechanism, long out of favour, to explain some recombination events.

Session IV, Organelle Biogenesis/Metabolic Regulation

Function and assembly of the preprotein translocase of the outer mitochondrial membrane (the TOM complex).
Frank E. Nargang and Rebecca D. Taylor. Department of Biological Sciences, University of Alberta, Edmonton, Alberta.

The TOM complex recognizes mitochondrial preproteins synthesized in the cytosol and translocates them across, or into, the outer mitochondrial membrane. The complex contains receptor proteins with domains that extend into the cytosol as well as integral membrane proteins that form the core TOM complex. The major component of the core complex is Tom40, which forms the translocation pore. The *tom40* gene has been shown to be essential in both yeast an *N. crassa*. Cells with reduced levels of Tom40 grow very slowly and contain small mitochondria that lack cristae. Mitochondria isolated from such strains are deficient in their ability to import mitochondrial preproteins and are deficient in the core TOM complex components Tom22 and Tom6 suggesting that the import and/or stability of these proteins is dependent on the presence of Tom40. We have analyzed mutant variants of Tom40 for in vivo function and for their ability to be assembled into the TOM complex in vitro. Assembly of Tom40 into the complex occurs through a series of intermediates. Our analysis of Tom40 variants suggests that a high molecular weight intermediate on the assembly pathway contains only newly imported subunits and that conserved regions in the N-terminus of the protein play an important role in the assembly process.

Protein translocation into mitochondria: Purification and characterization of the inner membrane protein translocases of *Neurospora crassa*.
Christian Kozany, Dejana Mokranjac, Andreja Vasiljev, Walter Neupert, and Holger Prokisch. Institut für Physiologische Chemie, Munich, Germany.

Mitochondria are essential organelles of eukaryotic cells. The large majority of the proteins required for mitochondrial structure and function is encoded by nuclear genes which have to be imported into mitochondria. Import of mitochondrial preproteins is mediated by a general translocase in the outer membrane, the TOM complex, and by two distinct translocases in the mitochondrial inner membrane, the TIM23 complex and the TIM22 complex. The TOM complex must specifically recognise mitochondrial precursor proteins synthesized in the cytosol and translocate them across the outer membrane. For further import into or across the inner membrane the TOM complex cooperates with both TIM complexes. The TIM23 complex mediates import of preproteins with N-terminal presequences, whereas hydrophobic proteins with internal targeting signals are inserted via the TIM22 complex. The translocation pathway has been studied in detail in the yeast system, however, not much is known about the precise composition and molecular structure of the TIM complexes. For a detailed biochemical characterisation of the TIM complexes we cloned the genes from *N. crassa* and generated via different methods strains expressing histedinyl-tagged Tim components. The possibility to obtain large amounts of *Neurospora* organelles allowed the purification of the low abundant TIM complexes in substantial amounts. The characterisation of the TIM complexes will be presented.

Transcripts and transcript-binding proteins in mitochondria of *Neurospora crassa*.
Robert Brambl, Nora Plesofsky, and Jill Kleidon. Department of Plant Biology, University of Minnesota, Saint Paul.

We compared expression elements of three disparate groups of mitochondrial genes in *Neurospora crassa, COB, COX1,* and the clustered *ATP8-ATP6-mtATP9-COX2*. To identify promoter sequences we employed the published *N. crassa* consensus sequence for *COB,* and we found closely related sequences within the 5'-UTRs of both *COX1* and

the *ATP8-COX2* transcriptional units. The *COX1* RNA includes two flanking URF sequences, but the more distant 3'-flanking *ND1* and 5'-flanking *tRNA^{cys}*are not included in the mature *COX1* transcript. The *ATP8, ATP6, mtATP9,* and *COX2* are expressed as a single transcript which does not include adjacent 5'-URF sequence. Primer extension analysis of the 5'-UTR of the *COX1* transcript showed that there was only one 5'-end for the *COX1* gene transcript, which is 73 nt downstream of the consensus promoter sequence and is the first nt 3' of the sequence for the intervening *tRNA^{cys}*. Primer extension analysis of the 5'-UTR *ATP8-COX2* sequence showed that the 5'-end for this transcript was the first nt 3' of the consensus promoter sequence. We per49 formed gel-shift experiments to detect proteins in mitochondria that bind to transcripts as possible regulatory proteins. Results with the 5'-UTR RNAs of *COB, COX1,* and *ATP8-COX2* suggest that they may be binding both unique proteins and an overlapping group of two to four proteins of ~155 to 45 *M*r. We successively deleted regions of the RNA 5'-UTRs to identify sequences that bound these proteins. We identified similar predicted stem-loop secondary structures in the protein-binding regions of all three UTRs.

Complex I, sex and alternative NADH dehydrogenases in Neurospora mitochondria. Arnaldo Videira. Instituto de Biologia Molecular e Celular and Instituto de Ciências Biomédicas de Abel Salazar, University of Porto, Portugal.

Respiratory chain complex I couples electron transfer with proton translocation through the inner membrane of mitochondria, thus participating in oxidative phosphorylation. The transfer of electrons from NADH to ubiquinone is mediated by protein-bound prosthetic groups. Complex I contains about 40 proteins of dual genetic origin distributed in two major domains, the peripheral and membrane arms. While 14 of these subunits are conserved in prokaryotes and constitute a "minimal structure" for activity, the others are present exclusively in eukaryotes. Most of the Neurospora polypeptides have been cloned and/or identified. A thoroughly genetic analysis has provided insights into the biogenesis, structure and function of complex I. An overview of the effects of disruption and site-directed mutagenesis of specific complex I genes, including the generation of models for human mitochondrial disease, will be presented. Complex I seems to be dispensable for Neurospora grown under vegetative conditions but it is required for sexual development. The sexual phase of the fungal life cycle is blocked in homozygous crosses between complex I mutants. In addition to complex I, organisms also contain non-proton-pumping alternative NADH dehydrogenases with varying number and specificity. Like plants, *N. crassa* contains 3-4 alternative NAD(P)H dehydrogenases in the mitochondrial inner membrane. One of them is located in the outer face of the membrane (external enzyme), working as a calcium-dependent oxidase of cytosolic NADPH. At least another external enzyme and a NADH dehydrogenase working with matrix substrates (internal enzyme) are present in the fungus.

Translational control by upstream open reading frames in mRNA. Matthew Sachs. Department of Biochemistry and Molecular Biology, OGI School of Science and Engineering, Oregon Health & Science University, Beaverton, OR.

Translational control by upstream open reading frames (uORFs) in the 5'-leaders of prokaryotic mRNAs and eukaryotic mRNAs is an increasingly well-documented form of genetic control. Arg-specific translational regulation by the arginine attenuator peptide (AAP), encoded by a uORF in the *Neurospora crassa arg-2* specifying the small subunit of carbamoyl phosphate synthetase, represents a clear example of such a control phenomenon. Using cell-free translation systems and a primer extension inhibition (toeprint) assay, we showed that synthesis of the 24-residue *N. crassa* AAP in high Arg causes ribosomes to stall at the uORF termination codon, blocking ribosomes from scanning to the downstream initiation codon. The AAP also causes Arg-regulated stalling of ribosomes involved in elongation when it is fused directly to the N-terminus of a luciferase reporter, suggesting that it blocks a step common to elongation and termination. The peptide sequence is important, and mutation of an evolutionarily conserved Asp residue to Asn eliminates regulation in vivo and in vitro in both *N. crassa* and *Saccharomyces cerevisiae*. Regulation appears independent of the charging status of tRNA. We have further developed the cell-free *N. crassa* system so that we can radiolabel nascent AAP-containing peptides and directly assess the regulatory effects of Arg on polypeptide synthesis. The data demonstrate that, when Arg causes ribosomes to stall during elongation following synthesis of the AAP, the nascent peptide remains associated with ribosomes, and stalled ribosomes can subsequently resume polypeptide synthesis. To characterize new mutations potentially affecting Arg-specific translational control, we have adapted a microtiter plate assay to monitor the affects of mutations affecting the growth of an *N. crassa arg-12^{s} pyr-3 arg-2-hph* through their effects on the expression of the *arg-2* and *arg-2-hph* genes.

Sugar sensing in Neurospora.
Daniel Ebbole and Xin Xie. Department of Plant Pathology and Microbiology, Texas A & M University, College Station Tx.

The ability to sense the presence of a preferred carbon source is a fundamental property of growing cells. In yeast, several mechanisms are involved in glucose sensing and controlling gene expression. One mechanism is to employ glucose transporter homologs that have evolved as glucose receptor/sensors but that are not themselves functional glucose transporters. We have analysed the *N. crassa rco-3* gene and conclude that it functions as a sugar sensor rather than a sugar transporter. To gain further evidence for the role of *rco-3* as a regulator we have isolates several suppressors of *rco-3* and are examining the effect of the *rco-3* mutant on gene expression. The characterization of the suppressed *rco-3* mutants and transcriptional profiling of the *rco-3* mutant strain is helping us define the genetic pathway responsible for sugar sensing.

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

The sulfur regulatory system of *Neurospora crassa* is composed of a group of structural genes (e.g., *ars-1*, arylsulfatase) that are under coordinate control of the CYS3 positive regulator and SCON negative regulators. We are constructing a comprehensive model of sulfur metabolism and have begun by further defining the role of the CYS3 regulator. CYS3-dependent sulfur regulated expression has been found in a number of genes involved in cysteine and methionine metabolism (e.g, methionine synthase, cystathionine gamma-lyase, homoserine transacetylase); thereby expanding the role of CYS3. Further, *in vitro* binding site studies have been used to more accurately determine the CYS3 recognition sequence. The combination of gene expression profile data, based on the availability of the genomic sequence, along with binding site data will allow for greater selectivity in a genome-wide analysis for genes controlled by this system.

Session V, Cell Biology and Morphogenesis

Analysis of dynactin-membrane interaction in Neurospora.
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Dynactin has been proposed to link the microtubule-associated motor cytoplasmic dynein with membranous cargo; however, the mechanism by which dynactin-membrane interaction is regulated is unknown. Actin-related protein 1 (Arp1) is the most abundant subunit of dynactin, and it forms a short filament to which additional subunits associate. A pointed-end binding subcomplex binds to one end of the Arp1 filament, while a shoulder/sidearm complex containing p150Glued binds to other end. p150Glued of dynactin has been shown to mediate dynein-dynactin interaction; however, it has not been found to play a direct role in membrane- binding. We analyzed 43 p150Glued mutants, and we found that C-terminal deletions that remove the terminal domains result in constitutive dynactin-membrane binding. We propose that the C-terminal domains of p150Glued regulate dynactin- membrane binding through a steric mechanism that controls accessibility of the Arp1 filament of dynactin to membranous cargo. We also examined mutants defective in the Arp1 pointed-end subcomplex. We found that these mutants show stronger dynein/dynactin-membrane interaction relative to wild-type suggesting that the Arp1 pointed-end complex may also play a role in regulating interaction of dynactin with membranous cargo.

Crystal structure of Hex-1 reveals its mechanism of self-assembly and evolutionary origin.
Greg Jedd[1], Ping Yuan[2], D. Kumaran[3], S. Swaminathan[3], Nam-Hai Chua[1] and K. Swaminathan[2]. [1]Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10021-6399, USA,[2]Macromolecular X-ray Crystallography Laboratory, Institute of Molecular Agrobiology, National University of Singapore, 1 Research Link, Singapore 117604, [3]Biology Department, Brookhaven National Laboratory, Upton, NY 11973-5000, USA.

The Woronin body is a specialized peroxisomal vesicle that is restricted to the filamentous Ascomycotina and its major protein component, Hex-1, self-assembles to form the vesicle's crystalline core. We present the crystal structure

of Hex-1 at 1.78 Å and provide the structural basis for its self-assembly. The protein consists of two mutually perpendicular B-barrels. The N-terminal barrel contains six B-strands and the C-terminal domain contains a five-stranded barrel and a flanking Alpha-helix. The crystal reveals three primary intermolecular contacts that promote self-assembly through the formation of crosslinked Hex-1 filaments. Self-assembly is abolished in vitro and in vivo by mutations in intermolecular contact residues, indicating that the crystal structure is a valid representation of the Woronin body-core. In spite of sharing a weak sequence homology, the tertiary structures of Hex-1 and the eukaryotic translation initiation factor 5a (eIF-5A) are very similar, suggesting an ancestral link between them. Since eIF-5A does not self-assemble, comparison of these two protein structures suggests how a crystalline protein complex evolved from a soluble precursor.

Morphological miscellany.
Tony Griffiths. Botany, UBC, 6270 University Blvd., Vancouver, BC. Canada V6T 1Z4.

Recent work has focused on trying to understand growth and branching of Neurospora through morphological mutants. One project (A. Virag) attempts to isolate mutations of the actin gene by obtaining cytochalasin resistant mutants. Preliminary indications suggest that at least one resistant mutant of this type is at the actin locus. Another approach (O. Gavric) is to piece together the available morphological mutants into groups that might represent developmental hierarchies, by studying interactions in double mutants, and by structural and physiological similarities between mutants. Several novel types of gene interaction have been observed. Some overall patterns are emerging. Finally we are extending earlier studies on morphological mutants by obtaining chemical profiles of the mutants (E. Jovel).

Antibiotic drugs and the vacuolar ATPase.
Barry Bowman and Emma Jean Bowman. MCD Biology, University of California, Santa Cruz. CA.

The vacuolar ATPase generates an electrochemical gradient across membranes of the Golgi, vacuole and other organelles in the endomembrane system. The enzyme is composed of 13 types of subunits and functions as a rotary motor. Several different kinds of antibiotics have evolved that are highly potent, specific inhibitors. To identify the binding site of the antibiotics we have selected mutant strains of *Neurospora crassa* that are resistant to the toxic effects of bafilomycin. One class of mutant strains has single point mutations in the *vma-3* gene, which encodes a 16 kDa proteolipid subunit of the vacuolar ATPase. Three different residues were altered among the bafilomycin-resistant strains. The mutated sites appear to be on the outer face of the "rotor" sector of the enzyme. The positions of two mutated residues corresponds precisely to the positions of mutated residues in the homologous subunit of the mitochondrial ATPase. In the mitochondrial ATPase the mutations confer resistance to oligomycin, an antibiotic that has no effect on the vacuolar ATPase. These results suggest that vacuolar and mitochondrial ATPases have an ancient, conserved antibiotic binding site. As the sequence of the polypeptides diverged, new antibiotics have arisen that target the same vulnerable site in this family of enzymes.

Poster Abstracts

1
Characterization of efflux of basic amino acids from the vacuole of *Neurospora crassa*.
Kelly A. Keenan, Ryan Cinalli and Chris Sondey. Richard Stockton College, Pomona, NJ.

The vacuole stores large amounts of the basic amino acids--arginine, lysine and ornithine--and it has been observed that conditions of nitrogen starvation cause an efflux or movement of these amino acids out of the vacuole. The amino acids presumably serve as a reserve nitrogen source. The components involved in efflux--the proteins as well as the signals-have never been characterized. The cupric ion permeabilization method has recently been developed to measure efflux and several strains with an increased level of efflux have been identified. RSC-44 has an increased arginine efflux compared to wild type while both arginine and ornithine efflux are increased in RSC-63. These strains

offer a way to characterize this efflux process. The strains were characterized by backcrossing with wild type and mapping the location of the mutation. The number of genes involved in efflux can be identified this way as well as the chromosomal location. The response of the strains to nitrogen starvation was also characterized since this is known to produce increased efflux. Unlike wild type, there was no increase in activity of the arginase during nitrogen starvation in the strains and an altered response in the vacuolar amino acids.

2

Photoactivation of con-10 gene expression in *Neurospora crassa*.

Luis M. Corrochano, Maria Olmedo, and Laura Navarro-Sampedro. Departamento de Genética, Universidad de Sevilla, Spain

The gene con-10 of Neurospora is expressed during conidiation and following illumination of mycelia with light. The photoactivation of con-10 disappears after two hours of illumination (light adaptation). To investigate the molecular nature of light adaptation in Neurospora, we have designed a protocol to isolate mutants altered in the adaptation of con-10 photoactivation. We are using a strain of Neurospora with a fusion of the con-10 promoter to the gene conferring resistance to hygromycin. This strain is sensitive to the drug when the promoter is inactive, i.e. during vegetative growth either in the dark or under continuous light. We have isolated 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. The promoter of con-10 is composed of DNA segments involved in repression in the dark and during mycelial growth, and DNA segments required for the activation during conidial development. We are using a series of fusions between specific segments of the con-10 promoter and the lacZ gene to investigate in detail the DNA sequences involved in regulating the expression of con-10.

3

RIP of *pac*C-1 affected both the glycosylation of secreted alkaline phosphatase and conidiation in *Neurospora crassa*.

S.R. Nozawa[1], M.S. Ferreira-Nozawa[2], M. Duarte[3], N.M. Martinez-Rossi[2], A. Videira[3] and A. Rossi[4]. [1]Departamento de Química, FFCLRP-USP, [2]Departamento de Genética, FMRP-USP and [3]Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal and [4]Departamento de Bioquímica e Imunologia, FMRP-USP, Ribeirão Preto, Brazil.

A conserved *pac*C-dependent pH response pathway has been identified in many fungi like *N. crassa* and *A. nidulans*. Gene *pac*C codes for a Zn-finger transcription factor that undergoes proteolysis at alkaline pH, yielding a functional protein responsible for the induction of genes expressing products with optimal activity at alkaline pH (e.g. alkaline phosphatases) and repression of those with optimal activity at acidic pH (e.g. acid phosphatases). Transcription of *pac*C is itself induced under alkaline growth conditions, and the *pal* genes promote the proteolytic activation of PacC, because expression of C-terminal truncated PacC derivatives suppresses *pal* mutant effects. Thus, if *pac*C gene governs positively the expression of alkaline genes, *pac* C mutants inactivated by RIP should not secrete alkaline phosphatase and the properties of the secreted acid phosphatase should be identical to that secreted by wild-type strains. Indeed, *pac*C-null mutants (RIP) were affected in conidiation at pH 4.0, and secreted alkaline phosphatase at pH 7.8 with altered thermal inactivation, electrophoretic and isoelectric focusing patterns, and reduced sugar content, as compared to the properties of the wild-type enzyme, indicating additional roles for *pac*C gene. Financial support: FAPESP, CNPq, CAPES and FAEPA.

4

Characterization of *pac*C-1, a wide domain regulatory gene responsive to ambient pH in *Neurospora crassa*.

N.M. Martinez-Rossi[1], S.R. Nozawa[2], G.S. May[3], M.S. Ferreira-Nozawa[1] and A. Rossi[4]. Departamentos de [1]Genética and [4]Bioquímica e Imunologia, FMRP-USP, Brazil, [2]Departamento de Química, FFCLRP-USP, Brazil and [3]University of Texas, M.D. Anderson Cancer Center, Div. Pathol. L.M., Houston, Texas, USA.

An essential step in the conversion of PacC, a conserved wide domain Zn-finger transcription factor, into its fully active form in response to ambient alkaline pH is the proteolytic removal of a C-terminal inhibitory region of the protein as mimicked in the *pac*C14 mutant of *A. nidulans*, which was characterized as a gain-of-function mutation. However, *pac*C14 behaved as a loss-of-function mutation because we have shown that this mutation alters posttranslational glycosylation of both acid and alkaline phosphatases secreted at pH 5.0, indicating that proteolytic cleavage of PacC may not be necessary for its activation. With the aim of characterizing further this adaptive response to ambient pH we cloned gene *pac*C-1 from *N. crassa* by screening a genomic library with fragments of genomic DNA generated by PCR amplification. One of the recovered sub-clones of 6.5 Kb revealed a full-length open reading frame of 1960 bp having 100% identity to the open reading frame generated by the *N. crassa* genomic project. This clone complemented the *pac*C14 mutation of *A. nidulans*, including remediation of the glycosylation of both the acid and alkaline Pi-repressible phosphatases secreted at pH 5.0, which confirms the involvement of gene *pac*C in the glycosylation of both secreted enzymes.
Financial support: FAPESP, CNPq, CAPES and FAEPA.

5
Characterization of the Neurospora "pathogenicity" signaling pathway.
Daniel Ebbole, Piotr Bobrowicz and Dan Li. Department of Plant Pathology and Microbiology, Texas A & M University, College Station Tx.

A major pathogenicity MAP kinase has been characterized from many plant pathogenic fungi. Although it has been relatively simple to generate MAP kinase mutants in a number of these pathogens it has been less simple to characterize the downstream targets of this pathway that contribute to disease initiation and progression. *N. crassa* contains all of the pathogenesis signalling components that have been characterized in fungal pathogens to date. Several targets of MAP kinase regulation in *N. crassa* have been identified in our laboratory and a number of these resemble virulence factors found in pathogens. Based on our interpretation of this data, we argue that it is straightforward for fungi to evolve the major requirements for pathogenesis through minor modifications of the basic pathways found in saprophytic fungi, such as *Neurospora*.

6
Cloning of ccr100, a *Neurospora crassa* cytochalasin A resistant mutant.
A. Virag, A.J.F. Griffiths. University of British Columbia, Vancouver BC, Canada.

Although the mechanisms involved in branching regulation are still elusive, branching is clearly affected by a multitude of gene products in filamentous fungi. Among these products actin, in the form of microfilaments, is consistently present at sites of hyphal tip growth and sites of branch emergence. We used this observation as a starting point in a genetic approach to identify genes involved in branching in *Neurospora crassa*. In a screen for cytochalasin A resistant mutants we isolated a strain that beside cytochalasin A resistance has a different branching pattern than the wild type strain, and is cold sensitive. The mutated locus was mapped to linkage group V. Sib-selection showed that the fragment that complements the ccr100 phenotype contains the gene encoding actin. A collection of mutant strains of the *ccr100* locus was obtained by RIP. The analysis and comparison of various characteristics of the obtained mutants is presented.

7
An on-line comprehensive gene list of *Neurospora crassa*.
Alan Radford, School of Biology, The University of Leeds, Leeds, UK

While compiling "The Neurospora Compendium", it became apparent that ambiguities had arisen, making our work more difficult where two totally different genes had been given the same symbol and where the same gene had been given more than one. Table 1 in the Compendium lists all the symbols that have been replaced and that are now obsolete synonyms, covering up to mid-2000. Use of these symbols should be avoided. Although the normal rules of precedence apply, there was no comprehensive list of gene symbols and names to use for verification that a symbol or name was available, or in a series of genes with the same root symbol to discover what was the next available number in the series. The FGSC lists only those genes for which it has strains. I have compiled an updatable list in XML format, with sufficient information for identification, which currently requires a recent version of Internet Explorer. The list gives gene name and symbol, plus map location, phenotype, gene product and sequence code if

available. It is being updated as information on new loci and relevant new data on existing loci become available. Updating would be greatly assisted if Neurospora workers routinely sent me details of new genes (a.radford@leeds.ac.uk). A form for convenient data submission is being developed.

8

Locating genes of the cellulase complex of *Neurospora crassa*.

A Radford, School of Biology, The University of Leeds, Leeds, UK.

Yazdi et al (1991) Enzyme Microb Technol 12:120-123 demonstrated the cellulolytic potential of *Neurospora crassa*, and partially characterised members of the complex. Taleb and Radford (1995) Gene 161:137-138 subsequently cloned and sequenced one gene of the complex, the cellobiohydrolase *cbh-1*. Neurospora being a close relative of *Trichoderma reesei*, the cellulase enzyme sequences of the latter were used to probe version 2 of the Neurospora genome database for homologues, using the integral BLAST facility. Five homologues for the EG1/CBH1 family were identified, on contigs 2.521, 2.343, 2.341, 2.681 and 2.236, with the first being the the best match for CBH1 and the second the best for EG1. The other three homologues lacked a cellulose-binding domain, a feature also absent in some members of the complex in other species. Contigs 2.585, 2.732 and 2.237 were homologues of CBH2 with the first of the three being the closest match. Contig 2.20 was the only homologue for EG2. A number of additional homologues were found for the cellulose-binding domain itself.

9

Microsampling and mass spectroscopy of cytoplasm from single fungal hyphae.

Richard J.A. Goodwin[1], C. Logan Mackay[2], Patrick R.R. Langridge-Smith[2], Frank Moffatt[3], Dave Bartlett[3], and Nick D. Read[1]. [1]Fungal Cell Biology Group, Institute of Cell and Molecular Biology, University of Edinburgh, Rutherford Building, Edinburgh EH9 3JH, UK[2]The Experimental Measurement Science Group, Department of Chemistry, University of Edinburgh, EH9 3JJ, UK[3]Syngenta, Jeallot's Hill International Research Centre, Bracknell, Berkshire, RG42 6ET, UK

We have developed techniques to analyse the biochemistry of *Neurospora crassa* at the single cell level. This radically new approach to fungal biochemistry is fundamentally different from the more traditional "mince-and-measure" analytical techniques. Procedures for microsampling from a single hypha, transferring the microsampled cytoplasm to a capillary electrophoresis column and analysis of the separated sample using mass spectrometry, have been accomplished. Both the *Neurospora* wild type and *hex-1* mutant, which is unable to block its septal pores allowing larger volumes of cytoplasm to be microsampled, have been used in these studies. Typically the volume of cytoplasm analysed has varied between 100 and 1000 pl.

10

Measuring calcium dynamics in living *Neurospora* hyphae using recombinant aequorin.

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Indirect evidence in the literature has suggested the involvement of Ca^{2+} signalling in regulating hyphal branch formation in *Neurospora crassa*. For example, hyperbranching can be induced by various Ca^{2+} modulators, or by genetical impairment or pharmacological perturbation of calmodulin/calcineurin. To obtain more direct evidence regarding the role of Ca^{2+} signalling in hyphal branching we have developed a technique using the Ca^{2+}-sensitive luminescent protein aequorin, which allows cytosolic free Ca^{2+} ($[Ca^{2+}]c$) measurement in living hyphae. Aequorin, originally from the jellyfish *Aequorea victoria*, responds to free Ca^{2+} by emitting light in a dose-dependent manner. Several strains of *N. crassa* were transformed with the codon optimised *gnaeqS* gene encoding aequorin. Based on their *in vivo* aequorin activity, the successful transformation of wild type, *cot-1*, *frost*, *spray*, and *T3* (harboring an inducible *cna-1* antisense expression cassette) strains of *N. crassa* has been shown. These strains are now being used to analyse the role of Ca^{2+} signalling in regulating hyphal branching.

11

Meiotic Silencing by Unpaired DNA (MSUD).

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To defend against the invasion of virus, eukaryotes have evolved a number of mechanisms to deal with DNA segments that are present in an inappropriate number. An example is Post-Transcriptonal Gene Silencing, in which a dsRNA species (produced by a RNA-directed RNA polymerase) is cut into fragments of 21-23 nucleotides (siRNA). These fragments act as guide RNAs for the cleavage of homologous cytoplasmic mRNA. We have discovered a related system of targeted gene silencing that operates after karyogamy, Meiotic Silencing by Unpaired DNA (MSUD; Cell 107:905-916). In the MSUD system, DNA unpaired with a homologous sequence during meiotic prophase generates a sequence-specific signal, presumably an RNA. This signal destroys existing transcripts originating both from unpaired and paired DNA sequences homologous to it. We have isolated a mutant that fails to perform MSUD. *Sad-1* suppresses several classical ascus-dominant mutants, suggesting that these, too, owe their ascus dominance to the MSUD mechanism. In addition, *Sad-1* suppresses the near-absolute barren phenotype of crosses between wild type and a variety of strains in which a segment of DNA is duplicated. Finally, interspecies crosses within the genus Neurospora that are normally almost completely infertile become much fertile if the *N. crassa* parent is a *Sad-1* mutant, suggesting that MSUD due to numerous small mispairings play a role in reproductive isolation of these species. *sad-1+* encodes a RNA-directed RNA polymerase.

12

A quick and easy way to determine gene function in meiosis using MSUD.

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DNA unpaired in meiosis causes silencing of all DNA homologous to it, by a process called Meiotic Silencing by unpaired DNA (MSUD). MSUD can be triggered in a cross containing three copies of a gene, i.e., between a wild-type strain and a strain containing two copies of a gene. Silencing by an ectopic transgene using the MSUD system is potentially a quick and easy tool to determine the role of genes in meiosis and ascospore development. The advantages can be summarized as follows. 1) Isolating a strain containing a duplicated gene segment is considerable faster and easier than isolating a RIP or gene-replacement mutant. 2) MSUD can be performed on genes for which a knockout construct would be lethal in the vegetative phase. 3) The effect of meiotic silencing will not begin until after karyogamy, allowing the investigators to determine the true role of a gene during sexual development. Clearly, this method can used to idendity gene functions in Neurospora that govern meiosis, post-meiotic mitoses and ascospore maturation. The stage at which development is arrested has been determined for several genes using the MSUD system. They include *asm-1* (white aborted ascospores), *mei-3*, which encodes the RecA/RAD51 protein (blocked in meiosis I), and genes that encode beta-tubulin (arrest before metaphase I), actin (lollipop asci and giant spores), histones H3/H4 (ascospores do not mature), and plasma membrane ATPase (bubble asci that do not mature).

13

Cloning and characterization of *scon-3*$^+$: A new member of the *Neurospora crassa* sulfur regulatory system.

Steven T. Sizemore and John V. Paietta. Biochemistry and Molecular Biology, Wright State University, Dayton, OH, USA.

In *Neurospora crassa*, a group of sulfur-regulated structural genes (e.g., arylsulfatase) are under the coordinate control of the CYS3 positive regulator and several SCON (sulfur controller) negative regulators. The negative regulator SCON2 encodes a protein containing the F-box motif; a motif involved inprotein-protein interactions. In *S. cerevisiae*, and other organisms, an F-box protein along with Cdc53p (cullin), and Skp1p form a complex, referred to as the SCF, which recruits specific substrates for ubiquitin-mediated proteolysis. The F-box protein provides target specificity for these complexes and the F-box motif is required for interaction with Skp1p, and therefore complex formation. SCON2's regulatory role may be attributed to its ability to form an SCF-complex and target specific substrates (e.g., CYS3) for rapid degradation. We have cloned the *N. crassa* homolog to SKP1, which we have designated *scon-3*$^+$. The *scon-3*$^+$ gene encodes a polypeptide of 171 amino acids interrupted by two introns of 80 bp and 98 bp respectively. Through yeast two-hybrid experiments, we demonstrate that SCON3 interacts with SCON2 in a manner dependent

upon the F-box motif of SCON2. Further, Northern blot analysis provides evidence that expression of *scon-3*$^{+}$ is regulated by sulfur availability.

14

Antioxidant defence systems of *Blakeslea trispora* and *Neurospora crassa*.

Tatyana Belozerskaya, Alexander Sokolov, Natalya Gessler. A.N.Bach Institute of Biochemistry, Moscow, Russia.

Comparative investigation of antioxidant defence mechanisms of *B.trispora* and *N.crassa* has been performed. Activation of constitutive SOD was observed in the mycelium of *N.crassa* in the dark in presence of trace amounts of carotenoids upon introduction of menadione. Neurosporaxanthin increase was found in *N.crassa* under the influence of light together with activation of SOD and CAT. SOD activity was significantly lower in mixed (+/-) cultures of *B.trispora* able to synthesize b-carotene in the dark. Further decline in SOD activity, and a decrease in CAT activity together with an increase of b-carotene was observed in *B.trispora* under oxidative stress. Thus, in *N.crassa* neurosporaxanthin acted mainly as photoprotector whereas in *B.trispora* b-carotene functioned as the main antioxidant on the background of inactivation of enzymes detoxifying active oxygen species. In the nap strain of *N.crassa* with increased level of carotenoids SOD activity was much lower and varied from 11 to 17% of the wild type.

15

Construction of *Neurospora* microarrays and its application for evolutionary study

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Rates of adaptation and speciation are generally not correlated with rates of molecular evolution at the genome level. A resolution to this paradox is that in order to adapt to new environments, organisms may modify a small number of regulatory genes, which consequently alter the timing of expression of a number of key genes, rather than de novo adaptive mutations. Until recently, however, it was virtually impossible to identify and quantify which genes in the genome are responsible for changes in fitness and speciation. Microarray technology enables us to assess the expression of thousands of genes simultaneously, and thereby potentially reveals the differences in gene expression between closely related species or subpopulations. A cDNA library of *N. crassa* is being constructed at the UNM. Using a portion of this library we have generated a preliminary cDNA microarray of approximately 550 genes. Natural isolates of *Neurospora* may be found growing on burned substrates over a wide geographic range. We are currently analyzing the differences between *N. crassa* isolates from different geographical regions and different *Neurospora* species in response to a particular stress or growth condition. These results will allow us to assess how genetic differences contribute to gene expression differences under identical laboratory conditions. Eventually, we intend to assess gene expression patterns of different *Neurospora* isolates in nature. Such information will help to understand the mode of evolution of *Neurospora* in the environment.

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Sterol Fingerprints of *pk* a complementary tool to Genetic Analysis.

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Among the fungi *Neurospora crassa* is one of the best-characterized genetic model. It is also one of the fastest growing fungi with a lateral branching system. Tip growth and branching in *N. crassa* remain poorly understood. Although molecular and morphological characterization of this fungus has been well researched, few studies have been devoted to biochemical analyses. Sterol glycosides and cerebrosides are an important component of the fungal cell wall, and may be involved in morphogenesis and branching. These compounds have been associated with the biological processes of growth, development, and signal transmission in many organisms. We are developing chemical fingerprints to determine whether mutant strain *pk* and similar strains are deficient in certain steps in ergosterol biosynthesis. Chemical analyses will include TLC, HPLC, GC, and other physico-chemical methods. Mutant strains deficient in ergosterol biosynthesis share morphological characteristics with pk. The deficiencies include slow growth,

sterile females, and poor conidia formation. Backcrossing and examination of double mutants obtained form *pk* and similar strains, may provide insight on the metabolic blocks affecting *pk* branching.

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Interactions of genes during *Neurospora crassa* development
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There are more than 100 loci that encode products that can affect tip growth and branching in *Neurospora crassa*. In order to dissect the genetics of branching we have examined the functional relationship between 35 of these loci. By using epistasis analysis some of these genes were grouped into common developmental pathways. We obtained double mutants through ascus analysis. In most cases,double mutants show a phenotype more severe than either single mutant alone, indicating that each mutant probably affects separate pathways involved in growth and branching. However, several double mutants were found to closely resemble one of the parental strains in morphology. This suggests both genes are part of a single developmental pathway, with the epistatic gene acting before the hypostatic one. The presented analysis gave a rudimentary characterization of a pathway involved in tip growth and branching.

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The identification of *vib-1*, a gene involved in vegetative incompatibility mediated by *het-c* locus in *Neurospora crassa*.
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Vegetative incompatibility is ubiquitous in filamentous fungi. It is controlled by *het* loci. One way to explore the molecular mechanisms controlling vegetative incompatibility is to isolate suppressors. In this study, three suppressor deletion mutants, *ahc* (Aerial hyphae, Hyphae fusion and Conidiation), *vc1* (Vegetative incompatibility and Conidiation) and *vc2* were identified from the strains that "escaped" from *het-c* vegetative incompatibility. The deletions in *ahc*, *vc1* and*vc2* mutants are ~26kpb, 19442bp and ~7kbp long, respectively. There are also unknown insertional fragments in *ahc* and *vc2* deletion regions. The three deletions map to chromosome V, between *lys-2* and *ilv-2*. They overlap each other in an ORF named as *vib-1* (Vegetative Incompatibility Blocked). Single *vib-1* mutants were also generated by RIP mutagenesis. Mutations in *vib-1* fully relieve inhibition of mycelial growth and suppression of conidiation conferred by *het-c* vegetative incompatibility and significantly reduce the rates of hyphal compartmentation and death. The *vib-1* mutants have a copious conidiation pattern, suggesting that VIB-1 is a conidiation suppressor. VIB-1 has a region sharing high similarity to PHOG, a possible phosphate non-repressible acid phosphatase in *Aspergillus nidulans.* Our native gel analysis, however, shows that *vib-1* is not a structural gene for non-repressive acid phosphatase. It may be, however, involved in the regulation of the activity of phosphate non-repressible acid phosphatase. VIB-1 is a putative nuclear protein due to the presence of a bipartite NLS (nuclear localization signal). We are conducting experiments to determine whether or not VIB-1 acts at a transcriptional level to mediate vegetative incompatibility, suppress conidiation and activate phosphate non-repressible acid phosphatase.

19 ***Neurospora* G gamma subunit (GNG-1) identification and characterization.**
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The G-protein-linked pathways evolved to respond to extracellular agonists in cells ranging from yeast, filamentous fungi and slime molds to mammals. In filamentous fungus *Neurospora crassa* genetic studies and genomic sequencing revealed at least 7 G-protein coupled receptors (GPCRs), three G alpha subunits (GNA-1, GNA-2, GNA-3), one G beta subunit (GNB-1), and one G gamma subunit (GNG-1). The previous genetic and biochemical studies of all three G alpha subunits, and the G beta indicate their roles in apical growth rate, aerial hyphae formation, nutritional sensing, female fertility and mating. The putative protein sequence of GNG-1 shows a COOH-terminal CAAX motif of isoprenylation commonly found in G gamma subunits (CVVM). GNG-1 is closely related to *Saccharomyces cerevisiae*, STE18 (35% identity) and non-visual mammalian G gamma subunits (33% identity). A delta *gng-1* mutant has been constructed and characterized. Phenotypic analysis of *gng-1* mutant shows a slow apical growth rate on solid medium, inappropriate conidiation in submerged culture, short aerial hyphae formation in standing liquid cultures and inability to develop female reproduction structures. The *gng-1* deletion impacts levels of all G alpha proteins and G beta protein in plasma membrane fraction, and causes reduced intracellular cAMP levels in cultures grown on solid medium.

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Identification and characterization of a *crnA* related nitrate transporter gene in *Neurospora crassa*.

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Neurospora crassa genome database was searched for sequence similarity to *crnA*, a nitrate transporter in *Aspergillus nidulans*. A 4 kb fragment (contig 2.585, subsequence [183125, 187125]) was cloned by PCR and was termed nit-10. In *Aspergillus nidulans*, *crnA* is clustered together with *niaD*, encoding nitrate reductase, and *niiA*, encoding nitrite reductase. However, our RFLP mapping results indicated that nit-10 is not linked to any genes known so far involved in nitrate assimilation such as nit-3 (nitrate reductase), nit-6 (nitrite reductase), nit-2, nit-4 (both are positive transcription regulators of nit-3), and nmr (negative regulator of nit-3). A nit-10 rip mutant was generated by crossing a normal wild type strain with a derived wild type strain carrying two copies of nit-10 the endogenous copy and a second one obtained by transformation. The mutant showed poor growth when nitrate was used as the sole nitrogen source. In addition, it showed strong sensitivity to cesium in the presence of nitrate and resistance to chlorate in the presence of nitrate, alanine, proline, or hypoxanthine. The transcripts of nit-10 were analyzed by Northern blots. The expression of nit-10 requires nitrate induction and is subject to feedback repression by nitrogen metabolites such as glutamine or ammonium. The transcripts of nit-10 could not be detected in either nit-2 or nit-4 mutants. This suggested that the products of nit-2 and nit-4 mediate the expression of nit-10. The half-life of nit-10 mRNA was determined by Northern blots after transferring wild type cells from an induction (nitrate) to a repression (glutamine) environment. It was found that half-life of nit-10 mRNA is approximately 4 minutes. In a similar experiment, except order of incubation was reversed (repression to induction), we found that nit-10 transcripts were induced after 30 minutes exposure to nitrate.

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Hyphal fusion pathway of *Neurospora crassa*

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Cell fusion is presumably an important factor in the growth and development of multicellular organisms. During the vegetative growth of filamentous fungi, there are many cell (or hyphal) fusion events within a colony or between colonies. Because filamentous fungi grow rapidly and extensively, hyphal fusion may be necessary to facilitate the sharing of resources or serve as a means of communication within the colony. In the model filamentous fungus, *Neurospora crassa*, the processes of hyphal fusion have been described microscopically. It is apparent that the hyphae respond and grow towards each other before hyphal fusion occurs. However, the molecular mechanism has not been characterized. Recently, a hyphal fusion mutant gene, *ham-2* (for hyphal anastomosis) has been cloned. The mutant has a greatly reduced ability to fuse with itself or with wild type strains. The ability of the *ham-2* mutant to fuse is reduced by a factor of a thousand fold when compared to wild type. Other phenotypes of the *ham-2* mutant strains include a reduced growth rate, short aerial hyphae and female sterility. These phenotypes may be caused by a defect in hyphal fusion. Currently, we are working to understand how HAM-2 is involved in hyphal fusion by looking at the localization of the protein and the mRNA expression pattern. Computer prediction models predict that HAM-2 is localized to the plasma membrane. We hypothesize that HAM-2 could be involved in the signaling pathway of hyphal fusion. Genetic analysis has indicated that a MAP kinase pathway mediates the process of hyphal fusion. We are assessing whether or not HAM-2 is regulated by or regulates a MAP kinase signaling cascade.

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Genetic analysis of temperature-sensitive mutant for *het-c* mediated vegetative incompatibility in *Neurospora crassa*

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Different fungal individuals are able to undergo hyphal fusion with each other. This event results in the vegetative heterokaryons containing two different nuclei in a common cytoplasm. The stability of heterokaryotic cell is controlled by *het* loci. When individuals have one or more different specificity at *het* loci, these heterokaryotic cells are unstable and usually undergo hyphal compartmentation and cell death. This phenomenon is called vegetative incompatibility. In forced heterokaryons and partial diploids at *het* loci, suppressor mutants of vegetative incompatibility are frequently

generated by spontaneous mutation called "escape". One suppressor mutant of *het-c* mediated vegetative incompatibility was identified from incompatible transformants of a *het-c*^{PA} strain transformed with a *het-c*^{OR} allele. This escape strain showed temperature-dependent vegetative incompatibility with *het-c*^{OR} strains in heterokaryon tests. Heterokaryons were incompatible at 20ºC, but compatible at 34ºC. Heterokaryon tests with *het-c*^{PA} strains showed incompatibility at both temperatures. Progeny containing the *het-c*^{PA} allele were isolated from a cross with a wild-type *het-c*^{OR} strain. These progeny had no functional *het-c*^{OR} allele and maintained temperature-dependent vegetative incompatibility with *het-c*^{OR} strains. When these progeny were crossed with wild-type *het-c*^{OR} strains, temperature-dependent phenotype co-segregated with *het-c*^{PA} phenotype. This phenotype was not complemented by introduction of a wild-type *het-c*^{PA} allele. These results indicate that the locus associated with temperature-dependent vegetative incompatibility is closely linked to the *het-c* locus.

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Comparing biological and phylogenetic species recognition in *Neurospora.*

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We are using *Neurospora* to provide a benchmark for recognizing fungal species. Under Simpson's Evolutionary Species Concept, we have compared Biological Species Recognition using mating tests with Phylogenetic Species Recognition using concordance of gene genealogies. The foundation for recognizing phylogenetic species is a comprehensive phylogeny of 147 isolates of all the conidiating *Neurospora* species (presented separately at this meeting; Dettman, et al.). Simultaneously, Biological Species Recognition was examined by crossing a subset of 73 strains of *N. crassa, N. intermedia,* and putative *N. crassa/N. intermedia* hybrids in an experiment designed to be independent of any phylogenetic results. Mating success from 894 crosses was used to circumscribe reproductively isolated groups. Statistical comparisons showed a generally strong correlation between species recognized by biological and phylogenetic criteria with at least five species detected within the *N. crassa/N. intermedia* complex. However, two important areas of incongruence should be noted. First, in both *N. crassa* and *N. intermedia,* two or more phylogenetic species were recognized in a single biological species, indicating that genetic isolation can precede reproductive isolation. Second, two biologically recognized species were found in a single *N. crassa* phylogenetic species, indicating that reproductive isolation can occur before genetic isolation can be detected, even though the marker loci were polymorphic within each biologically recognized species. When reproductive isolation occurs in a distinct geographic region, it provides support for the controversial concept of sympatric speciation. Moreover, the incongruence of phylogenetically and biologically recognized species suggests that speciation mechanisms within *Neurospora* are complex. While neither method of species recognition is superior to the other, when combined results suggest that species recognized by either methodare, in nature, on a trajectory to genetic and reproductive isolation and deserving of the species rank.

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Spore killer sensitive isolates become the killers in heterokaryons.

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Meiotic drive acts during or after meiotic divisions to diminish the transfer of a sensitive gene or chromosome region to the progeny. In Neurospora the Spore killer variants perform this activity. In matings between Spore killer and Spore killer sensitive strains, the progeny are almost exclusively Spore killers. The four ascospores with sensitive linkage group III regions abort after the second mitotic division and the four ascospores with the killer chromosome region survive (Turner and Perkins, Genetics 93: 587-606, 1979). When heterokaryons of Spore killer-2 (*Sk-2K*) and sensitive (*Sk-2S*) are formed using forcing auxotrophic markers, the conidia which germinate and form colonies are almost exclusively *Sk-2S*. This is the reverse of the mating challenge. Spore killer-3 and *Sk-3S* do not have this interaction. Equal types of both *Sk-3K* and *Sk-3S* heterokaryon constituents are observed as well as heterokaryons with both nuclear types if colonies are grown from plated conidia. When Spore killer-2 and Spore killer-3 are mated, there is almost exclusively mutual destruction with very few survivors and those are not either parental type. In contrast,

when *Sk-2K* with *Sk-3K* heterokaryons are formed and conidiospores are plated, the *Sk-3K* class is almost exclusively the survivor and *Sk-2K* colonies are rare. The mechanism of killing in either ascospores or conidia is unknown.

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Phylogenetic species recognition in *Neurospora*.
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Under an Evolutionary Species Concept, we compared phylogenetic species recognition with existing biological species recognition in the outcrossing, conidiating species of *Neurospora*. The five described biological species were represented by 147 strains from a wide geographic distribution; 128 of these were from the *N. crassa/N. intermedia* species complex, including nine strains described as putative *N. crassa/N. intermedia* hybrids. All strains previously had been identified by mating with designated tester strains. A comprehensive molecular phylogeny was constructed from four non-coding nuclear loci, and phylogenetic species were delineated by the concordance of gene genealogies. Several phylogenetic species were well supported, but the relationships among them were not. In general, the five described biological species corresponded well with phylogenetic species. The *N. crassa/N. intermedia* species complex, unresolved in previous phylogenetic studies, was resolved here into a large clade of each *N. crassa* and *N. intermedia*, and at least three additional phylogenetic species. None of the putative *N. crassa/N. intermedia* hybrids appeared to be true hybrids. While some clades showed geographic correlation, no major phylogeographic trends were consistent across all species. Simultaneously, biological species recognition within the *N. crassa/N. intermedia* complex was examined in a subset of 73 strains by nearly 900 mating tests (Jacobson et al. poster). Whereas phylogenetic and biological species recognition were generally congruent, there were several cases where phylogenetic methods recognized species in the *N. crassa/N.intermedia* complex not recognized by mating experiments, and one reverse case. These results also show that neither phylogenetic nor biological species recognition, alone, can uncover all the *Neurospora* species; both are needed, especially if isolates will be used in subsequent genetic and/or population studies.

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***Neurospora* in western North America.**
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Recent surveys have clearly established that species of *Neurospora* are common primary colonizers of trees and shrubs killed by wildfires in forests and woodlands of western North America. These studies substantially expand the known distribution and habitats of a genus best known from the tropics and subtropics. *Neurospora* species have been observed at 33 fire sites in habitats ranging from cottonwood stands along the Rio Grande to mountain forests in New Mexico, California, Nevada, Idaho, Montana and Alaska. Colonization occurs beneath the bark of diverse deciduous and conifer hosts. The combined 2000-2001 collection includes more than 500 isolates from 35 degrees to 63 degrees north latitude and from 515 m to >2400 m elevation. Approximately 95% of the collected isolates have been identified as *N. discreta.* Important questions remain regarding the reservoirs of inoculum, modes of colonization and dispersal. Perithecia have been observed at only one burn site, despite the fact that isolates can be recovered from soils within and adjacent to burn sites using plating procedures that select for ascospore-derived individuals. Within a site, mating type among individuals is sometimes significantly skewed from a 1:1 ratio. Nevertheless, evidence suggests substantial diversity on small and large spatial scales, as has been observed previously for isolates from Florida and Louisiana. Both mating types are present at western sites, ascospores can be obtained from crosses in the laboratory, and genetic variation independent of mating type exists within and between sites (at the *het-c* locus, for example).

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Molecular analysis of hyphal anastomosis.
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Growth in filamentous fungi occur through combinations of hyphal tip extension, branching and fusion(anastomosis). Initial live cell imaging as well as mutational analysis indicate the possible role for MAPK signaling pathway in hyphal

fusion. We have four hyphal fusion defective mutants-*so-1*, *ham-2* ,*nrc-1* and *mak-2*. All show pleiotropic effects on growth and reproduction.These include slower growth rate, lack of aerial hyphae, female sterility and reduction or complete lack of self fusion or formation of a heterokaryon with a wild type strain. A wild type copy of *nrc-1* is able to complement conidiation defect, growth rate, aerial hyphae production as well as restoration of self hyphal fusion and form heterokaryons with wild type strain. Two mutants, *nrc-1* and *mak-2* are known to have MAPKK and MAPK homologs respectively in yeast. We have created a dominant activated allele of NRC-1 by expressing only the carboxy terminal kinase domain. This allele will be introduced into wild type, *nrc-1* null mutant , *ham-2* and *mak-2* to see what the effect(s) of expression of the activation domain is. Future work will also investigate the levels of phosphorylation of MAK-2 in a dominant activated *nrc-1* mutant using phospho-specific antibodies to ERK-2. These experiments will indicate the relationship between *nrc-1* and *mak-2*-whether they are involved in the same signaling pathway or whether these mutations affect different pathways but result in a similar mutant phenotypes.

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Comparative analysis of two-component histidine kinases in *Neurospora crassa* and the fungal pathogen *Cochliobolus heterostrophus*.

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Two-component signal transduction systems have been found in bacteria, plants, fungi, and slime molds. These signaling systems often function to allow the organism to sense and adjust to changing environmental conditions. These systems consist of a histidine kinase component, that autophosphorylates in response to an environmental stimulus, and a response regulator component. Most eukaryotic histidine kinases are hybrid, containing both the histidine kinase and the response regulator within the same protein. *N. crassa* (WICGR 2nd assembly) contains at least eight putative hybrid histidine kinases while *C. heterostrophus* contains at least twelve. Only six of these histidine kinases appear to be orthologs common to both *C. heterostrophus* and *N. crassa*. The remaining six *C. heterostrophus* histidine kinases may be unique to *Cochliobolus* or to fungal pathogens. Deletions strains for each *C. heterostrophus* histidine kinase are under investigation.

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A further study of the *cr-5* mutant of *Neurospora crassa*.

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The *crisp-5 (cr-5)* mutant (allele 123 CJ13-6A) of *N. crassa* was isolated in the Georgia Southern Neurospora Laboratory and was found to be linked to *ad-4* in LG III. The present study mapped *cr-5* to the right of *ad-4* with 1.3% to 5% recombination. Both *cr-5* and wild type 74A were grown on minimal medium in which the carbon source was 2% sucrose, 2% glucose, 2% fructose, 2% lactose, 2% mannitol, 2% glycerol, or 2% sorbose. The *cr-5* mutant grew better on glucose and fructose separately than it did on sucrose. Both the mutant and wild type grew very poorly on lactose, mannitol, and glycerol. Neither strain tested grew on sorbose as the sole carbon source. Observations utilizing SEM indicated that, compared to wild type 74, *cr-5* has a reduced mycelium containing clusters of conidia formed by highly branched conidiophores carried on short aerial hyphae. The mutant *cr-5* conidiates prematurely and profusely. Conidia of *cr-5* consistently germinated earlier than those of wild type, but the percent of conidia germinating one hour after the first observation of germination was higher for wild type.

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Characterization of osmotic sensitivity of *os-8, os-9, os-10,* and *os-11*.

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Osmotic-sensitive mutants of *N. crassa* are identified by their failure to grow on medium with elevated concentrations (4% or 6%) of NaCl. A number of osmotic-sensitive mutants have been isolated in our laboratory. Two of these, *os-9*

(alleles SS-788 and SS-462) and *os-11* (allele SS-18) had been found to fail to grow on medium with elevated NaCl, but to grow on medium with elevated KCl or glucose. This was in contrast to the first described osmotic mutant, *os-1*, which was sensitive to all three osmotica. In the present study two more of our mutants *os-8* (allele SS-931) and *os10* (allele SS-1018) were examined. The mutant *os-8* failed to grow on medium with 2% NaCl, with 4% KCl or 6% glucose. The mutant *os-10* failed to grow on medium with 5% NaCl, with 3% KCl or 3% glucose. To further characterize the osmotic sensitivity of *os-9* and *os-11*, revertants of the mutants were obtained. Both mutants were crossed to the *trp-1* tester strain and double mutants were recovered. Double mutants of *os-9* and of *os-11* were each subjected to UV irradiation. A total of 51 and 47 putative revertants of *os-9* and *os-11*, respectively were recovered.

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Neurospora proteins that bind methylated DNA and DNA mutated by RIP.
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Using gel-mobility-shift assays we have detected proteins in *Neurospora crassa* that bind methylated DNA. One such factor binds most efficiently to DNA that is both methylated and contains mutations induced by RIP. We refer to this factor as MRBP-1 (Methyl/RIP Binding Protein 1). MRBP-1 was purified and peptide sequence data obtained using mass spectrometry. These data suggest that MRBP-1 might be a complex of several proteins. Methyl-DNA binding proteins may function "downstream", exerting their effects after methylation has been set up (eg. repressing gene expression). These factors may also be involved in maintaining methylation patterns in Neurospora. To test these possibilities we are introducing mutations in genes encoding the potential MRBP-1 complex components.

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Analysis of M-134, a crisp-like morphological mutant of *Neurospora crassa*.
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M-134, a mutant strain of *N.crassa*, was isolated using ultraviolet irradiation in the Georgia Southern University Neurospora Laboratory. Preliminary mapping of M-134 indicated that the mutant is in Linkage Group I and linked to *lys-4* by 9% recombination. Scanning electron microscopy was used to compare differences between M-134 and wild type *N. crassa*. Both strains were grown on dialysis tubing coated with minimal medium, fixed, dehydrated, and sputter coated. Examination of the specimens showed that M-134 had reduced mycelium and highly developed conidiophores with primary, secondary, and tertiary branching when compared to the wild type *N. crassa*. Microscopy also revealed flask shaped clusters of conidia and moderate proconidial chains. Following 6 days of growth, clusters of densely packed conidia were observed on a reduced mycelial mat.

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Characterization of cobalt-sensitive mutant of *Neurospora crassa*.
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Cobalt-sensitive mutants of *N.crassa* (CSM) were isolated by mutation enrichment protocols. CSM isolates showed differences in growth, morphology and sporulation. Single spore isolates of CSM showed that most of them to be 5-fold sensitive to cobalt. A CSM with good sporulation and growth was chosen for further experiments. CSM is 5-fold more sensitive to cobalt ions (I-50 = 0.1mM) on ammonium-N medium as compared to wild type N.crassa (I-50 = 0.5mM). Cobalt sensitivity was also observed on nitrate-N medium. Cross-sensitivity to Ni was also observed in CSM. Cobalt taken up by wild and CSM at their respective I-50 values was found to be similar, indicating that CSM accumulates more cobalt than wild type. Most of cobalt accumulated by wild type *N.crassa* was on surface (70%) while in case CSM it was in intracellular fraction. Fractionation of cell free extracts by DEAE-cellulose chromatography resolved cobalt in to protein-bound and free ionic fractions. No significant differences in cobalt

distribution were observed between wild and CSM *N.crassa*. Mg ions that reverse the toxic effects of cobalt ions by suppressing transport in wild type were unable to do so in CSM. The above data suggest that the mechanism of increased toxicity of Co in CSM is due to hyperaccumulation of these ions resulting from modifications in cell wall and membrane transporter(s). In preliminary experiments some of the mutants showed features related to vacuolar mutants and hence *N.crassa vma-1* was tested and found to be sensitive to cobalt, suggesting the role of Ca homeostasis in CSM. Mapping of CSM loci is in progress. The utility of CSM in bioremediation of toxic metal ions from low concentrations will be presented.
Acknowledgement: This work was supported by DAE grant No. 2000/37/20/BRNS

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The search for roles in gene silencing and cell differentiation of the asd-2 gene, an essential gene for sexual development in Neurospora crassa.

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Investigation into genes responsible for Neurospora crassa sexual development has led to the discovery of the asd-2 (ascus development) gene. The protein product of this gene has been found to be similar to the AGO/eIF2C2/Zwille family of proteins. This family has been implicated in two major processes: involvement in cell differentiation and involvement in posttranscriptional gene silencing. One member of this family, the N. crassa QDE-2 (quelling deficient) protein has been shown to be involved in quelling (posttranscriptional gene silencing). Initially, the asd-2 gene was identified as essential for sexual development; homozygous asd-2 crosses are blocked shortly after karyogamy. Ascospores are never produced and asci are short and few in number. Small perithecia are formed that lack perithecial beaks. Recently, we have shown that the asd-2 gene is not essential for quelling as quelling does occur in the asd-2 mutant strain. The mutant strain exhibits slow vegetative growth with an aberrant hyphal branching pattern as compared to wild-type. We are investigating the role of the asd-2 gene product in cell signaling and/or cell differentiation and the relationship of this role to sexual development.

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A report on the recent activites of the Fungal Genetics Stock Center.

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The Fungal Genetics Stock Center has continued to support both traditional genetic approaches and emerging molecular approaches towards working with Neurospora and other fungi. The FGSC has distributed thousands of fungal strains to researches around the world and continues to develop its holdings of Neurospora and related fungi. While our traditional focus has been on fungal strains, the inclusion of cloning vectors, gene libraries and cloned genes has enabled the FGSC to expand its reach into areas not served by its traditional materials. Beyond this, the FGSC has become the recognized home of fungal genetics on the internet. In our continuing effort to serve the fungal genetics research community, the FGSC always welcomes input and ideas from our constiuency.

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