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Abstract

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TWENTIETH FUNGAL GENETICS CONFERENCE SCIENTIFIC PROGRAM

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Plenary session abstracts

Session I: REGULATION OF GENE EXPRESSION Chair: Eric Selker

Vegetative incompatibility in *Podospora anserina*, characterization of genes induced during non-allelic incompatibility. Joël Bégueret, Institut CNRS de Biochimie et Génétique Cellulaires, Bordeaux, France.

In *P. anserina*, nine *het* loci have been identified by genetic analysis of wild-type isolates. Five of these loci are involved in allelic incompatibility systems as described in *Neurospora. crassa* and other fungi. However in *Podospora*, genetic interactions between non-allelic *het* genes have also been described. In these cases, the incompatibility reaction is triggered by the coexpression of *het* genes that belong to different loci. Five *het* genes involved in such non-allelic incompatibility systems have been identified, they define three incompatibility systems, het-R/het-V, het-C/het-E and het-C/het-D. We have used the thermosensitivity of the het-R/het-V system to identified genes whose expression is induced during the progress of the incompatibility

reaction. Four genes, named *idi* (for induced during incompatibility) have been characterized. *idi-1*, *2* and *3* encode small polypeptides that contain a signal peptide. Using GFP labelling, the idi-1p was found to be located in the septa. *idi-4* encodes a transcription factor containing a b-ZIP domain. The expresion of *pspA*, a gene coding for a subtilisine-like protease is also highly enhanced during incompatibility. We observed that the expression of most of these genes is also induced when incompatibility is released by the other non-allelic incompatibility system, het-C/het-E, but not by the allelic system het-s/het-S. Some of these genes are also induced under glucose and/or nitrogen starvation suggesting a functional relationship between the cell death reaction induced during starvation or by the coexpression of non-allelic *het* genes.

Translational control of gene expression.
Matthew S. Sachs, Department of Biochemistry and Molecular Biology, Oregon Graduate Institute, Portland OR 97291-1000.

Short peptide coding regions (upstream open reading frames or uORFs) in the 5'-leaders of eukaryotic mRNAs can serve critical regulatory functions. uORFs are found in many mRNAs in filamentous fungi. A single uORF with an evolutionarily conserved peptide sequence is found upstream of the structural genes for the small subunit of arginine-specific carbamoyl phosphate synthetase from *Neurospora crassa*, *Magnaporthe grisea*, *Trichoderma virens*, *Aspergillus nidulans* and *Saccharomyces cerevisiae*. The *N. crassa* uORF specifies a 24-residue peptide named the arginine attenuator peptide (AAP) because it is involved in negative, Arg-specific translational regulation. *In vivo*, the *N. crassa* AAP down-regulates translation of ARG2 in response to Arg by reducing the average number of ribosomes associated with the *arg-2* mRNA. AAP-mediated translational regulation has been reconstituted in an *N. crassa* cell-free translation system. A primer extension inhibition assay has been used to map the positions of ribosomes on capped and polyadenylated synthetic RNAs added to this system. Arg causes ribosomes to stall soon after they have translated the AAP. Arg-specific ribosome stalling is proposed to result in Arg-specific negative regulation because such ribosomes would block ribosomal scanning from the 5'-end of the mRNA and therefore block trailing ribosomes from translating ARG2. The AAP amino acid sequence, but not the RNA sequence encoding it, is critical for regulation. AAP translation can cause stalling of ribosomes involved in termination or elongation. Regulation by these evolutionarily conserved fungal leader peptides represent a novel mechanism of *cis*-acting translational control.

Chromatin modulation and regulation of pathogenic development in the smut fungus *Ustilago maydis.*
Joerg T. Kaemper, Michael Reichmann, Claudia Quadbeck-Seeger, and Regine Kahmann. Ludwig-Maximilian-University, Genetics, Munich, Bavaria, Germany.

In the phytopathogenic fungus *Ustilago maydis* the multiallelic *b* mating-type locus represents the central control locus for sexual and pathogenic development. The *b* locus encodes a pair of unrelated homeodomain proteins termed bE and bW that form heterodimers when originating from different alleles; the heterodimer is presumed to regulate pathogenicity genes, either directly by binding to cis regulatory sequences (class 1 genes), or indirectly via a *b*-dependent signal cascade (class 2 genes). Using a bE/bW fusion protein we were able to isolate the first direct target for the bE/bW heterodimer in the promoter of *lga2*, a gene located in the *a* locus.

This sequence is bound by the bE/bW heterodimer and mediates the *b*-dependent regulation of the gene *in vivo*. In a screen for components of the *b*-dependent signal cascade we have isolated two different genes, one coding for a histone deacetylase (Hda1), the other one for a protein (Rum1) with similarities to the human retinoblastoma binding protein 2. Both genes are essential for the establishment of a repressed state of several class 2 genes in the absence of the b heterodimer. We propose that both Hda1 and Rum1 are in a complex with other proteins with at least one of them allowing sequence specific DNA binding. In this model, the regulation of gene activity is achieved by modulation of the chromatine structure mediated by the action of the histone deacetylase Hda1.

Genome defense in Neurospora.
Eric Selker, Joseph Dobosy, Michael Freitag, Shan Hays, Greg Kothe, Elena Kuzminova, Brian Margolin, Johanna Swanson and Hisashi Tamaru. Institute of Molecular Biology, University of Oregon, Eugene, OR 97405.

We wish to identify the important features of eukaryotic genomes and the forces responsible for shaping them. In organisms with small genomes, such as fungi, a large fraction of their DNA appears functional and may be maintained by natural selection alone. Even small genomes, however, are littered with sequences that look like selfish DNA. Although it is difficult to demonstrate that a particular sequence is useless, mechanisms well suited to fight selfish DNA have been found suggesting that genome structure is important. Neurospora has at least three distinct, but somewhat interrelated gene silencing systems that probably serve in genome defense: 1. RIP and RIP-associated methylation, 2. quelling, and 3. RIP-independent methylation of "foreign" DNA. We are using several approaches to investigate the control and mechanism of gene silencing systems - most particularly DNA methylation. One involves identification and characterization of mutants defective in methylation (*dim*). So far, we have identified four *dim* genes. Mutation of *dim-2*, which encodes a DNA methyltransferase, eliminates all methylation in vegetative cells and can derepress the transcription of some sequences and repress others. In the cases examined so far, methylation interferes with transcription elongation without affecting initiation. We have detected Neurospora proteins that bind to methylated sequences and may mediate these effects, potentially by influencing chromatin structure. Methylation-dependent silencing can be relieved by Trichostatin A (TSA), an inhibitor of histone deacetylases. Selective loss of methylation is associated with TSA treatment, suggesting that acetylation of chromatin proteins can directly or indirectly control DNA methylation. To explore the connection between DNA methylation and chromatin we are testing the effects of mutations in genes for histones H2A, H2B, H3, H4 and H1 and other candidate components of the methylation machine, including PCNA and a histone deacetylase.

Deletion of the gene encoding a typical histone H1 has no apparent phenotype in *Aspegillus nidulans*.
Ana Ramón, Ramón González, María Isabel Muro-Pastor, Irene García and Claudio Scazzocchio. Institut de Génétique et Microbiologie, Université Paris-Sud, URA D2225, 91405 Orsay Cedex, France.

A typical histone H1 can be purified from *Aspergillus nidulans* We have cloned, sequenced and deleted the gene (*hhoA*) coding for this protein. This gene comprises six introns. The position of

one of the introns is identical to that found in a number of H1 plant histones. The peptidic sequence show the three typical domains including a recognisable globular domain. The closest similarity is found with the two globular domains of the H1-like protein of *Saccahromyces cerevisiæ*. Thus, the gene coding for the latter protein probably originated by internal duplication of the globular domain in a typical H1-coding gene. Deletion of *hhoA* results in complete disappearance of the H1 protein defined by electrophoretic mobility and PCA solubility. The deleted strain has no apparent phenotype. We have analysed growth, conidiation, conidial viability, UV and DMSO sensitivity, the appearance of resting and mitotic nuclei, the sexual cycle and ascospore viability. The nucleosomal repeat is identical in *hhoA*+ and *hhoA*- strains. We have analysed the nucleosomal structure of a number of promoters, including the *niiA-niaD* and *prnD-prnB* bidirectional promoters. In both expression is associated with important chromatin rearrangements The former shows complete nucleosome loss under conditions of full expression. This nucleosome loss is independent from transcription and strictly dependent on the activity of the GATA factor AreA. The deletion of *hhoA* does not change either the structure of the resting promoters nor the alterations associated with expression. The role of linker histones in fungi remains an open question.

Gene silencing, methylation and chromatin conformation in *Ascobolus*.

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The mechanisms by which cytosine methylation affects transcription are not completely elucidated. We took advantage of the process of methylation induced premeiotically (MIP) acting in *Ascobolus* to target *in vivo* methylation to different portions of two genes. Methylation of the promoters rarely led to gene silencing and inhibited transcription with variable efficiencies. In contrast, methylation of the coding sequences always led to gene silencing and resulted in the production of truncated transcripts with the length expected if methylation blocked transcript elongation. The analysis of the chromatin of the native *met2* gene in unmethylated and methylated regions revealed that methylation led to a change in chromatin which was independent of the transcriptional state. The chromatin change was coextensive with methylation and started at the position where transcripts were truncated. These results suggest that the methylation-associated gene silencing process in *Ascobolus* could be mediated *via* a change in chromatin which would affect transcript elongation.

We also asked whether the linker histone H1 played a role in this *in vivo* methylation-associated chromatin modification observed in *Ascobolus*. It remains controversial whether histone H1 is also a methylated DNA-binding protein. We cloned the histone H1 gene from *Ascobolus*. We used MIP to create strains in which the native H1 gene was methylated. This resulted in its silencing as revealed by the complete disappearance of histone H1 protein, showing that H1 is not necessary for the MIP gene silencing process. The loss of histone H1 was associated with a hypermethylation of genomic DNA. In addition, strains lacking histone H1 stopped growing a few days after germination. The analysis of the chromatin of the *met2* gene revealed that the

methylation-associated chromatin modification was similar in strains in which the histone H1 gene was either active or silenced. These results provide a direct *in vivo* demonstration that histone H1 protein does not participate in methylation-associated chromatin modification in *Ascobolus*.

Inter-nuclear gene silencing in *Phytophthora infestans*.
Pieter van West[1], Sophien Kamoun[1,3], John W. van 't Klooster[1], Neil A.R. Gow[2], and Francine Govers. WAU, Phytopathology, Wageningen, The Netherlands. [2]Univ of Aberdeen, Molecular Biology, Aberdeen, Scotland. [3] Present address: Department of Plant Pathology OSU, Wooster OH, USA.

Transformation of the diploid oomycete *Phytophthora infestans* with antisense, sense and promoter-less constructs of the coding sequence of the elicitin gene *inf1* resulted in transcriptional silencing of both the transgenes and the endogenous gene. To investigate the mechanism of gene silencing we took advantage of the fact that *P. infestans* has coenocytic mycelia and that mycelial cells may contain multiple nuclei that can differ genetically, resulting in heterokaryotic strains. It appeared that: (i) transcriptional gene silencing is dominant in multinucleated cells, (ii) the silenced state can be transmitted from nucleus to nucleus in heterokaryotic strains, and (iii) gene silencing is maintained in a non-transformed nucleus after nuclear separation (van West *et al*., 1999). In addition, we showed that upon fusion of a silenced non-transgenic strain with a strain containing wildtype nuclei, the silenced state could be transmitted again to the wild type nuclei. Transcriptional gene silencing in *P. infestans* apparently involves inter-nuclear transfer of signals from silenced (transgenic and non-transgenic) nuclei to wild type nuclei, leading to stable gene silencing in the wild type nuclei. These findings support a model reminiscent of paramutation and involving a trans-acting factor that is capable of transferring a silencing signal between nuclei.

Session II: SEXUAL AND ASEXUAL DIFFERENTIATION Chair:Giuseppe Macino

Light, mating types and other factors in regulation of development in *Coprinus cinereus*.
Ursula Kües, Alan P.F. Bottoli, Robert P. Boulianne, José D. Granado, Michaela J. Klaus, Simon Kuster, Yi Liu, Eline Polak, Piers J. Walser and M. Aebi. Inst. f. Mikrobiologie, ETH Zürich, Switzerland.

Coprinus cinereus alternates in its lifecycle between two mycelial stages, the monokaryon and the dikaryon with two genetic distinct nuclei per cell. Monokaryons form constitutively uninucleate asexual spores (oidia) within the aerial mycelium and may generate thickwalled chlamydospores and multicellular sclerotia. Dikaryons commonly form only few oidia, but abundant chlamydospores and sclerotia. Sclerotia develop from hyphal knots, areas of intense localized branching. Hyphal knots alternatively mature into fruitbody initials. Development of these different structures depends on the interplay between environmental signals such as light, temperature and nutrition, and internal factors such as the products of the mating type loci. The A mating type transcription factors play a central role in regulation. Blue light overrides A mediated repression of oidia production and A induced formation of chlamydospores, hyphal knots and sclerotia. At least in oidiation, the B mating type products modify the effect of A and light. In contrast to sclerotia formation, both A and light are needed to induce fruitbody initials.

With fruitbody specific galectin genes we developed a read-out system to analyse the function of these and other regulators on regulation of fruitbody development. Currently, we study the role of RAS, cAMP and nutrition in fruiting. A collection of UV and REMI mutants helps us to unravel regulatory processes. Analysis of a mutant defective in the transversion of hyphal knot into fruitbody initials revealed a link to lipid metabolism.

The circadian system of Neurospora: A great model for the time of your life.
Jay C. Dunlap. Dartmouth Medical School, Biochemistry, Hanover, NH, USA.

Circadian rhythms are widespread among eukaryotes. Those molecularly understood appear to work in similar ways, and one of the best understood systems is that of Neurospora. The circadian clock appears to be a negative feedback loop wherein the *frq* gene, a known component of the clock in *Neurospora crassa*, encodes two FRQ proteins which travel to the nucleus to block the activity of the heterodimeric activator of *frq* comprised of the WC-1 and WC-2 proteins. Given appropriate delays in the synthesis, action, and turnover of FRQ, this negative feedback loop yields oscillations in *frq* transcript and FRQ protein levels; this cycling of clock components is characteristic of circadian oscillators. Light delivered at any point within the cycle acts rapidly through the WC proteins to increase the level of *frq* transcript thereby resetting the clock; mammalian circadian rhythms are reset in a similar manner. Temperature acts posttranscriptionally to determine the absolute level of FRQ in the cell and the site of initiation of translation within the *frq* transcript, thereby dictating the ratio of long FRQ versus short FRQ. Resetting of the clock by changes in temperature can be understood in terms of changes in these set points. More than a dozen circadianly expressed genes are known to act downstream of the clock. These clock-controlled genes (*ccg*s) include a hydrophobin (*eas=ccg-2*), trehalose synthase (*ccg-9*), and glyceraldehyde 3-P dehydrogenase (*ccg-7=gpd*) and play roles in clock regulation of development, stress responses, and intermediary metabolism.

WC-1 and WC-2 form a transcription complex that acts as a photoreceptor.
Giuseppe Macino, Paola Ballario, Claudio Talora, Lisa Franchi, Hartmut Linden Universita' di Roma La Sapienza.

The filamentous fungus *Neurospora crassa* is currently considered an important model for the study of blue light signal transduction, owing to the simple genetics of the pleiotropic responses to blue light stimuli. The two central components of blue light response, White Collar-1(WC-1) and White Collar-2 (WC-2), appear to encode zinc-finger putative transcription factors, essential for light activated transcription (Ballario *et al*., 1996; Linden and Macino, 1997). The products of the wc genes are required for all processes that are under control of blue light such as mycelial carotenogenesis, circadian rythm of conidiation, and phototropism of perithecial beaks (for a review Ballario and Macino, 1998 and Linden *et al*., 1998). PAS, the multifunctional domain found in eubacteria, archaebacteria and eukaryotes involved in dimerization (Ponting and Aravind, 1997) is present in both WC-1 and WC-2. Furthermore a PAS degenerated domain called LOV (for Light, Oxygen and Voltage), identified in prokaryotic proteins (like PYP, Bat, and NifL) and the plant blue light photoreceptor NPH1 (Huala *et al*., 1998), where it seems to act as a versatile flavin-binding domain, is present in WC-1. The in vitro ability of the White Collar proteins to form hetero and homodimers via their PAS domain has been demostrated (Ballario *et al*., 1998). Now we report the biochemical analysis by antibodies immunodetection

of the proteins WC-1 and WC-2 under dark and light growth conditions. WC-1 and WC-2 are both present in the dark. Their fate, upon light irradiation, has been investigated in wild type Neurospora strains and in *wc-2* and *wc-1* genetic backgrounds. We show that WC-1 and WC-2, undergoes a light-induced hyperphosphorylation. We observe by coimmunoprecipitation the presence of a WC-1 WC-2 complex (WCC) in the dark and in the light. A new model of Neurospora blue light transduction, based on WCC complex phosphorylation is presented.

The fluffy gene of *Neurospora crassa* encodes a Cys6-Zn2 cluster protein that regulates macroconidiation.

Daniel J. Ebbole, and Lori Bailey Shrode. Texas A&M; University, Plant Pathol. & Microbiol, College Station, Texas, USA.

Macroconidiation provides *N. crassa* with a means to efficiently and rapidly disperse itself. *Fluffy* (*fl*) is a regulator of macroconidiation and encodes a member of the Gal4 class of transcription factors. Null mutations of *fl* block the switch from filamentous growth to the budding growth characteristic of proconidial chain formation. These mutants are also blocked in expression of many of the known conidiation-specific genes. The pattern of *fl* mRNA expression is consistent with its role as a regulator of morphogenesis. A basal level of *fl* expression is observed in undifferentiated mycelia. *fl* mRNA levels are induced during development at approximately the time when budding growth initiates and *fl* mRNA levels declines at later stages of development. *acon-2* and *acon-3* also are regulators of conidial morphogenesis and *acon-2* is required for induction of *fl* mRNA while *acon-3* is not. This finding is consistent with the view that *acon-2* precedes *fl* in the pathway regulating conidiophore development and that *acon-3* functions at the same time as *fl* or later. Elevated expression of *fl* from a constitutive promoter was sufficient to induce conidiophore morphogenesis in minimal medium. However, not all conidiation-induced genes were expressed. Although *fl* is necessary and sufficient to induce conidiophore morphogenesis, additional factors are required to coordinate activation of some of the conidiation-induced genes.

Analysis of an opsin gene from *Neurospora crassa*.

Jennifer A. Bieszke[1], Katherine A. Borkovich[1], Donald O. Natvig[2], Laura E. Bean[2], Edward L. Braun[3], and Seogchan Kang[4]. [1]University of Texas Medical School, Micro. and Mol. Genetics, Houston, TX, USA. [2]Univ. of New Mexico, Department of Biology, Albuquerque, NM, USA. [3]Ohio State University, Dept. Plant Biology, Columbus, OH, USA. [4]Pennsylvania State Univ., Dept. Plant Pathology, University Park, PA, USA.

Opsins are a class of retinal-binding, seven-helix transmembrane proteins that function as light-responsive ion pumps or sensory receptors. Previously, genes encoding opsins had only been identified in animals and the archaea. Here, we report the identification and mutational analysis of an opsin gene, *nop-1*, from the eukaryotic filamentous fungus *Neurospora crassa*. The amino acid sequence of *nop-1* predicts a protein that shares up to 81.8% amino acid identity with archaeal opsins in the 22 retinal binding pocket residues. Furthermore, NOP-1 contains the conserved lysine residue that forms a Schiff base linkage with retinal in other rhodopsins, and two acidic residues essential for H+ transport in bacteriorhodopsin. Evolutionary analysis revealed clear relatedness between NOP-1 and archaeal opsins, as well as between NOP-1 and several fungal opsin-related proteins. The results provide evidence for a eukaryotic opsin family

homologous to the archaeal opsins, providing a plausible link between archaeal and visual opsins. Functional residues conserved between NOP-1 and archaeal opsins suggest a role for NOP-1 in photobiology. Results from Northern analysis support conidiation and light-based regulation of *nop-1* gene expression. delta *nop-1* strains exhibit a synthetic light-dependent effect upon conidiation in the presence of the mitochondrial H^+-ATPase inhibitor oligomycin. We propose that eukaryotic opsins represent a relatively ancient group of proteins involved in light perception.

Session III: SECONDARY METABOLISM AND PATHOGENICITY Chair:Nancy Keller

A Protein Pathogenicity Factor from *Pyrenophora tritici-repentis*

Linda Ciuffetti. Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon.

Pyrenophora tritici-repentis is the causal agent of tan spot of wheat and known to be a destructive pathogen worldwide. Certain isolates of the fungus have been shown to produce in culture a host-specific toxin(s) (HST) that induces typical tan spot necrosis upon infiltration into tissue of susceptible wheat cultivars. Purification of the major necrosis-inducing toxin (Ptr ToxA) enabled us to clone the gene (*ToxA*) for this HST. Fungal transformation studies confirmed that this gene functions in the plant as a primary determinant of pathogenicity in the *Pyrenophora*-wheat interaction.

The *ToxA* open reading frame (ORF) encodes a preproprotein (19.7 kD), with an N-terminal signal peptide, followed by a dual domain (N + C) protoxin. Analysis of the mature Ptr ToxA by mass spectroscopy indicated a size of 13.2 kD. Additional proteolytic processing is involved in the production of the mature Ptr ToxA. Treatment of the mature toxin with the enzyme pyroglutamate amino peptidase confirmed the amino-terminal position of Ptr ToxA to be amino acid residue Gln-61.

Following purification of Ptr ToxA and the cloning of the *ToxA* gene, we have recently directed our efforts toward the elucidation of the site- and mode-of-action of this toxin in sensitive wheat. One approach to investigate possible protein-protein interactions between Ptr ToxA and sensitive wheat, is a genetical approach utilizing the yeast two-hybrid system. The cDNAs of a toxin-sensitive wheat cultivar were directionally cloned into a lambda vector. Following extensive screening of the library for positive interacting proteins, we identified a cDNA clone with a coding sequence of 426bp. Database searches did not reveal any significant homology to identified proteins. Attempts to identify the complete ORF of this putative interacting protein are in progress. Additionally, labeled derivatives of the toxin have been produced that should be useful for a biochemical approach to the identification of the toxin's site-of-action. To produce biologically active derivatives of Ptr ToxA that could be used for receptor-binding experiments, functional Ptr ToxA was expressed and purified from *Escherichia coli*. Polyhistidine-tagged, fusion protein (NC-FP) consists of both the N- and C-domains of the *ToxA* ORF and elicits cultivar-specific necrosis in sensitive wheat genotypes, with a specific activity similar to native toxin. A fusion protein consisting of the C-domain only is far less active (*ca.* one-fifth that of the native protein). These studies indicate that the N-domain is necessary for efficient folding of toxin and that post-translational modifications of Ptr ToxA are not essential for activity. Labeled

NC-FP retains significant activity as compared to the unmodified NC-FP. Labeled NC-FP is currently being utilized in both *in vitro* and *in vivo* binding assays to identify potentially interacting wheat proteins. Comparison of the results from the yeast-two hybrid screen and the binding assays with labeled NC-FP will potentially identify wheat proteins that play a significant role in the signal transduction pathway of this host-pathogen interaction.

A pathogenicity island in *Nectria haematococca*.

H. Corby Kistler[1], Yinong Han[1], Ulla Benny[1], Xiaoguang Liu[2], Esteban Temporini[2], Hans VanEtten[2]. [1]University of Florida, Gainesville, FL USA; [2]University of Arizona, Tucson, AZ USA.

Host range determinants of fungal plant pathogens are poorly understood. In some instances it appears that the ability to cause disease on a particular host plant species is determined by a single gene with large effect (e.g. the avenacinse gene from *Gaeumannomyces graminis*, *PWL2* from *Magnaporthe grisea*). However in *Nectria haematococca* MPVI, pathogenicity to pea is conferred by a cluster of genes, each individually having a small but significant effect. These "*PEP*" genes are within 25 kb of each other and located on a conditionally dispensable (CD) chromosome. The *PEP* gene cluster contains six genes that are expressed during infection of pea tissue but the biochemical function of only one of the genes is known with certainty. This gene, *PDA1*, encodes a specific cytochrome P450 that confers resistance to pisatin, an antibiotic produced by pea plants. Three of the *PEP* genes, in addition to *PDA1,* can independently confer some level of virulence to an isolate lacking the CD chromosome; functions for two of these three genes are hypothesized, based on predicted amino acid sequences. The deduced amino acid sequence of another transcribed portion of the *PEP* cluster, as well as three apparently non-transcribed open reading frames, have a high degree of similarity to known fungal transposases. Both the G+C content and codon usage of the six genes in the *PEP* cluster differ from that of genes on other *N. haematococca* chromosomes. Several of the features of the *PEP* cluster - a cluster of pathogenicity genes, the presence of transposable elements and suggestions of exogenous origin - are shared by pathogenicity islands in pathogenic bacteria of plants and animals.

Melanin and Fe(II) as an extracellular redox buffering system.

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Melanin is a fungal extracellular redox buffer which, in principle, can neutralize antimicrobial oxidants generated by immunologic effector cells, but its source of reducing equivalents is not known. We wondered whether the large quantities of Fe(II) generated by the external ferric reductase of fungi might have the physiologic function of reducing fungal melanin and thereby promoting pathogenesis. We observed that exposure of a melanin film electrode to reductants decreased the open-circuit potential and reduced the area of a cyclic voltammetric reduction wave, whereas exposure to oxidants produced the opposite effects. Exposure to 10, 100, 1,000 or 10,000 µM Fe(II) decreased the open circuit potential of melanin by 0.015, 0.038, 0.100 and 0.120 V, respectively, relative to a silver-silver chloride standard, and decreased the area of the cyclic voltammetric reduction wave by 27, 35, 50 and 83%, respectively. Moreover, exposure to

Fe(II) increased the buffering capacity by 44%, while exposure to millimolar dithionite did not increase the buffering capacity. The ratio of the amount of bound iron to the amount of the incremental increase in the following oxidation wave was approximately 1.0, suggesting that bound iron participates in buffering. Light absorption by melanin suspensions was decreased 14% by treatment with Fe(II), consistent with reduction of melanin. Light absorption by suspensions of melanized *Cryptococcus neoformans* was decreased 1.3% by treatment with Fe(II) ($P < 0.05$). Cultures of *C. neoformans* generated between 2 and 160 μM Fe(II) in cultural supernatants, depending upon the strain and the conditions [the higher values were achieved by a constitutive ferric reductase mutant in high concentrations of Fe (III)]. We infer that Fe(II) can reduce melanin under physiologic conditions; moreover, it binds to melanin and cooperatively increases redox buffering. The data support a model for physiologic redox cycling of fungal melanin, whereby electrons exported by the yeast to form extracellular Fe(II) maintain the reducing capacity of the extracellular redox buffer.

Session IV: CELL BIOLOGY Chair:Walter Neupert

Motors and intracellular organelle movements.
Gero Steinberg. Ludwig-Maximilians-Universitaet, Institut fuer Genetik, Muenchen, Germany.

Polarized secretion and intracellular transport of membranous organelles are crucial requirements for fungal growth and morphogenesis. Organelle movements are mediated by fibrous elements of the cytoskeleton, which serve as tracks for mechanoenzymes that convert chemical energy into motion. Recently, several fungal representatives of microtubule-dependent kinesins and dyneins, as well as several actin-based myosins were identified, and genetic and cell biological investigations greatly extended our knowledge of their biological role. However, most work was done on the yeast *S. cerevisiae*, and besides nuclear migration our understanding of the molecular machinery for long distance transport of membranous organelles in dimorphic and filamentous fungi is still fragmentary. Therefore, we started to study the biological role of several potential organelle motors of the kinesin, dynein and myosin protein family in the dimorphic plant pathogen *Ustilago maydis*. Our data indicate a role of dynein and myosin in nuclear migration and secretion, respectively, which is in agreement with findings for *S. cerevisiae*. However, in contrast to yeast, microtubules play a central role in polarized secretion, endocytosis and tip growth of *U. maydis* sporidia and hyphae. The central role of the microtubule cytoskeleton is confirmed by the existence of two kinesin motors that are conserved among higher eukaryotes but are absent from *S. cerevisiae*. The existence of a complex microtubule cytoskeleton establishes *U. maydis* as a simple model system for cytoskeleton analysis.

Novel proteins required for nuclear distribution in *Neurospora crassa.*
Michael Plamann[1], In Hyung Lee[1], Santosh Kumar[1], Peter Minke[2], and John Tinsley[2]. University of Missouri-Kansas City, School of Biol. Sciences, Kansas City, MO, USA. [2]Texas A&M; University, Biology, College Station, TX, USA.

Cytoplasmic dynein is the most complex of the cytoplasmic microtubule-associated motor proteins, and it has been shown to be required for the movement and positioning of nuclei in fungi. We have developed a genetic system for the analysis of cytoplasmic dynein in the filamentous fungus *Neurospora crassa*. We have shown previously that the *N. crassa ro-1*, *ro-3*,

and *ro-4* genes encode subunits of either cytoplasmic dynein or dynactin, a dynein-associated complex. We have isolated hundreds of *ro* mutants, and the availability of multiple independent alleles of *ro* genes encoding known subunits of cytoplasmic dynein and dynactin provide us with the opportunity to do a detailed analysis of protein interactions and specific functions within the motor complex. We present our analysis of a specific *ro-4* allele and our initial analysis of various *ro-1* and *ro-3* alleles. We also report that five additional *ro* genes encode novel proteins. *ro-7* is predicted to encode a 70 kD protein distantly related to actin. In *ro-7* mutants, cytoplasmic dynein and the dynactin complex accumulate at spindle pole bodies suggesting that RO7 is required for proper intracellular targeting of cytoplasmic dynein and dynactin. *ro-10* is predicted to encode a novel 24 kD protein that may be required for stability of the dynactin complex, because $p150^{Glued}$,the largest subunit of the dynactin complex, is not detectable in a *ro-10* deletion strain. Our results suggest that at least some of these novel proteins are required for the proper function of cytoplasmic dynein or dynactin.

Protein import into mitochondria.

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The majority of mitochondrial proteins are encoded by the nucleus, are synthesized in the cytoplasm as preproteins and are imported in a post-translational manner into the mitochondria. Transport of proteins into the mitochondria is catalyzed by protein translocation machineries located in both the outer and inner mitochondrial membranes, the TOM and the TIM complexes, respectively.

The TOM complex mediates the recognition of preproteins, their transfer through the outer membrane and the insertion of resident outer membrane proteins. We have recently purified the TOM complex from *N. crassa*. Reconstitution studies shows it forms cation selective, high conductance channels. Electron microscopy revealed the isolated TOM complex particles are about 138 in diameter and appear to contain two or three pores per particle. Further translocation across the inner membrane requires a membrane potential and is mediated by one, of at least two, import machineries, the TIM complexes. Translocation of presequence-targeted proteins is facilitated by the Tim17-Tim23 machinery, which operates closely with Tim44, mt-Hsp70 and Mge1p, to drive import into the matrix in an ATP-dependent manner.

A second independent, translocation machinery, termed the Tim22-Tim54 translocase mediates the insertion of members of the mitochondrial carrier family into the inner membrane. Three related proteins, of the intermembrane space, Tim9, Tim10 and Tim12, small metal binding proteins, interact directly with the incoming carrier proteins at the TOM complex, to mediate their passage through the intermembrane space. Together with Tim22-Tim54, Tim9, Tim10 and Tim12 facilitate the insertion of the carrier proteins directly into the inner membrane. Finally, a subset of inner membrane proteins, some nuclear encoded, others mitochondrial encoded, reach their correct orientation in the inner membrane, via a membrane potential-dependent export step from the matrix. We have recently identified a novel translocase in the inner membrane, the Oxa1p complex, which mediates this export step.

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Mitochondrial fusion and the transmembrane GTPase, FZO1P.

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Membrane fusion is required to establish the morphology and cellular distribution of the mitochondrial compartment. In Drosophila, mutations in the fuzzy onions (Fzo) GTPase block a developmentally-regulated mitochondrial fusion event during spermatogenesis (Hales, K. G. and M. T. Fuller. 1997. Cell. 80:121-129). The predicted fuzzy onions protein belongs to a novel family of high molecular weight GTPases also present in yeast, nematodes, and mammals. We have shown that the yeast ortholog of fuzzy onions, Fzo1p, plays a direct and conserved role in mitochondrial fusion. A conditional fzo1 mutation causes the mitochondrial reticulum to fragment and blocks mitochondrial fusion during yeast mating. Fzo1p is a mitochondrial integral membrane protein with its GTPase domain exposed to the cytoplasm. Point mutations that alter conserved residues in the GTPase domain do not affect Fzo1p localization but disrupt mitochondrial fusion. Suborganellar fractionation suggests that Fzo1p spans the outer and is tightly associated with the inner mitochondrial membrane. This topology may be required to coordinate the behavior of the two mitochondrial membranes during the fusion reaction. We propose that the fuzzy onions family of transmembrane GTPases act as molecular switches to regulate a key step in mitochondrial membrane docking and/or fusion.

Regulation of Gene Expression

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Regulation of Gene Expression

1. Pyrimidine biosynthesis genes in *Aspergillus nidulans* and the evolutionary origin of fungal dihydroorotases. Alexei Aleksenko[1], Wenguang Liu [2], Zoran Gojkovic [2], Jens Nielsen [1],

and Jure Piskur [2]. [1] DTU, CPB, Lyngby, 2800, Denmark. [2]DTU, Microbiology, Lyngby, 2800, Denmark

The six biochemical steps of the de novo pyrimidine biosynthesis pathway are conserved in all known organisms. However, unlike in prokaryotes, in animals and fungi at least the first two activities are grouped on a multifunctional enzyme. In *A. nidulans*, the first two steps of the pathway are performed by a multifunctional enzyme comprising the activities of carbamoyl phosphate synthetase (CPSase) and aspartate transcarbamylase (ATCase). This polypeptide is encoded by a 7 kbp cluster gene, *pyrABCN*. The enzyme of the third step, dihydroorotase (DHOase), is encoded by a separate locus *pyrD*. However, the *pyrABCN* gene contains an evolutionary remnant of a DHOase-encoding sequence, which arrangement is similar to that in yeast. Comparison of amino acid sequences of active dihydroorotases with related enzymes indicates that the monofunctional dihydroorotases from fungi are more similar to ureases and enzymes of the pyrimidine degradation pathway, from which they have probably originated, than to DHOases of other organisms. The *pyrABCN* gene is transcribed as a single 7 kb mRNA species. The level of transcripts of *pyrABCN, pyrD* and, to a lesser degree, *pyrF* genes responds to the presence of exogenous pyrimidines and to the conditions of pyrimidine starvation. Derepression of *pyrABCN* and *pyrD* under pyrmidine starvation is noticeably enhanced in *pyrE* mutants which accumulate dihydroorotic acid. The data suggest that dihydroorotate is probably an inducer of at least some genes of the pathway, while UMP is a likely repressor. The cluster gene *pyrABCN* contains an upstream short open reading frame which may be involved in regulation. Some common features have been identified in promotor regions of *pyr* genes.

2. The *cpcA* gene of *A. nidulans* encodes the transcriptional activator of the general control system and is additionally involved in sexual development. Meike Andermann, Bernd Hoffmann, Gerhard H. Braus. University of Göttingen, Molecular Microbiology, Göttingen, Lower Saxony, Germany.

The *cpcA* gene of the filamentous fungus *Aspergillus nidulans* encodes a protein of 245 amino acids in length with high similarity to the general amino acid control transcriptional activator Gcn4p of yeast which are able to complement each other. The mRNA level of *cpcA* is regulated under amino acid starvation resulting in a fourfold increase after eight hours of limitation. Deduced cpcA protein binding sites (GCRE´s) in its own promoter imply a transcriptional autoregulation. Gel retardation assays showed binding activity of cpcAp in its promoter which was abolished when point mutations were integrated into the GCRE´s. Two upstream open reading frames in the 5´region of the *cpcA* mRNA were identified suggesting an additional translational regulation. An increase of cpcA protein was observed to a factor of three after amino acid limitation. Deletion of *cpcA* causes a reduced growth rate to approximately 30% of wild-type. Additionally, *cpcA* mutant strains are unable to derepress general control regulated genes as *trpC* and *argB* resulting in an unability to grow under amino acid starvation conditions. Overexpression of *cpcA* results in a specific increased transcription of general control regulated genes. The sexual developmental program of *A. nidulans cpcA* overexpression strains was affected. High amounts of cpcA protein resulted in a block in cleistothecia formation at a defined timepoint and therefore in sterility. The same phenotype was observed after overexpression of the yeast *GCN4* in *A. nidulans*. We suggest a function of the cpcA protein as general control

transcriptional activator. In addition, overexpression of *cpcA* results in a direct or indirect manner in sexual sterility.

3. Inducer dependent nuclear localisation of the FacB transcriptional activator of *Aspergillus nidulans*. Alex Andrianopoulos, Meryl A. Davis, and Michael J. Hynes. University of Melbourne, Department of Genetics, Parkville, Victoria, Australia

Gene expression can be regulated at the transcriptional, translational or post-translational level. While structural genes are predominantly controlled at the transcriptional level, regulators also show a number of post-translational modes of regulation including ligand dependence, nuclear exclusion and interactions with coactivating or antagonistic regulatory proteins. Understanding the mechanisms which control the activity of regulatory factors is important to our understanding of regulatory networks and their action.The *facB* gene of *Aspergillus nidulans* encodes a $C_6Zn(II)_2$ binuclear cluster DNA binding domain protein and is the major transcriptional activator of genes required for acetate utilisation. FacB positively regulates the expression of the glyoxylate bypass genes *acuD* and *acuE* as well as *facA* and *facC*. Their products are required for the anaplerotic glyoxylate bypass which converts acetyl-CoA from acetate or fatty acids into TCA cycle intermediates. In addition, FacB activates the expression of the *amdS* gene.The *facB* gene and its product are regulated at multiple levels. The gene is controlled transcriptionally by acetate induction, but not by FacB, and carbon catabolite repression by the negatively acting CreA. Full transcriptional activation of FacB also requires acetate.Here we show that the cellular localisation of FacB is regulated. In the absence of acetate, FacB as a LacZ or GFP fusion is cytoplasmically localised. Addition of acetate causes the rapid translocation of FacB to the nucleus. A 142 amino acid region of FacB, spanning the $C_6Zn(II)_2$ DNA binding motif is sufficient for nuclear localisation. A mutation which destroys DNA binding by FacB does not inhibit nuclear localisation.

4. The *vvd* gene is required for light-adaptation of the *N. crassa* conidiation-induced genes, *con-6* and *con-10*.Lori Bailey Shrode, Lori D. White, Daniel J. Ebbole. Texas A&M; University, Plant Pathology & Microbiology, College Station, Texas, USA.

con-10 and *con-6* are two of the conidiation-induced (*con*) genes of *N. crassa* that were identified on the basis of their preferential expression during macroconidiophore development. They are also directly regulated by a number of environmental stimuli independent of development, including blue light photoinduction. We identified an allele of *vvd*, in a mutant screen designed to obtain strains with elevated expression of *con-10*. *vvd* mutants display enhanced carotenogenesis in response to light. *con-10* and *con-6* expression was induced to much higher levels in the *vvd* mutant and light adaptation is apparently absent. Our initial characterization of two *vvd* alleles suggests that *vvd* is required for reducing expression of the light-induced *con-10* and *con-6* genes in response to light.

5. Evidence for 2,4-Dinitrotoluene degrading genes in *Phanerochaete chrysosporium*: In situ and DNA homology tests. Christine E. Barrow, Michelle M. Jackson, Gail P. Hollowell, and Sisir K. Dutta. Howard University, Biology, Washington, D.C., USA.

The nitroaromatic compound 2,4-dinitrotoluene (2,4-DNT) is a hazardous waste product with known toxicity to humans, animals, and plants. Increasing contamination of soil and ground water is a major environmental concern. HPLC analyses, in situ and liquid culture experiments suggest that the degradation of 2,4-DNT by genes in the fungus *P. chrysosporium*. The basidiomycete fungus is preferred in bioremediation because it can also degrade other toxic nitroaromatic compounds. The 2,4-DNT degrading gene clusters in bacteria are well characterized, however, similar genes in the fungus have yet to be isolated. We report on evidence of 2,4-DNT degrading genes in this fungus based on our in situ bioremediation and DNA homology studies. The fungal spores were applied to soil contaminated with 2,4-DNT. The resulting soil extractions were then analyzed by HPLC to determine residual 2,4-DNT concentrations at varying time intervals. The dioxygenase gene of the bacteria Burkholderia, also known to degrade 2,4-DNT was isolated and purified from a plasmid clone donated by Dr. J. Spain. This 6.8 kilobase gene sequence was used as a probe against the fungal genomic DNA template in Southern blot homology studies. The bacterial gene sequence was also used in primer selections and for PCR analysis. Fragments of 6.8 kilobases or more received priority due to the presence of introns in the fungal genome. The resulting PCR fragments will be sequenced and cloned for expression studies. These results may lead to use of *P. chrysosporium* in the large-scale bioremediation of 2,4-DNT contaminated soil and groundwater worldwide.

6. Chromatin structure of the *cbh1* promoter and the regulation of its expression in *Trichoderma reesei*. Nigel J. Belshaw[1], Marja Ilmén[2], Merja Penttilä[2]and David B. Archer[1]. [1]Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, UK. [2]VTT Biotechnology and Food Research, P.O. Box 1500, FIN-02044 VTT, Finland.

DNase I sensitivity analyses of wild-type and mutant *cbh1* promoters were performed under conditions that repress, induce or are neutral for *cbh1* expression. Previous studies have revealed a major role in the repression of *cbh1* expression for the global regulator CRE1 and sequence analysis revealed a number of putative binding sites for this transcription factor. Here we show that although several regions of the *cbh1* promoter contain consensus binding sites for CRE1 and these also bind to a GST-CRE1 fusion protein *in vitro*, only two of these putative binding sites are hypersensitive to DNase I (DH1 and 2) indicating that only these two regions are occupied by CRE1 *in vivo*. Deletion of the region around DH1 does not lead to derepression of *cbh1* expression, whereas mutation of the region at DH2 abolishes DNase I hypersensitivity and leads to derepression suggesting a pivotal role for this region in maintaining repression. A third DNase I hypersensitive site (DH3) was also detected, but only in inducing conditions. Micrococcal nuclease sensitivity was used to map the positions of nucleosomes in the *cbh1* promoter. No major nucleosome rearrangements occurred during derepression or induction.

7. Molecular cloning of spray gene in *Neurospora crassa*. Jin-Woo Bok[1], Teruo Sone[2], Fredrick J. Bowring[3], David E.A. Catcheside[3], and Anthony J.F. Griffiths[1]. [1]University of British Columbia, Department of Botany, Vancouver, BC, Canada. [2]Hokkaido University, Faculty of Agriculture, Sapporo, Japan. [3]Flinders University, Biological Sciences, Adelaide, Australia.

Ca^{2+} in Neurospora serves more a regulatory than a nutritive or structural function. A tip-high gradient of free Ca^{2+} prevails in growing hyphae, probably maintained by a constant redistribution within cells rather than by a regulation of uptake from the media. The decreased

uptake of Ca^{2+} caused by a verapamil was correlated with branching and the phenotype resembled that of the frost and spray mutants. High levels of Ca^{2+} added to the medium could reverse the effects of both the drug and the mutants. To figure out the relationship between Ca^{2+} and spray gene, we have tried to clone and analyse the gene. It was found in a cosmid clone X11: G10 by chromosomal walking from am locus and complementation of the mutant. The 5.7 kb DNA fragment which transformed the mutant into wild type successfully was subcloned into TOPO-XL vectors and sequenced. The spray mutant had a single base pair change (TTTAA (r) TTGAA). The size of mRNA was about 4.4 kb by Northern blotting analysis. The start site and intron mapping of the gene is in progress by RT-PCR and 5' RACE. Partially determined sequence showed homology (about 50 %) with hypothetical membrane proteins in ACS-1-GCV3, YPL221w, YGL139w, MRF1-SEC 27 and YOR 365c of *S. cerevisiae* and in SPAC1F7.03 of *Schizosaccharomyces pombe* from BLAST Search.

8. Copper-modulated gene expression in *Podospora anserina*.C. Borghouts, N. Averbeck, E. Kimpel, H.D. Osiewacz. Johann Wolfgang Goethe-Universität, Botanisches Institut, Frankfurt, Osiewacz, Germany.

Copper is an essential micronutrient and a cofactor of various enzymes (e.g., cytochrome oxidase, laccase, superoxide dismutase). On the other hand, increased levels of copper are cytotoxic. Consequently, there is a clear need to control cellular copper levels tightly. Previously, we have cloned a gene encoding GRISEA, a transcription factor involved in the control of cellular copper homeostasis (1,2). GRISEA was demonstrated to be an ortholog of MAC1 of *Saccharomyces cerevisiae* and appears to control the expression of a gene coding for a high affinity transporter of copper (3). The DNA binding domain of GRISEA was mapped to the first 168 amino terminal amino acids. The activation domain is located in the second half of the protein. At low cellular copper concentrations, GRISEA is involved in the activation of target gene expression. Increased copper levels lead to a repression of GRISEA, most likely via intramolecular interactions between different regions of the protein. The role of GRISEA in the molecular machinery involved in the control of cellular copper homeostasis in *P. anserina* will be discussed. References: 1. Osiewacz HD, Nuber U (1996) Mol Gen Genet 252:115-124 2. Borghouts C, Kimpel E, Osiewacz HD (1997) Proc Natl Acad Sci USA 94: 10768-10773 3. Borghouts C, Osiewacz HDMol Gen Genet (in press) Acknowledgment: The experimental work was supported by a grant of the Deutsche Forschungsgemeinschaft.

9. Cloning and sequencing of chitin synthase genes of four different classes from *Cochliobolus sativus*. Thierry G. Bruyere. Novartis Crop Protection, Research, Witterswil, Baselland, Switzerland.

In our screening for chitin synthase gene fragments from phytopathogenic fungi, we succeeded in getting four gene fragments from *Cochliobolus sativus* which belong to four different gene classes. To get full length clones, a genomic library prepared in Lambda ZAP II has been screened with each of the four gene fragments as a probe. Positive plaques have been passed through a second and a third screening run to assure the purity of the selected clones. The four clones, termed Csat1, Csat2, Csat3 and Csat4 have been completely mapped and sequenced following three different strategies: production of unidirectional nested deletions of double-stranded DNA clones, chromosome walking using customized primers and subcloning

particularly mapped fragments. In all cases, both strands were sequenced. Southern blotting experiments proved that the four Csat genes are single-copy genes. The existence of a fifth gene was not confirmed even by low-stringency hybridizations. For Csat1, a fragment of 4647 nucleotides has been sequenced. The coding region has been identified by comparing its reading frame to published sequences. One putative intron spanning 52 nucleotides has been localized on the open reading frame. The gene encodes for a 998 amino-acid protein. For Csat2, a fragment of 8153 nucleotides has been sequenced. An open reading frame of 3206 nucleotides has been identified (interrupted by one 43 bp intron) which encodes for a 1053 amino-acid protein. For Csat3, a 4549 nucleotide has been entirely sequenced, the open reading frame is interrupted by five introns and encodes for a 900 amino-acid protein. For Csat4, 4618 nucleotides have been sequenced. The gene has two introns of small size and encodes for a 1128 amino-acid protein. The expression of the four chitin synthase genes was studied with mycelium of *Cochliobolus sativus* grown in liquid culture or on barley plants. Primers specific for each gene and able to distinguish between cDNA and genomic DNA, were designed and used in RT-PCR experiments to monitor gene expression. Interestingly, the four messenger RNAs were present in the tested development stages excluding a differential expression of one particular gene. Furthermore, RT-PCRs produced with RNA samples from fungal spores gave similar results as with myceliums indicating that the regulation of chitin synthase may occur at a post-translational level. Gene disruption experiments have been performed in order to better characterize the role of these genes in fungal growth and eventually validate chitin synthase as a target for novel antifungal compounds.

10. *Agrobacterium tumefaciens*-mediated transformation of yeasts and filamentous fungi: Effect of the host on T-DNA integration.Paul Bundock [1], Marcel J.A. de Groot [2], Amke den Dulk-Ras [1], Haico van Attikum [1], Alice G.M. Beijersbergen [2], Aaron A. Winkler [1], Yde H. Steensma [1], Paul J.J. Hooykaas [1], and Cees A.M.J.J. van den Hondel[1]. [1]State Leiden University, IMP, Leiden, Zuid Holland, The Netherlands. [2]Unilever, Research Laboratory, Vlaardingen, Zuid Holland, The Netherlands.

Agrobacterium tumefaciens is a gram negative soil bacterium induces the formation of crown galls or tumours at plant wound sites. During tumorigenesis, *A. tumefaciens* transfers a part of its tumour inducing (Ti) plasmid, the T-DNA, to the plant cell where it then integrates. *A. tumefaciens* is also able to transfer T-DNA to *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. We have studied in these hosts the mechanism of T-DNA integration. When a T-DNA carrying sequences homologous to the yeast genome was transferred to *S. cerevisiae* it integrated efficiently via homologous recombination. This demonstrates that the mechanism of T-DNA integration is determined by the host cell. Furthermore, a T-DNA lacking homology with the *S. cerevisiae* genome can integrate via illegitimate recombination. We have been able to demonstrate that *A. tumefaciens* is able to transform the filamentous fungus *Aspergillus awamori*, demonstrating for the first time DNA transfer between a prokaryote and a fungus. Analysis of the transformed fungi showed that in most cases the T-DNA was present as a single intact copy in the fungal genome. The T-DNA had integrated into the genome via an illegitimate recombination mechanism, as shown by sequencing the genomic DNA flanking the integrated T-DNA copies.

11. Isolation and characterization of *hisB*, the *Aspergillus nidulans* homolouge of yeast *his3*. Silke Busch, Katja Starke, Bernd Hoffmann, and Prof. Dr. Gerhard Braus. University of Göttingen, Molecular Microbiology, Göttingen, Lower Saxony, Germany.

The yeast *his3* gene encodes the imidazole glycerolphosphate dehydratase (E.C.4.2.1.19) of the histidine biosynthetic pathway. We isolated the *his3* homologue of *Aspergillus nidulans*, named *hisB*, respectively. Sequence analysis revealed an open reading frame of 801 kb, interrupted by an intron of 58 kb. The cDNA clone complements the histidine biosynthetic defect of the yeast *his3* mutant, which indicates functional equivalence of these homologs. Further evidence derived from sequence similarities which range between 54 and 38 % identities to other imidazole glycerolphosphate dehydratases. A transcriptional regulation of *hisB* by histidine itself does not occur. The yeast *his3* gene is regulated by the mechanism of general amino acid control. We showed that the *hisB* gene in *Aspergillus nidulans* is transcriptionally activated after amino acid starvation. Two putative binding sites for the transcription factor CpcA of the general control pathway of *Aspergillus nidulans* could be identified 379 and 228 bp upstream of the translational start point.

12. The carboxy-terminal 23 amino acid region of *Aspergillus parasiticus* AFLR is a transcription activation domain. Perng-Kuang Chang, Jiujiang Yu, Deepak Bhatnagar, and Thomas Cleveland. Southern Regional Research Center, ARS-USDA, New Orleans, LA, USA.

AFLR, a zinc cluster protein, transactivates the expression of genes in the aflatoxin biosynthetic pathway gene clusters in *Aspergillus parasiticus*, *Aspergillus flavus* as well as the sterigmatocystin synthesis gene cluster in *Aspergillus nidulans*. We showed, by fusion of an *A. parasiticus aflR* coding region to the GAL4 DNA-binding coding region, that the AFLR carboxy- terminus contained a transcription activation domain. Compared to the AFLR carboxy-terminal fusion protein (AFLRC), a mutant AFLRC retained approximately 75% of the activation activity after deletion of three acidic amino acids, Asp365, Glu366 and Glu367, in a previously identified acidic stretch. Removal of the carboxy-terminal amino acid, Glu444, did not affect the activation activity. Substitutions of acidic Glu423, Asp439, or Asp436/Asp439 with basic amino acid(s) resulted in 10 to 15-fold lower activation activities. The Asp436His mutation abolished the activation activity. In contrast, substitutions of basic His428, and His442 with acidic Asp resulted in 20 % and 40 % decrease in the activation activity, respectively. Simultaneous substitutions of Arg427, Arg429, and Arg431 with Leu also significantly decreased the activation activity; the decrease was approximately 50-fold. Results suggest that the carboxy-terminal 23 amino acid region of *A. parasiticus* AFLR is a transcription activation domain and that total acidity in this region is not a major determinant of AFLR's activation ability.

13. Sequence analysis and characterization of genes expressed during appressorium formation in *Magnaporthe grisea*. Woobong Choi, Eric G.C. Fang, Maciek Sasinowski, and Ralph A. Dean.

Magnaporthe grisea, the rice blast pathogen, requires the formation of a specialized infection structure called an appressorium to infect host plants. We have initiated an EST (Expressed Sequence Tag) analysis utilizing an appressorium-stage specific cDNA library to identify and

characterize genes involved in appressorium formation. To date, 2717 cDNA clones have been sequenced from either 5' or 3' ends. Approximately 45% of the sequences significantly matched (p value < 10^{-3}) sequences in the GenBank database based on BLASTX. Twelve genes were found to be previously identified in *M. grisea* and several clones showed homology to genes associated with pathogenesis in other plant pathogenic fungi. Differential hybridization analysis using high density library filters with cDNA from different developmental stages identified 631 cDNA clones with appressorium stage specific/up-regulated expression patterns. Characterization and the possible role of these genes in appressorium formation will be presented.

14. Expression of heme proteins in *Aspergillus niger*. Ana V. Conesa-Cegarra, Peter Punt, Cees AMJJ van den Hondel. TNO Nutrition and Food Research Institute, MGG, Zeist, Utrecht, The Netherlands.

Filamentous fungi are widely exploited for their capacity of high level secretion of enzymes. Molecular genetic approaches for strain improvement have shown that this ability can also be extended to many other proteins of fungal origin. However, a major exception to this rule seems to be high level secretion of fungal metallo-proteins. So far, attempts to overproduce this type of enzymes in recombinant fungal systems have had limited success. To gain insight into the bottle-necks existing for the (over)production of metallo-proteins in filamentous fungi, we have started a project to study the expression of genes encoding heme-containing fungal peroxidases in *Aspergillus niger*. Three genes were chosen for this study: lignin peroxidase (*lipA*) and manganese peroxidase (*mnp1*) encoding genes from *Phanerochaete chrysosporium* and the chloroperoxidase (*cpo*) encoding gene from *Caldariomyces fumago*. Different expression cassettes for each of the three genes have been constructed and used to transform a protease deficient strain of *Aspergillus niger*. Analysis of the transformants shows expression for all three genes. Manganese peroxidase and chloroperoxidase are both produced as active extracellular enzymes while lignin peroxidase is incorrectly processed resulting in an inactive extracellular protein. Further analysis of the limiting factors for peroxidase overproduction is in progress and will be presented.

15. The Yeast Proteome Database: A research tool available to the fungal research community. Maria C. Costanzo, Peter E. Hodges, Brian P. Davis, Andrew H.Z. McKee, Michael E. Cusick, Kevin J. Roberg-Perez, William E. Payne, and James I. Garrels. Proteome, Inc., Beverly, MA, USA.

The Yeast Proteome Database (YPD™) is an integrated resource for *Saccharomyces cerevisiae* protein information encompassing the research literature, functional genomics, and proteomics. It contains about 6100 Yeast Protein Reports, one for each of the known or predicted *S. cerevisiae* proteins. YPD continues to grow rapidly in terms of size and features. It now contains more than 75,000 free-text annotations derived from a review of more than 9,800 research papers. Each YPD Protein Report presents an extensive tabulation of protein properties, annotations, and references for one protein in a convenient Web page format (see http://www.proteome.com/YPDhome.html). Each protein is now classified by biochemical function and cellular role, quickly alerting users to the biological significance of a protein and also providing a search tool for organizing groups of proteins into cellular processes and

molecular machines. Other properties tabulated in each report include mutant phenotype, gene and protein sequence, sequence similarities, protein-protein interactions, regulators of gene expression, and protein modifications. Relationships between genes are shown by BLAST alignments of each protein to other *S. cerevisiae* proteins, to Drosophila proteins, and to human proteins. Similarities to known proteins of other fungi are also noted in the text annotations. Pop-up windows expand on sections of the Protein Report, showing physical and genetic interactions for each protein/gene, membership in characterized protein complexes, post-translational modifications, and all known regulators (conditions, small molecules, and transcription factors) of expression. The Gene Expression pop-up window of each report also contains a graphical representation of transcription profiles generated using DNA microarray technology, which are accumulating at an ever-increasing rate. For fungal proteins similar to *S. cerevisiae* proteins, YPD provides a wealth of information that may contain clues as to their biochemical function or cellular role, or may suggest new experimental directions.

16. Activity of transposon Restless in homologous and heterologous hosts. Katarzyna Czechowska, Sabine Jacobsen, Frank Kempken, and Ulrich Kück. Ruhr-Universität Bochum, Allgemeine Botanik, Bochum, NRW, Germany.

The class II transposon Restless, a 4.1 kb hAT-like element from the hyphomycete *Tolypocladium inflatum*, was shown to be active in strain ATCC 34921 [1]. In order to analyse transposon activity in other *T. inflatum* strains or in heterologous species, such as *Penicillium chrysogenum*, we have constructed transposon vectors for DNA mediated transformations. The vectors were designed to confer phleomycin resistance after excision of the Restless element. We transformed different *T. inflatum* strains and *P. chrysogenum* with the transposon vectors and demonstrated the integration by PCR and Southern analysis. Transformed strains were further screened for excision events by isolating phleomycin resistant colonies. The molecular analysis of the corresponding isolates will be presented. [1] Kempken F, Kück U (1996) Mol Cell Biol 16: 6563-6572

17. Transposable elements and the organization of the *Fusarium oxysporum* genome: clusters of DNA transposons and chromosomal rearrangements. Marie-Josée Daboussi[1], Aurélie Hua-Van [1], Jean-Michel Davière [1], Thierry Langin[2]. [1]University Paris-Sud, IGM , Orsay, Essonne, France. [2]Université Paris-Sud, IBP, Orsay, Essonne, France.

Several families of transposable elements (TEs) are present in the genome of the phytopathogenic fungus *Fusarium oxysporum*. They are representative of the major groups of eukaryotic elements, retrotransposons, LINE-like element and DNA transposons. These elements are present in copy numbers ranging from a few elements to hundreds per genome. By cloning random DNA fragments and by sequencing contiguous stretches of genomic DNA, many other repetitive sequences with features of TEs were discovered. This finding reveals that earlier studies had uncovered only the tip of TEs in this species. These new elements, with varying copy number, are full-length or degenerate. They are interspersed in the genome but appeared not to be randomly distributed. Indeed, the analysis of their distribution on both chromosomes separated by CHEF and cosmids, showed that some chromosomes contained many different types of TEs and that elements of a particular family are concentrated in some genomic regions. Precise composition and arrangement of repetitive DNAs were investigated by sequencing three

regions of the genome surrounding different insertion sites of the *impala* element. The relative organization of genes and TEs in these regions showed that they are composed essentially of intermixed transposable elements of several types, e. g., at least 11 elements belonging to 6 different families can be found in a contiguous 40kb region. Some repeats are frequently reiterated and many of them are inserted into other elements. In addition, part of these regions corresponds to duplicated fragments. The mechanisms involved in the generation of the particular organization of these regions, as well as their significance, will be discussed.

18. Genes differentially expressed in a *U. maydis* mutant of the cAMP pathway include nitrogen utilization genes. Adriana C. De Maria, Yume Kohno, Mário Moniz de Sá, Seline Hayden. University of British Columbia, Biotechnology Lab, Vancouver, B.C., Canada.

U. maydis, the basidiomycete causing smut disease in corn plants, switches from a saprophytic yeast-like haploid phase to an infectious filamentous dikaryon after mating. The involvement of the cAMP pathway in morphogenesis was demonstrated by the fact that mutants of *uac1*, the gene encoding adenylate cyclase, show constitutive filamentous growth[1,2]. On the other hand, mutants in the gene encoding the regulatory subunit of PKA (*ubc1*) show a multiple budding phenotype, a defect in filamentous growth after mating and an inability to complete sexual development in the plant[2,3]. In an attempt to characterize new genes involved in the morphological switch and pathogenesis, we have constructed a library enriched for cDNAs differentially expressed in a *ubc1* mutant using the subtractive hybridization technique. Several clones for mRNAs that were up or down-regulated in the mutant were isolated and characterized. One of the up-regulated clones is 52% similar to *UGA4*, the gene encoding a GABA-specific permease from *S. cerevisiae*. The region of similarity includes one of the two PKA sites present in *UGA4*. Another up-regulated clone is highly similar to genes encoding the NADP-linked glutamate dehydrogenase from other fungi, including *GDH3*, a gene involved in the response to starvation in yeast[4]. *U. maydis* strains knocked-out for these two genes are being analyzed. The increased expression of genes related to nitrogen utilization in the *ubc1* mutant suggests that the cAMP pathway in *U. maydis*, as in other fungi, may mediate the response to extracellular nitrogen source. 1.Barret et al., 1993, Mol. Plant-Microbe Interac. 6:274 2.Gold et al., 1994, Genes Dev. 8: 2805 3.Gold et al., 1997, The Plant Cell 9:1585 4.Wilkinson et al., 1996, Microbiol. 142:1667

19. Regulation of a feruloyl esterase gene (*faeA*) from *Aspergillus niger*. Ronald P. de Vries, Jaap Visser. Wageningen Agricultural University, MGIM, Wageningen, The Netherlands.

Three factors have been identified involved in the regulation *faeA* from *Aspergillus niger*. The expression of the gene on D-xylose is mediated via *XlnR*, the xylanolytic transcriptional activator and depends on the xylose concentration. A decrease in the expression *faeA* and three other xylanolytic genes was observed with increasing xylose concentrations in a wild-type strain, whereas expression levels in a *CreA* mutant were not influenced. Xylose concentrations higher than 1 mM result in repression of the expression of xylanolytic genes mediated by the carbon catabolite repressor protein *CreA*. The expression levels of *faeA* and other xylanolytic genes on xylose are therefore not only determined by induction via *XlnR* but also by repression via *CreA*. A third factor involved in *faeA* regulation responds to the presence of certain aromatic compounds with a defined ring structure such as ferulic acid and vanillic acid. The structural

requirements necessary for induction have been identified. A benzene ring is present in all inducing compounds, which is substituted at C3 with a methoxy group and at C4 with a hydroxy group. C5 is not substituted, whereas different alifatic groups at C1 of the aromatic ring are allowed. Although the hydroxyl function at C4 is strongly preferred, low levels of expression have also been found with veratryl alcohol and veratric acid, two 3,4-dimethoxy compounds. Expression levels of *faeA* on combinations of ferulic acid and xylose are higher than on each compound alone, indicating a positive interaction between the activation by aromatic compounds and by *XlnR*. We acknowledge financial support of Danisco Ingredients, Brabrand, Denmark.

20.Construction of a sequence-ready framework for the rice blast fungus based on fingerprinting contigs and BAC-end sequencing. Ralph A. Dean. Clemson University, Plant Pathology, Clemson, SC, USA.

The rice blast fungus, *Magnaporthe grisea*, is an important model for fungal pathogenesis and is a major candidate for whole genome sequencing. To provide a framework for gene discovery, a strategy to survey the genome randomly was devised. This strategy involves the sequencing of the ends of large DNA fragments maintained as BAC clones. A large insert (130 kb) bacterial artificial chromosome (BAC) library with 25-fold coverage (9,216 clones) was previously constructed using a rice infecting strain 70-15. These end sequences (sequence tag connectors) in theory are distributed every 2-3 kb along the rice blast genome. Since the rice blast genome is estimated to contain ~10,000 genes over ~40 Mb, this strategy is anticipated to reveal the majority of the rice blast genes. Moreover, when coupled with BAC fingerprinting and contig assembly, this strategy will provide a physical layout of gene organization. The entire library has been fingerprinted and end-sequenced. The assembled contigs have been anchored using RFLP markers covering all seven chromosomes. Up to date progress on contig assembly, contig anchoring and sequence analysis will be presented. The data is publicly available at www.genome.clemson.edu.

21. Biochemical aalysis of WC-2, a transcription factor required for the biological clock. Deanna L. Denault [1], Liu Yi [1], Susan K. Crosthwaite[2], Chenghua Lou [1], Jay C. Dunlap [1], and Jennifer J. Loros [1]. [1]Dartmouth Medical School, Biochemistry, Hanover, NH, USA. [2]University of Manchester, Biological Sciences, Manchester, UK.

White-collar loci products (*wc-1 wc-2*) have been found to act as global regulators for light perception and the circadian clock in *Neurospora crassa*. Both WC-1 and WC-2 proteins contain Zn-finger domains with distinct similarity to other transcriptional activators within the GATA factor family. Additionally, both WC's contain PAS domains, evolutionary conserved regions mediating protein-protein interactions. WC-2 antibodies show WC-2 to be predominantly a nuclear protein that does not display an robust rhythm in either the nuclear or cytoplasmic fraction. Moreover, we do not detect a significant change in level following a light-pulse. This surprising lack of regulation prompted us to determine if regulation could be at the level of association with other proteins. On sucrose gradients we find WC-2 migrating in two peaks; the ~60 KDa low molecular weight peak corresponds to the monomeric form, whereas the ~200 kDa high molecular weight peak has a broad tail trailing towards ~540 kDa. As expression of a central component of the *Neurospora* clock, *frq*, requires WC-2 (Crosthwaite et al. Science 276:763-769, 1997) and FRQ is also known to repress it's own expression, one possibility is that

FRQ interacts directly with WC-2, thereby interfering with transcriptional activation. To address this we performed *in vitro* binding assays of GST-tagged WC-2 and radiolabelled FRQ proteins. Under these conditions, WC-2 specifically interacts with FRQ. Studies are currently underway to determine if this interaction can be demonstrated *in vivo*.

22. Cloning and characterization of two novel genes from *Aspergillus niger* encoding peptidyl prolyl cis-trans isomerases belonging to the cyclophilin family. Patrick M.F. Derkx, Susan M. Madrid. Danisco Ingredients, Danisco Biotechnology, Copenhagen, Copenhagen, Denmark.

Aspergillus, a filamentous fungus, is widely used for the production of homologous and heterologous proteins but, compared to homologous proteins the production levels of heterologous proteins are usually low. Low levels of secreted proteins may be due to limitations at the post translational level. Peptidyl prolyl cis-trans isomerase (PPI) is a foldase which catalyzes the *cis-trans*isomerization of peptide bonds preceding proline residues. Two *A. niger* genes encoding cyclophilin like PPIs have recently been cloned by heterologous screening of genomic and cDNA libraries. These genes encode a protein of approx. 20 kDa in size and both proteins contain a PPI specific catalytic core domain and a region involved in the binding of cyclosporin A. The CYPA protein, lacking signal sequence and ER retention signal, is most likely targeted to the cytosol. The CYPB protein however, contains a signal sequence and an ER retention signal which is responsible for targeting and retention of proteins in the ER. Transcription of *cypB* was shown to be induced by stress caused by unfolded proteins and heat shock whereas the transcription of *cypA* only increases moderately after heat shock. It is therefore likely that CYPB plays an important role in the folding of secretory proteins. Acknowledgments This work was funded by an EC Biotechnology program grant BIO2 CT-942045

23. Construction of a plasmid for expression of glycosylated bovine beta-casein in *Aspergillus oryzae*. Todd Z. DeSantis[1], Gabe Overbay[1], Rafael Jimenez-Flores [2], Susan Elrod[1]. California Polytechnic State University, Biological Sciences, San Luis Obispo, CA, USA. [2] CalPoly State University, Dairy Prod. Tech. Center, San Luis Obispo, CA, USA.

Beta-casein is a non-glycosylated, monomeric milk phosphoprotein with a hydrophilic domain in its N-terminal region and a contrasting hydrophobic domain at its C-terminal end. This amphiphilic property makes beta-casein a good surface active agent that has applications in the food industry as well as the potential for use in biodegradation of marine petroleum spills. The bovine beta-casein gene has been previously cloned and mutated to contain a novel glycosylation site at Asn_{73}, near the N-terminus. This glycosylated protein has been shown to possess enhanced emulsifiying properties. It has also been demonstrated to act as an antifreeze agent. Previous attempts to produce enough protein for further study have not been very successful. Expression in a transgenic mouse system yielded 2 mg/ml, however the high cost of maintaining this system makes it impractical. Expression in a *Pichia* yeast system yielded <1 mg/l and the protein was not secreted. Here, we report the construction of a plasmid for expression of glyco-beta-casein cDNA in *Aspergillus oryzae.* The 5' end of the cDNA was fused in frame to the promoter and secretion signal from the *A. oryzae* TAKA amylase gene while the 3' end was fused to the glucoamylase transcriptional terminator from *A. awamori*. Fusions were accomplished using the

"Splicing by Overlap Extension" (SOE) PCR technique and cloned into pCR-Script. This plasmid is being used to transform *A. oryzae*. Glyco-beta-casein expression levels will be measured using protein gel electrophoresis and Western blots with bovine beta-casein-specific antibodies.

24. A novel ATP-binding cassette transporter involved in multidrug resistance in the the filamentous fungus *Aspergillus nidulans*.Adriana M. do Nascimento[1], Maria F. Terenzi[1], Maria Helena S. Goldman [2], Gustavo H. Goldman[1]. FCFRP-Universidade de Sao Paulo, Ciencias Farmaceuticas, Ribeirao Preto, Sao Paulo, Brazil. [2]FFCLRP-USP, Biologia, Ribeirao Preto, Sao Paulo, Brazil

Multidrug resistance can be caused by increased ATP-dependent efflux of toxic compounds from cytoplasm and plasma membrane mediated by membrane-bound ATP-dependent transporters of the ABC (ATP-Binding Cassette) superfamily. As a preliminary step to characterizing genes encoding ABC-proteins that confer multiple drug resistance in *Aspergillus nidulans*, we are using a PCR-based approach. Five degenerated primers designed based on the coding regions of the ATP-binding cassette were used to identify such genes. DNA fragments in the expected size were amplified. These fragments were cloned and several subclones were sequenced. Sequence and Southern blot analysis showed that these fragments correspond to four different genes encoding ABC-transporters (named abcA-D, respectively). Northern blot analysis showed that the *abcD* gene transcript size is around 6.0-kb and this gene is induced about five-fold by miconazole, three-fold by ethidium bromide, and two-fold by camptothecin. Financial support: FAPESP, CNPq, and CAPES, Brazil, and ICGEB-UNIDO.

25. Biochemical and molecular responses to exogenous sterols by Phytophthora spp. W. David. Dotson, Shirley R. Tove, Leo W. Parks. North Carolina State University, Microbiology, Raleigh, NC, USA.

Phytophthora species are eukaryotic natural sterol auxotrophs. Sterol biosynthesis is blocked at the level of squalene epoxidation in *Phytophthora*. Remarkably however, vegetative growth of *Phytophthora* can occur even in the complete absence of sterols. Growth of these organisms is enhanced, often dramatically, when an exogenous source of sterols is provided. Furthermore, sterols may be required for the induction and development of sexual spores in *Phytophthora*. Using differential display, we have seen evidence for altered gene expression patterns in response to the presence of sterols. Using a low stringency Southern blot method, we have looked for homologs of yeast sterol metabolic genes in *P. cactorum*and *P. parasitica*. Hybridization patterns have implicated *Phytophthora* sequences similar to the sterol C5 desaturase but not to the C22 sterol desaturase gene of *Saccharomyces cerevisiae*. Through sterol feeding experiments and chromatographic analysis we have confirmed the existence of both C5 desaturase and delta 7 reductase activities in *Phytophthora* and the absence of C22 desaturase activity. Evolutionary conservation of these sterol conversions indicates their physiological significance to *Phytophthora*. While the physiological consequences of these conversions remain unclear, further elucidation of sterol function and metabolism in *Phytophthora* should facilitate more rationally designed approaches towards the control of these important pathogens.

26. Longevity and stability of the mitochondrial chromosome in *Podospora anserina* greatly depends on the respiratory metabolism.Eric Dufour, Odile Begel, Jocelyne Boulay, Béatrice Albert, and Annie Sainsard-Chanet. C.G.M, C.N.R.S, GIF-sur-YVETTE, 91190, FRANCE

The vegetative growth of the filamentous fungus *Podospora anserina* is limitated. This senescence process is always associated with the accumulation of circular molecules (senDNAs) containing specific regions of the mitochondrial chromosome. Although the link between senescence and mitochondrial instability is well established in *Podospora* and in different fungi, the nature of this link and the control of the mitochondrial DNA integrity are not clearly understood at the present time. Whereas some data suggested that the mobile group II intron alpha (the first intron of the cytochrome c oxidase subunit I gene,cox1) has a prominent role in the senescence process in *Podospora anserina*, others indicated that it is not the case for some nuclear mutants. We describe the first mutant of *Podospora anserina* precisely deleted for this intron and show that it displays a senescence syndrome similar to that of the wild-type though its lifespan is increased about two-fold. We also describe a nuclear mutant in which subunit 5 of cytochrome c oxidase is inactivated. This mutant uses an alternate respiratory pathway. We show that it escapes senescence as the spontaneous mitochondrial mutants mex, deleted for a part of intron alpha and of the upstream exon. Interestingly, these results indicate that « immortality » in *Podospora anserina* can be acquired not by the lack of intron a but by the lack of active cytochrome c oxidase and that the respiratory metabolism plays a major role in the control of the mitochondrial DNA integrity. The factors involved in this control (possible antioxygen defense role of the alternate oxidase, reduced energetic statement, modification in the import mitochondrial apparatus) will be discussed.

27. The [URE3] prion of *Saccharomyces cerevisiae* is an aggregated form of Ure2p that can be cured by overexpression of Ure2p fragments. Herman K. Edskes, Kimberly T. Taylor, Vaughn T. Gray, Reed B. Wickner. NIDDKD, NIH, Laboratory of Biochemistry and Genetics, Bethesda, MD, USA.

Genetic evidence identified [URE3] as the prion form of Ure2p. Ure2p functions in blocking assimilation of poor nitrogen sources in the presence of a good nitrogen source (e.g. ammonia or glutamine). In its prion form this function is lost but the protein has acquired the ability to convert its normal form into the altered prion form. The N-terminal region of Ure2p (Ure2p1-65) has been designated the prion domain as it is sufficient to propagate [URE3]. Its overexpression induces the de novo appearance of [URE3] by 1000-fold. The C-terminal region of Ure2p carries out the nitrogen regulatory function. A fusion between Ure2p or Ure2p1-65 and green fluorescent protein (GFP) is aggregated in cells carrying [URE3] but evenly distributed in cells lacking the [URE3] prion. The Ure2p C-terminus when fused to GFP is evenly distributed regardless of the presence or absence of [URE3]. Overexpression of fragments of Ure2p or Ure2-GFP fusion proteins efficiently cure the prion. We suggest that incorporation of fragments or fusion proteins into a putative [URE3] 'crystal' of Ure2p poisons its propagation.

28. Characterization of promoter elements involved in the expression of early and late aflatoxin pathway genes. Kenneth C. Ehrlich, Jeffrey W. Cary, and Beverly G. Montalbano. Southern Regional Research Center/USDA, Food and Feed Safety, New Orleans, LA, USA.

Aflatoxins are the most potent fungal carcinogenic metabolites. Enzymes involved in aflatoxin biosynthesis in *Aspergillus parasiticus* are encoded by as many as 17 clustered genes. Most of these genes are co-regulated by the C_6-zinc cluster DNA-binding protein, AFLR, as evidenced by the presence of binding sites for AFLR in the promoter regions of those so far characterized. Expression of the gene encoding AFLR, and the genes for polyketide formation,*fas1A, fas2A*, and *pksA*, occurs prior to expression of genes encoding oxidative enzymes involved in later steps in the biosynthetic process, such as *avnA, ver1* and *omtA*. The *aflR* and *avnA* promoters were analyzed using Beta-glucuronidase reporter assays to elucidate regions involved in transcription control. Maximal promoter activity for both genes was found in *A. parasiticus*transformed with constructs in which the promoter was truncated at -118 bp. Approximately 20% of the maximal activity occurred when the promoter was truncated at -100, thereby removing putative AFLR binding sites. Based on electrophoretic mobility shift assays, only AFLR bound to sequences from -100 to -118 in the *avnA* promoter, whereas, in addition to AFLR, a protein or proteins in nuclear extracts from *A. parasiticus* grown on inducing medium bound to an adjacent or overlapping site in the *aflR* and *pksA* promoters. Sites for binding the transcription factor that mediates regulation by ambient pH, PACC, were detected in the *aflR* and *pksA* promoters, but not in the *avnA* promoter. PACC binding could be involved in negative regulation of gene expression when the fungi are grown in aflatoxin non-inducing media.

29. Effect of site-directed mutagenesis of the *magB* gene on G protein signaling in *Magnaporthe grisea*. Eric G.C. Fang, Ralph A. Dean. Clemson University, Plant Pathology, Clemson, SC, USA.

MagB, encoding a G alpha subunit, is involved in signaling pathways that regulate a number of cellular responses in *M. grisea*, including appressorium formation, conidiation, sexual development, mycelial growth, and surface sensing. Site-directed mutagenesis was used to further dissect the pleiotropic effects controlled by *magB*. Conversion of glycine 42 to arginine ($magB^{G42R}$) was predicted to abolish GTPase activity, which in turn constitutively activates G protein signaling. This mutation caused autolysis in the aged cultures, misscheduled melanization, reduction in both sexual and asexual reproduction, and reduced virulence. Furthermore, $magB^{G42R}$mutants were able to produce appressorium on both hydrophobic and hydrophilic surfaces, although the development on the hydrophilic surface was delayed. A second dominant mutation $magB^{G203R}$ (glycine 203 converted to arginine) was expected to block dissociation of the G beta gamma from G alpha subunit thus producing a constitutively inactive G protein complex. This mutation did not cause drastic phenotypic changes in the wild type genetic background, but complemented the conidiation defect in *magB*- mutants. These results strongly suggest the involvement of the G beta gamma subunit in signaling pathways regulating cellular development in *M. grisea*.

30. The sequence requirements for arginine attenuator peptide function in arginine-specific translational regulation. Peng Fang, Zhong Wang, and Matthew S. Sachs. Oregon Graduate Institute, Biochem & Mol Biol, Portland, OR, USA.

The *Neurospora crassa arg-2* mRNA contains an evolutionarily conserved, 24-codon upstream open reading frame(uORF)in its 5'-leader. This uORF encodes the arginine attenuator peptide (AAP) which, when translated in the presence of high [Arg] causes ribosomes to stall at its

termination codon (codon-25), reducing translation of the mRNA. We examined the role of AAP peptide and RNA sequence on translational control using an *N. crassa* cell-free translation system and primer-extension inhibition assay to map ribosomes on the mRNA. Deletion of the relatively non-conserved AAP N-terminus had no significant effect on AAP-mediated translational regulation, but continued deletion into the highly conserved region eliminated regulation. Parallel introduction of silent mutations at each possible codon where substitution was possible in a functional, shortened AAP coding region(28/63nt changes) did not significantly affect AAP function, although single nucleotide changes altering the conserved peptide sequence eliminated function. Finally, when a rare Leu codon (UUA) but not a common Leu codon (CTC) was inserted at position 25 in an AAP-luciferase fusion polypeptide, substantial Arg-regulated ribosome stalling was seen at the rare Leu codon but not the common Leu codon. These data indicate that the sequence of the evolutionarily conserved nascent peptide, but not the sequence of the mRNA that encodes it, is responsible for arginine-specific translational attenuation. They suggest that the mechanism by which Arg and the nascent AAP serve a regulatory function is enhanced when the ribosome encounters termination or rare codons immediately after AAP translation. Possibly such encounters provide more time or a more favorable environment for the AAP to exert regulatory function. (Supported by NIH GM47498.)

31. Expression of *Nit-3* gene of nitrate catabolic pathway in *N. crassa* is under general negative control. Bo Feng. The Ohio State University, Biochemistry, Columbus, Ohio 43210, USA.

The activated expression of *nit-3* gene encoding nitrate reductase in *Neurospora* is regulated by the synergistic action of the global positive regulator NIT2 and the pathway-specific positive regulator NIT4. The physical NIT2-NIT4 interaction is critical for the synergy between these two proteins. A mutant NIT2 that failed to interact with NIT4 resulted in defective *nit-3* gene activation and nitrate utilization. Reverse genetics was used to isolate nitrate utilization revertants in the background of a NIT2 zinc finger mutation lacking the ability to interact with NIT4. The revertants, snnb1 and snnb2 (Suppresser of NIT2 Non-NIT4 Binding mutation) regained the ability to activate *nit-3* expression independent of NIT2-NIT4 interaction, suggesting that mutation in either snnb1 or snnb2 bypassed the requirement of NIT2-NIT4 interaction for activating *nit-3* gene. Snnb mutants also resulted in the abnormal exression pattern of nit-3, rendered it partially insensitive to nitrogen repression and independent of nitrate induction. Further analysis showed that mutation in snnb2 also resulted in defects in glucose repression of beta-galactosidase expression. Moreover, snnb mutants exhibited either delayed conidiation or aconidiation, female sterility and semicolonial morphology, indicating a general role of snnb loci in regulating cell functions. This study suggests that the *nit-3* gene in nitrate assimilation pathway is subjected to general negative regulation. The NIT2-NIT4 interaction may conteract the negative effects of snnb, thus allowing regulated, yet high level, nit-3 expression. (This work was supported by grant GM23367 from the National Institute of Health)

32. *Schizophyllum commune*'s "Scooter" is a member of an active transposon family and has disrupted *thn*, a homolog of fungal regulatory genes. Thomas J. Fowler, Micheal F. Mitton, and Carlene A. Raper. University of Vermont, Microbiology & Mol.Genet., Burlington, VT, USA.

Several DNA sequences from *Schizophyllum commune* that represent a family of transposable elements called *sct* (***S****chizophyllum* ***c****ommune* **t**ransposon- nicknamed "Scooter") have been characterized. The original isolation of *sct* was from an insertional mutation of the mating-type receptor gene, *bbr2*. A nonfunctional *bbr2* was cloned and shown to contain a 647 base pair (bp) insertion. The inserted DNA had two features of a transposable element: an 8 bp target site duplication (TSD) and 32 bp inverted repeats at each end. The *sct* DNA sequence had neither significant similarity with other sequences in DNA databases nor any long open reading frames. Genomic Southern blot analysis of several *Schizophyllum* strains, using *sct* as a probe, showed up to twenty DNA fragments with significant hybridization. PCR was used to recover other possible members of the *sct* family. One amplified product, of approximately 1200 bp, contained nearly all of the *sct* sequence and had additional sequence located between the inverted repeats. This larger element, *sct2*, was used as a probe to screen a cDNA library. Two positive clones are being characterized for their potential to encode a *sct*transposase. In a related experiment, a colony was identified that had a sector of the frequently occurring mutant phenotype known as thin. We determined that the thin sector was the result of a *sct* insertion into a gene designated *thn*. The inserted DNA caused 8 bp TSDs and was 98% identical to the original *sct*. The *thn* mutation was complemented by DNA-mediated transformation with a wild-type allele of *thn*. The *thn* gene product is related to a regulator of conidiation in *Aspergillus nidulans* and a negative regulator of signal transduction in *Saccharomyces cerevisiae*.

33. Regulation of the *fmdS* gene encoding formamidase in *Aspergillus nidulans.* James A. Fraser, Meryl A. Davis, and Michael J. Hynes. University of Melbourne, Genetics, Parkville, Vic, Australia.

Fungi are capable of utilising numerous unrelated carbon and nitrogen sources, with the expression of the genes involved often strictly regulated. A major form of this regulation occurs as the phenomena of nitrogen metabolite repression (NMR) and carbon catabolite repression (CCR). Amide utilisation by *A. nidulans* has been extensively studied in our laboratory through analysis of the complex regulation of *amdS*. The primary substrate of *amdS* is acetamide, with expression affected by NMR, CCR and induction by acetate and omega-amino acids. The utilisation of formamide is mediated by a different structural gene, *fmdS*. We have cloned the *fmdS* gene and found that it encodes a protein belonging to the urease family of enzymes, rather than the amidase signature group. Unlike *amdS*, *fmdS* expression is primarily regulated via NMR and does not require addition of exogenous inducer. Despite the role of formamide as a nitrogen source only, *fmdS* displays a novel form of carbon regulation. Under carbon starvation conditions expression decreases dramatically, the opposite response to genes regulated by CCR. To further analyse *fmdS* regulation a series of reporter gene fusions were created, helping to determine specific sites of action for AreA (the major NMR regulatory protein). A binding site required for the *A. nidulans* CCAAT-binding factor has also been identified, with *fmdS* expression reduced twofold in a *hapC* deletion mutant. Sequence analysis and isolation of cDNAs show that a gene of unknown function lies directly 5' of *fmdS*, with 936bp of overlap with the *fmdS* transcript. The 5' gene has been disrupted through insertion of the *riboB* gene, with no phenotype yet discovered. The role of this gene may potentially be in nitrogen catabolism, as RT-PCR has shown it to be regulated in response to nitrogen limitation.

34. Characterization of signals for de novo DNA methylation in vegetative cells of *Neurospora crassa*. Michael Freitag, Vivian Miao, and Eric U. Selker. University of Oregon, Inst. of Mol. Biol., Eugene, OR, USA.

Like DNA of many other eukaryotes, Neurospora DNA can be modified by cytosine methylation. Our goal is to understand how specific cytosines are targeted for methylation. Most DNA sequences that are subject to de novo methylation in vegetative cells of Neurospora have previously undergone repeat-induced point mutation (RIP). RIP introduces C:G to T:A mutations and enriches DNA for A+T in general and TpA dinucleotides specifically. We carried out a detailed dissection of a relic of RIP, the zeta-eta region, to elucidate which mutations induced by RIP and/or which features of mutated DNA create methylation signals. We report results from tests of hybrid constructs involving segments of eta and its unmutated homologue, theta, and of studies with methylation signals created by in vitro mutagenesis. We show that the polarity of RIP (C:G to T:A mutations) is essential for creating signals for de novo methylation from unmutated Neurospora DNA. Whereas both increases in A+T content and TpA density of DNA contribute to the strength of methylation signals, a larger effect was found by increasing the TpA density without altering A+T content when compared to fragments that were very A+T-rich but had low TpA densities.

35. Resection of the frequency promoter of *Neurospora crassa*. Allan C. Froehlich, Yi Liu, Jay Dunlap, and Jennifer Loros. Dartmouth Medical School, Biochemistry, Hanover, NH, USA.

The circadian system in *Neurospora crassa* is among the best understood of any organism. The oscillator consists of an autoregulatory feedback cycle, wherein the frequency gene encodes for two forms of the FRQ protein which negatively feed back on their own expression resulting in rhythmic levels of both frq RNA and protein. The level of action of the negative feedback, transcriptional versus post-transcriptional, is being investigated using frq::hph reporter constructs. The *N. crassa* circadian system can also be entrained, both by light and by temperature. Temperature entrainment is translationally regulated, whereas light resets the *N. crassa* clock by rapidly inducing frq at the transcriptional level. A small region of the promoter responsible for the light response has been isolated using cis-analysis, and factors interacting with this region are currently being examined.

36. *Ashbya gossypii* as a model system for fungal functional genomics. Thomas D. Gaffney[1], Albert Flavier[1], Krista Gates[1], Michelle Kirksey[1], John Marhoul[1], Joann Gardner[1], Steve Goff[1], Fred Dietrich[2], Peter Philippsen[2]. [1] Novartis AgBiotechResearch, Res. Tri. Pk., NC, USA. [2] U. of Basel, Biozentrum, Applied Microbiology, Basel, Switzerland

The filamentous ascomycete *Ashbya gossypii* was first identified in 1915 as a pathogen of cotton, and described more extensively in 1926 by Ashby and Nowell (Annals of Botany 40:69-86). It has been noted as a particularly destructive pathogen, capable of inflicting severe damage on developing cotton bolls and making it impossible to grow cotton in certain parts of the world (U.S. Department of Agriculture Technical Bulletin No. 1469 (1973)). Further reports indicate that Ashbya is also a pathogen of tomato and various citrus fruits, and is vectored by sucking insects such as Antestia and Dysdercus. Additional features of *Ashbya gossypii* make it an appealing microorganism for a functional genomics approach: 1) One of the smallest known

eukaryotic genomes - 8.8 million base pairs. 2) Very efficient homologous recombination, allowing simple gene knockout strategy and precise positioning of reporter gene constructs. 3) Yeast replicons function in Ashbya, allowing efficient introduction of heterologous DNA. 4) Lack of the extensive duplication of chromosomal segments observed in yeast - should allow identification of phenotypes "masked" by duplication in yeast. 5) Useful model system for identification of genes and pathways required for normal filamentous growth. Here we report on a sampling of the results obtained thus far with the Ashbya model system.

37. Cytochrome c expression in *Aspergillus nidulans.* Rebecca E. Gardiner[1], Rosemary E. Bradshaw[1], and Simon C. Brown[2]. [1] Massey University, Inst. of Mol. Biosciences, Palmerston Nth, New Zealand. [2]Massey University, Inst. of Fundamental Sci., Palmerston Nth, New Zealand.

The filamentous fungi *Aspergillus nidulans* is an obligate aerobe, and therefore generates its main energy requirements by means of oxidative respiration, using the cytochrome *c* respiratory pathway. This is in contrast to the yeast *Saccharyomyces cerevisiae*, which can grow without oxygen. *S. cerevisiae* switches off the production of many oxidative respiratory components (such as cytochrome *c*) when energy can be produced by alternative means (fermentation) which do not require oxygen. Surprisingly, *A. nidulans* appears to regulate the production of cytochrome *c* in a similar manner, even though it appears to have an absolute requirement for oxygen. A functional analysis of the *A. nidulans* cytochrome *c* gene (*cycA*) promoter is currently being carried out to determine the molecular basis of regulation of the gene. In particular, the focus will be on the HAP1 and HAP2 regulatory proteins, which are known to affect cytochrome *c*expression in yeast, since consensus sequences for the binding sites of these proteins have been found in the *cycA* promoter, and the gene is known to be transcriptionally induced by oxygen. Another intriguing observation is that cytochrome *c* deficient mutants of *A. nidulans* which were created at Massey University by targeted gene disruption (Bird, 1996) are viable upon fermentable carbon sources. These results suggest the mutant strains must be using alternative means of energy production which do not require cytochrome *c*. The extent to which these mutant strains (as well as wildtype strains) are utilising an "alternative" respiratory pathway and fermentation has been investigated using respiratory measurements and ethanol assays, respectively.

38. Attempts at cultivating wild strains of various Agaricus species.József Geml[1] and Dr. Imre Rimóczi[2].[1]Korona Spawn Plant and Research Laboratory, H-3395 DEMJÉN Pf. 1., Hungary and University of Horticulture and Food Industry, Department of Botany, BUDAPEST. [2]University of Horticulture and Food Industry, Department of Botany, H-1118 BUDAPEST Ménesi u. 44., Hungary.

The mushroom produced in the greatest amount today is *Agaricus bisporus*. Beside this species some other Agarics have been examined by mushroom breeders, although growing them is not in practice yet (perhaps there is one exception: *A. bitorquis*, but its cultivation takes place in much smaller scale). The importance of using wild varieties of *A. bisporus* in breeding new commercial strains has been realized by several researchers in the last decade. These wild types can be used to improve the commercial strains' growing characteristics, resistance to pests and diseases etc. In our laboratory we made the first steps of this long way, collecting wild varieties

of several species, bringing them into cultivation and making some initial observations which can be useful in the future. In this paper we are going to introduce the main Agaricus species of Hungary, including their descriptions and habitats with some experiences in culturing and cultivating tribes of them.

39. Catalase activity is necessary to heat-shock recovery in *Aspergillus nidulans* germlings. Gustavo H. Goldman [1], Maria A. Noventa-Jordão [1], Ricardo M. Couto [1], Maria H. Goldman[2], J. Aguirre[3] Suresh Iyer[4] Allan Caplan[4]and Hector F. Terenzi[2]. [1]FCFRP-Universidade de Sao Paulo, Ciencias Farmaceuticas, Ribeirao Preto, Sao Paulo, Brazil. [2]FCLRP-USP, Biologia, Ribeirao Preto, Sao Paulo, Brazil. [3]AM, Inst.Fisiol.Cel., Mexico, Mexic. [4]University of Idaho, MMBB, Moscow, Idaho, USA.

To understand the molecular mechanisms induced upon stress that contribute to the development of tolerance in eukaryotic cells, we have chosen the filamentous fungus *Aspergillus nidulans* as a model system. Here, we report the response of *A. nidulans* germlings to heat-shock. The heat treatment dramatically increased the concentration of both mannitol and trehalose. We have found that the defense against the lethal effects of heat exposure depends on the activity of the defense system against oxidative stress. We show that treatment with hydrogen peroxide increases *A. nidulans* germling viability after heat shock. In addition, mutants deficient in the key antioxidant enzyme catalase were more sensitive to a 50 ºC heat exposure. Under the tested conditions, the *cat*A mRNA accumulated upon heat-shock while *cat*B mRNA levels remained unaltered. Financial support: FAPESP and CNPq, Brazil, and ICGEB-UNIDO

40. Tagging of genes that confer multidrug resistance in *Aspergillus nidulans* by restriction enzyme-mediated integration (REMI). Gustavo H. Goldman [1], Cristiane C. de Souza, and Maria Helena S. Goldman[2]. [1]FCFRP-Universidade de Sao Paulo, Ciencias Farmaceuticas, Ribeirao Preto, Sao Paulo, Brazil. [2]FFCLRP-USP, Biologia, Ribeirao Preto, Sao Paulo, Brazil.

As a preliminary step to characterizing genes that confer pleiotropic drug resistance in *Aspergillus nidulans*, we isolate transformants by REMI (Restriction Enzyme-Mediated Integration) that show pleiotropic drug sensitivity. We have used a plasmid containing the *pyr*4 gene to transform an *A. nidulans pyr*G mutant in the presence of *Bam*HI. One thousand two-hundred sixty-seven transformants were isolated using the plasmid pRG3 digested with *Bam*HI. Southern analysis of these mutants, and of randomly selected transformants was consistent with the occurrence of single plasmid integration events in about 70 % of the cases. Approximately 900 of these transformants were examined for sensitivity to fourty-seven drugs or stress agents with different and/or the same mechanism of action. Thirty-five transformants displayed sensitivity to a single drug (either itraconazole, miconazole, hygromycin or cycloheximide) while six of them displayed multidrug-sensivity. The *pyr*4 marker was shown to be tightly linked to the mutant phenotype in only one from these multidrug-sensitivity transformants. Financial support: FAPESP and CNPq, Brazil, and ICGEB-UNIDO

41. Using time lapse video to analyze the circadian rhythm of Neurospora. Van D. Gooch, and Cory D. Loxtercamp. University of Minnesota-Morris, Div of Science and Math, Morris, MN, USA.

Neurospora crassa expresses a clearly defined circadian rhythm of conidiation with a period of 21.5 hours. We have monitored this rhythm using time-lapse video under constant red light. It was found that under these rhythmic conditions the growth front would proceed past the eventual location of conidiation with no conidiation at the front; once the growth front was at the end of the area of eventual conidiation, then development of conidia in all areas of that band occurred in unison. Densitometry analysis and curve fitting using individualized frames was used to determine peak times of conidiation. The accuracy of this technique was found not to be significantly different than the classical interpolation technique use by Sargent and others. *Neurospora* were subjected to a 1 hour 38C pulse during different times of the 21.5 hour period and a time lapse video of the phase response curve was produced.

42. Identification, characterization and chromosomal localization of two putative histone deacetylases from *Aspergillus nidulans.* Stefan Graessle, Peter Loidl, Hubertus Haas, Markus Dangl, and Gerald Brosch. Innsbruck, Microbiology, Med. School, Innsbruck, Tirol, Austria.

Growth and development of prokaryotic and eukaryotic cells depends on a coordinated gene expression. Thereby regulation on the level of transcription plays an essential role in all organisms. In eukaryotes, transcriptionally active genes are preferentially localized in genomic regions enriched in acetylated histones. The dynamic equilibrium of core histone acetylation is maintained by histone acetyl-transferases and deacetylases. Both enzymes often function as components of regulator complexes targeted to particular genes by DNA binding transcription factors. Using PCR approaches, we have cloned and sequenced cDNA fragments of *arpd3* and *ahos2*, two putative histone deacetylases from *Aspergillus nidulans*, which are the first deacetylases to be analyzed from a filamentous fungus. Comparisons of the cloned sequences with the GenBank database revealed high similarity to RPD3-type deacetylases from *Saccharomyces cerevisiae*. Hybridization of these cDNA fragments with a chromosome-specific cosmid library of *A. nidulans* allowed the chromosomal localization of both genes and led to the genomic sequence of *arpd3*. Moreover, comparison between this sequence and the corresponding cDNA revealed 3 introns interrupting an open reading frame of 2028 bp, which encodes a protein of 676 amino acids with a predicted molecular weight of 75 kDa. Compared to different yeast RPD3-type deacetylases the deduced ARPD3 amino acid sequence reveales a considerable extension of the C-terminus. Currently, we are using northern hybridization analysis in order to determine the expression levels of ARPD3 and AHOS2 in different developmental states and distinct growth conditions of the fungus.

43. Homologous transformation of *Fusarium venenatum.* Alison M. Griffen, Marilyn G. Weibe, Geoff D. Robson, and Anthony P.J. Trinci. University of Manchester, Sch. Biological Sciences, Manchester, Manchester, UK.

Although *F. venenatum* has been transformed successfully using heterologous vectors the transformation rate is low. *F. venenatum* is a good host for heterologous protein production as it is already cultured in 150 m^3 fermenters for the production of Quorn. We have been developing a homologous transformation system based on the hygromycin resistance plasmid pAN7-1 and ribosomal DNA. A 1.2 kb rDNA fragment was amplified by PCR from *F. venenatum* genomic DNA. This rDNA fragment was cloned into pAN7-1 to generate pAGF-37 and pAGF-48 which contain one and two copies of the rDNA fragment respectively. These plasmids have been used

to transform protoplasts of *F. venenatum* giving 4.9 transformants per ug DNA. The transformants obtained have been assessed for their ability to grow on varying concentrations of hygromycin B. Southern analysis of genomic DNA has determined whether the vectors have integrated at the rDNA locus and given an indication of copy number. We have now introduced the *glaA* gene from *Aspergillus* into pAN7-1 containing an rDNA fragment. Using this vector we hope to determine whether previously low heterologous glucoamylase production was the result of poor expression from a heterologous promoter or the result of poor integration.

44. Breeding and cultivating wild Pleurotus strains in hungary.Csaba Hajdú. Korona Spawn Plant and Research Laboratory, H-3395 DEMJÉN Pf. 1., Hungary.

The oyster mushrooms (Pleurotus sp.) are cultivated in the largest quantity in the world after *Agaricus bisporus*. The cultivation of this mushroom has been increased in some areas of the world, so its importance is undoubted. In Hungary, the total quantity of all cultivated mushrooms was about 33,000 tons in 1997 and around 10 % of it was *Pleurotus ostreaus*. Hungary was one of those countries where oyster mushroom cultivation started as early as in the 60's. Due to our famous researchers and growers, since that time we have obtained lots of worldwide important pioneer results (successful new strains, improved growing methods etc.). This paper gives an overview about the research and breeding work of the Korona Spawn Plant and Research Laboratory; including the collecting wild strains of *P. ostreatus*, *P. pulmonarius* and other species, selecting the best ones, creating and growing new hybrids of them.

45. Mutagenic DNA-repair genes in *Aspergillus nidulans*: The uvsI gene encodes a REV3 homologue, a subunit of the non-essential DNA polymerase zeta. Kyu-Yong Han [1], Suhn-Kee Chae [2], and Dong-Min Han [1]. [1]Wonkwang University, Division of Life Science, Iksan, Chonbuk, South Korea. [2]Paichai University, Division of Life Sciences, Taejon, South Korea.

Reductions of spontaneous and UV-induced reversion of certain mutant alleles have been shown in *uvsI* mutant strains of *Aspergillus nidulans*. To facilitate cloning of the *uvsI* gene on MMS containing plates, *uvsI, uvsA* double mutants exhibiting very high MMS-sensitivity were used as a transformation host, since either single mutant was no more than slightly sensitive to MMS. An *uvsI*-complementing clone was obtained from a chromosome III specific library. Sequence determination of a minimally localized DNA fragment having the *uvsI*-complementing activity within the clone revealed an ORF with the highest amino acid identity to yeast REV3, a subunit of the DNA polymerase zeta involved in translesion DNA synthesis. UV-survival of heterozygous diploids of *uvsI501* with a disruptant of the cloned gene demonstrating the same UV-survival curve as that for homozygous *uvsI501* diploids confirmed that the cloned gene is the *uvsI*. The *uvsI*ORF encodes a polypeptide of 1,681 amino acids with calculated MW of 191.4 KDa. One small intron of 54 bp at near the N-terminus is confirmed by sequencing of RT-PCR products. A Northern blot band of about 5.3 kb was detected. In UVSI, the well- conserved regions, I-VI, among DNA polymerases were present in the correct order. In addition, two zinc-finger motives, [C-X2-C]-X11-[C-X2-C] and [C-X2-C]-X10-[C-X4-C], existed similarly to REV3. Further sequencing of an upstream region of *uvsI* revealed another ORF of 1,401 bp without putative intron. This ORF resides very close to *uvsI* in opposite direction and encodes a putative polypeptide exhibiting high amino acid similarity to two hypothetical *Arabidopsis* proteins. A possible relationship between *uvsI* and the ORF is currently being carried out.

46. Regulation of the expression of the xylanolytic enzyme system in *Aspergillus niger*.
Alinda A. Hasper, Alinda A. Hasper, and Leo H. De Graaff. Wageningen Agricultural University, Molecular Genetics of Industrial Microorganisms, Wageningen, Gelderland, The Netherlands.

Little is known about the mechanism of pathway-specific induction of extracellular enzyme systems in fungi. Induction of polysaccharide degrading enzyme systems, as e.g.xylanases, depends on low molecular weight inducers, which can be taken up by the organism. In Aspergilli, has been shown that in addition to xylobiose, D-xylose is able to induce the xylanolytic system. However, it is not clear whether the induction is the direct effect of D-xylose or that the inducing compound is the result of a transglycosylation reaction, e.g catalysed by b-xylosidase. The gene encoding *A. niger* b-xylosidase, *xlnD*, has been cloned and the role of b-xylosidase in the induction process studied. Furthermore, *A. niger* mutants with decreased xylanolytic gene expression were isolated by using a xylan induction-responsive element of the endo-xylanase encoding gene xlnA of *A. tubingensis*. Endo-xylanase activity of these mutants decreased a 300-500-fold in comparison to the wild-type strain. Also a strong decrease is found for b-xylosidase activity in these mutants. By mutant complementing, the transcriptional activator *xlnR* was isolated. XlnR encodes a protein of 875 amino acids with a domain capable to form a Zn binuclear cluster. Besides this region no extensive homology was found to other transcription activators. By sequencing the *xlnR* allele of three loss-of-function mutants, a mutation was found in *xlnR* in all cases, excluding that the isolated gene is a suppressor. Using in vitro binding assays and footprinting techniques, the target sites in the promoter of *xlnA* have been determined to be 5'GGCTAA-3. Activation of transcription by XlnR is not limited to genes involved in xylan degradation, also genes encoding endoglucanases are activated.

47. Regulation of ornithine decarboxylase synthesis in *Neurospora crassa*.
Martin A. Hoyt. University of California, Irvine, Mol. Biol. and Biochem., Irvine, CA, USA.

Ornithine decarboxylase (ODC), encoded by the *spe-1* gene of *Neurospora crassa*, catalyzes the initial step in the synthesis of polyamines. In *N. crassa*, polyamines repress the synthesis and increase the degradation of ODC. Changes in the rate of ODC synthesis correlate with similar changes in the abundance of *spe-1* mRNA. This polyamine-mediated regulation of *spe-1* mRNA requires a sequence element downstream of the transcription start site. This polyamine responsive element (PRE) is required for polyamine-mediated repression of *spe-1* mRNA abundance, and can confer polyamine regulation to a downstream reporter coding region. Use of the beta-tubulin (*tub*) promoter to drive expression of the *spe-1* transcribed region demonstrates that polyamine regulation imparted by the PRE is promoter-independent. Neither deletion of the PRE nor changes in cellular polyamine status alter the half-life of *spe-1* mRNA. In addition to effects on *spe-1* mRNA abundance, sequences within the PRE impede the translation of a downstream coding region. This impediment is relieved by deletion of those sequences or by polyamine starvation. The sequences imparting translational effects lack either an upstream open reading frame or obvious secondary structure.

48. Distribution and evolution of the impala transposable element family in the *Fusarium oxysporum* species.
Aurélie Hua-Van[1], Catherine Gerlinger[1], Thierry Langin [2], and Marie-Josée

Daboussi[1]. [1] Université Paris-Sud, IGM, Orsay, Essonne, France. [2]Université Paris-Sud, IBP, Orsay, Essonne, France.

Impala is an active Class II transposable element, first identified in a strain of *Fusarium oxysporum* that is pathogenic on melon. The nine copies present in this strain have been sequenced and grouped in three divergent subfamilies, differing by an important nucleotide polymorphism (about 20 %). This situation can be explained by two non mutually exclusive hypotheses: (i) an ancestral polymorphism associated to vertical transmission, and/or (ii) horizontal transmission of one or more subfamilies from another species. To gain insights on the molecular evolution of *Impala* elements, we have investigated their presence in different host-specific forms, by Southern blot, PCR and sequencing. We showed that *Impala* elements are present in most of the *F. oxysporum* strains tested, indicating that they an ancient component of the *F. oxysporum* genome. Subfamily-specific amplifications revealed the coexistence of divergent subfamilies within the same genome, a situation in favour of the hypothesis of an ancestral polymorphism followed by vertical transmission and independent evolution in the specific-host forms. Phylogenetic analysis identified at least five subfamilies in which some elements showed particular features: internal deletions whose breakpoints are located at the same nucleotide position or high rate of transitions CG to TA. These particular sequences are found in strains with different host specificity, addressing questions about the evolutionary history of the strains. The use of *Impala* as a tool for tracing populations will be discussed.

49. Identification of an upstream gene that affects *aflR* expression. Yiting Huang, and Jae-Hyuk Yu. Clark University, Biology Department, Worcester, MA, USA.

A mutant hunt was conducted to identify and isolate genes which regulate the gene activator, *aflR*, in the sterigmatocystin (ST) / aflatoxin gene cluster in certain Aspergillus species. *aflR* resides within the ST gene cluster and is known to regulate the expression of all other genes within the cluster. A special mutant strain of *A. nidulans*, LY345, carrying multiple copies of the ST gene cluster, was mutagenized with 4-nitroquinoline-1-oxide. Mutants were screened for the production of ST intermediates. A mutant of LY345, YH7239, was unable to produce any ST intermediates. Northern analysis showed this strain produces no aflR transcript. Since all ST cluster genes in LY345 are buffered against mutations within the gene cluster by virtue of multiple copies, the mutant, YH7239, is presumed to carry a mutation in a possible regulatory gene located external to ST gene cluster. Future work will be directed at rescue and cloning of this presumptive regulatory gene.

50. Structure of genes for Hsp30 from the white-rot fungus *Coriolus versicolor* and enhancement of their expression by heat shock and exposure to a hazardous chemical. Yosuke Iimura, and Kenji Tatsumi. National Institute for Resouces and Environment, Hydrospheric Environmenta, Tsukuba, Ibaraki 3058569, Japan.

The white-rot fungus *Coriolus versicolor* is a ligninolytic basidiomycete and it has been the focus of considerable attention because of its ability to degrade hazardous chemicals. In this study, we isolated two genomic DNAs that encode the heat-shock protein Hsp30 from *C. versicolor*. The nucleotide sequences of the two genes differ at 37 positions within the open reading frames but these differences result in only three amino acid substitutions. Three small

introns interrupt the open reading frames. Two putative eukaryotic regulatory sequences, namely, a CAAT box and a TATA box, are present in the promoter regions. The promoter regions also contain the consensus heat-shock element, a xenobiotic-response element, a stress-response element, and a metal-response element. Northern blot hybridization indicated that the expression of these genes is constitutive at normal temperatures and enhanced at elevated temperatures. Expression was also enhanced in cells of *C. versicolor* that had been exposed to the hazardous chemical pentachlorophenol.

51. Characterization of alpha-amylase genes of industrial fungus *A. kawachii*. Kiyoshi Ito, and Yuji Miyamoto. National Research Institute for Brewing, Genetic Engineering Divison, Higashihirosima, Hiroshima, Japan.

Filamentous fungus *Aspergillus kawachii* is a nearly related strain to *A. awamori* and it is widely used for *shochu* (a Japanese traditional spirit) fermentation. *A. kawachii* produces acid stable alpha-amylase (AAA) along with neutral alpha-amylase (NAA) which is homologous to alpha-amylase of *Aspergillus oryzae* (Taka amylase, TAA). To examine the conditions of these alpha-amylase production, we characterized the alpha-amylase genes of *A. kawachii*. The genomic DNA library was screened using TAA cDNA as a probe. One AAA gene, two NAA genes, and one unidentified alpha-amylase (UAA) gene were cloned. From the results of Northern analysis and reporter gene analysis, it was known that NAA gene was expressed constitutively without the need of inducer such as starch or maltose and slightly repressed by the addition of glucose. The AAA gene was not expressed in liquid culture but strongly expressed in solid state culture.

52. Patterns of phylogeography and sequence evolution in the ribosomal intergenic spacer region (IGS) of the cosmopolitan mushroom *Schizophyllum commune*. Timothy Y. James, Jean-Marc Moncalvo, Shih-hon Li, and Rytas J. Vilgalys. Duke University, Botany, Durham, North Carolina, USA.

This study addresses gene flow and population structure in the common mushroom, *Schizophyllum commune*, by analysis of sequence variation in the intergenic spacer (IGS) region of the rDNA repeat. Over 180 strains of *S. commune* have been sequenced including two outgroup species for this fast-evolving gene region. Most of the strains were unique in sequence and dikaryotic strains often contained more than a single rDNA allele. Three major geographic clades were detected by phylogenetic reconstruction: a North American group, a Caribbean / South American group, and an Eurasian / African group. Patterns of phylogeography are consistent with a continental scale of population size, but there is also evidence for long distance gene flow. Most sequence polymorphism was clustered within a 50 base pair hypervariable region within the non-coding IGS region. The consistency index of several of these characters (nucleotides) was 1.00 suggesting that recombination in this gene region may be suppressed. The pattern of genetic inter-relatedness between geographic regions for the IGS data were very similar to data collected using allozyme markers. However, both IGS sequence data and allozyme data contrast strongly with the distribution of mating alleles in *S. commune* which shows no geographic patterns in distribution at any level (Raper et al. 1958, Am. Nat. 92:221).

53. Mutagenic DNA-repair genes in *Aspergillus nidulans*: Isolation and characterization of a RAD6 homologue gene. Young-Kug Jang[1], Hyen-Sam Kang[2], and Suhn-Kee Chae[1]. [1]Paichai

University, Division of Life Sciences, Taejon, Chungnam, South Korea. [2]Seoul National University, Dept. of Microbiology, Seoul, South Korea.

Mutagenic DNA-damage tolerance pathways have not been well understood. In *S. cerevisiae*, genes in the *RAD6* epistasis group have shown to be responsible for mutagenic DNA repair. The yeast RAD6 protein is an ubiquitine-conjugating enzyme and is required for mutagenesis and sporulation. In *A. nidulans*, defects in mutagenesis have been observed when genes (*uvsI*, *uvsC*, and *uvsE*) in two different epistasis groups, UvsI and UvsC, were mutated. The *uvsI* gene, a *REV3*homologue, has been cloned and shown to encode an error-prone DNA polymerase zeta. On the other hand, *uvsC* produces an *E. coli* RecA and yeast RAD51 homologue involving recombination and recombinational DNA repair. To understand more about mutagenic DNA repair pathways, an *A. nidulans* RAD6 homologue gene (temporally, named as *radB*) was isolated using the PCR based sib-selection method with degenerated PCR primers from the chromosome specific genomic DNA library. Sequence determination of genomic DNA and cDNA of *radB* revealed an open reading frame of 456 bp, interrupted by three introns (141 bp, 52 bp, and 72 bp, respectively), encoding a polypeptide of 151 amino acids with estimated molecular mass of 17 KDa. The deduced amino acid has 93%, 83%, and 75% sequence identity to MUS-8 of *N. crassa*, rhp6+ of *S. pombe*, and RAD6 of *S. cerevisiae*, respectively. The *radB* gene was assigned on the left arm of the chromosome V. Similarly in the case of RAD6 and RAD18 of yeast which were shown to work together, RADB and UVSH (a RAD18 homologue) of *A. nidulans* are also able to form a protein complex.

54. Unfolded protein response in *Aspergillus*. David Jeenes, Adrian Watson, Jane Morrice, Celina Ngiam, Donald MacKenzie and David Archer. Institute of Food Research, Norwich Research Park, Norwich NR4 7UA, UK.

The synthesis of foldases and chaperones, which are resident in the lumen of the endoplasmic reticulum (ER) and assist the folding of secretory proteins, is regulated at the transcriptional level by the unfolded protein response (UPR). We have shown that perturbation of the protein folding process in *Aspergillus niger*, whether by chemicals such as tunicamycin, dithiothreitol and a calcium ionophore or by the secretion of heterologous proteins, leads to up-regulation of the synthesis of foldases such as protein disulphide isomerase (PDI encoded by *pdiA*).We have also shown that, under the conditions of UPR, the transcription of the gene encoding acetyl-CoA carboxylase (ACC encoded by *accA* in *A.nidulans*), is up-regulated. ACC catalyses the first committed step in membrane fatty acid synthesis, suggesting that UPR coordinates the synthesis of ER membrane with the synthesis of ER lumenal proteins. UPR may have even wider regulatory roles which will also be presented.

56. Withdrawn

57. Reconstitution of an *Aspergillus oryzae* CCAAT-binding protein, AoCP, from purified recombinant subunits, AoHapB, AoHapC and AoHapE. Masashi Kato[1], Akimitsu Tanaka[1],Hideki Hashimoto[1], Fumiko Naruse[1], Peter Papagiannopoulos[2], Stefan Steidl [3], Olivier Litzka [3], Axel A. Brakhage [3], Meryl A. Davis[2], Michael J. Hynes[2], Tetsuo Kobayashi[1], and Norihiro Tsukagoshi [1]. [1]Nagoya University, School of Agriculture, Nagoya, Aichi , Japan.

[2]University of Melbourne, Department of Genetics, Melbourne, Victoria, Australia. [3]Tech. Univ. Darmstadt, Inst. Mikrobiol.& Genetik, Darmstadt, Germany.

Many fungal genes contain CCAAT sequence in their promoter regions. We have found CCAAT-binding proteins in *A. nidulans*: AnCF for *amdS* encoding the *A. nidulans* acetamidase, AnCP for *taa* encoding *A. oryzae* Taka-amylase A, and PENR1 for the *aatA* and bidirectionally oriented genes *acvA* and *ipnA*, encoding the *A. nidulans* penicillin biosynthetic enzymes. AnCF/AnCP/PENR1 has been shown to contain a polypeptide encoded by the *hapC*gene, a homologue of the *HAP3* gene of *S. cerevisiae*. Recently, two *A. nidulans* genes, *hapB* and *hapE*, encoding polypeptides with a central core showing high similarity to Hap2p and Hap5p have been isolated. HapB, HapC and HapE were shown to be necessary and sufficient for DNA binding by reconstitution of the complex *in vitro*. In this study, *A. oryzae* was found to contain a nuclear protein designated AoCP, which bound to the CCAAT sequence in the promoter region of the *taa* gene. AoCP contained a component immunologically similar to *A. nidulans* HAPC. A homologue of the *A. nidulans hapC* gene was isolated from *A. oryzae*, designated as *AohapC* and sequenced. The *AohapC* gene introduced into an *A. nidulans hapC* deletion strain was found to complement the *hapC* deletion and resulted in restoration of the CCAAT binding activity, leading to enhancement of *taa* gene expression. Furthermore, two genes, *AohapB* and *AohapE*, homologues of *A. nidulans hapB* and *hapE*were isolated from *A. oryzae*. We succeeded in reconstituting the CCAAT-binding complex from purified recombinant polypeptides, AoHapB, AoHapC and AoHapE.

58. Transcriptional regulation of the catalase B gene *(catB)* in *Aspergillus nidulans*. Laura Kawasaki, and Jesus Aguirre. Universidad Nacional Autonoma de Mexico, Molecular Genetics, Mexico City, Mexico, Mexico.

A. nidulans contains at least three catalases. Thus far, two genes have been cloned and characterized: *catA* and *catB* (Navarro *et al.*, 1996; Kawasaki *et al.*, 1997). Besides being developmentally regulated, CatB activity was induced by H_2O_2, paraquat or uric acid catabolism but not by osmotic stress, whereas the third catalase activity has been detected only during late stationary phase. *catB* transcriptional regulation was studied by using a *catB::lacZ* fusion containing 3.5 kb of *catB* 5'upstream regulatory sequences. The reporter gene activity was induced during the stationary phase of growth. Starting with the activity detected by 10h of growth, the beta-galactosidase activity was induced 4, 14 and 22 fold at 18h, 28h and 48h, respectively. Under oxidative stress conditions produced by a 2h paraquat treatment, the activity was induced 17 fold. A second *lacZ* fusion containing only 993 bp of *catB* 5' upstream regulatory sequences behaved similarly to the previous one. After we sequenced this region, a comparison analysis revealed several putative regulatory elements similar to consensus sequences shown to bind transcription factors involved in responses to different types of stress. These sequences are contained within a 580 bp region, whose deletion clearly reduced *catB::lacZ* induction during stationary phase and oxidative stress. A more detailed analysis of this region is underway. We are also trying to isolate *catB*-deregulated mutants using a strain with two copies of the *catB::lacZ* fusion. We expect this approach will allow us to define important regulators of the oxidative stress response in *A. nidulans*. Supported by grant IN-206097 from PAPIT-UNAM, Mexico.

59. Restless aided transposon tagging of a nitrogen regulator from *T. inflatum*. Frank Kempken, and Ulrich Kück. Ruhr-University Bochum, Allgemeine Botanik, Bochum, NRW, Germany.

In the past years several fungal transposable elements have been identified. We have isolated and characterized *Restless*, a new type of fungal class II transposons from *Tolypocladium inflatum* which so far has not been found in any other fungus (Kempken& Kück, 1996, MCB 16:6563-6572). The predicted amino acid sequence deduced from an open reading frame encoded by Restless shows significant homology to transposases of the hAT transposon family, e.g. the maize Activator element. We set out to proof the usefulness of *Restless* to tag genes by identifying regulatory genes of the nitrogen metabolism, which have not yet been characterized in *T.inflatum*. As a simple selection system we used chlorate resistance, which may occur by mutations in cofactor or uptake genes, the nitrate reductase gene or in an regulatory gene. Mutations of the first three types were excluded by physiological tests and PCR. Among the remaining seven mutations we successfully tagged and cloned a gene with a C6 zinc finger. The deduced amino acid sequence of this gene shows significant similarity to the *nit-4* gene of *Neurospora crassa*, which is a nitrogen metabolism regulator (Yuan et al., 1991, MCB 11:5735-5745). To our knowledge, this is the first fungal gene identified by transposon tagging. This method should be useful in any fungus which harbors known transposable element.

60. *CMR1*, a novel transcriptional activator with Cys2His2 type zinc-finger and Zn(II)2Cys6 binuclear cluster motifs regulates transcription of melanin biosynthesis genes *SCD1* and *THR1* of *Colletotrichum lagenarium*. Youki Kenmochi [1], Yoshitaka Takano[2], Gento Tsuji[1], James A. Sweigard[3], Iwao Furusawa[2], Osamu Horino[1], and Yasuyuki Kubo[1]. [1]Kyoto Prefectural University, Lab. of Plant Pathology, Kyoto, Kyoto, Japan. [2]Kyoto University, Lab. of Plant Pathology, Kyoto, Kyoto 606-8502, Japan. [3]E. I. Du Pont de Nemours, Central Research and Development, Wilmington, DE 19880-0402, USA.

Colletotrichum lagenarium is a phytopathogenic fungus that causes anthracnose disease of cucumber. Conidia of *C. lagenarium* differentiate melanized appressoria as an infection structure that are essential for penetration of the host plant. A gene, *pig1*involved in the amount of melanin production of *Magnaporthe grisea* was cloned by REMI insertional mutagenesis. *C. lagenarium CMR1* gene was then isolated using *pig1* as a probe. *CMR1* was a single copy gene and contained an open reading frame consisting of 984 amino acids with four introns. At the N terminal region of the deduced amino acid sequence, two Cys2His2 type zinc-finger and one Zn(II)2Cys6 binuclear cluster DNA binding motifs were recognized. Coexistence of those motifs in a transcriptional factor is novel and unique form and has not been reported in any fungal transcriptional factors. *CMR1*disruptant showed a phenotype with the defect of melanin biosynthesis during mycelial growth and accumulated melanin intermediate scytalone in the culture media. However appressorial melanization was normal as that of the wild type strain. Expression of melanin biosynthesis genes in *CMR1* disruptant was investigated by RNA blot analysis. In the wild type, accumulation of transcripts of melanin biosynthesis genes, polyketide synthase gene *PKS1*, scytalone dehydratase gene *SCD1* and trihydroxynaphthalene reductase gene *THR1* increased during mycelial melanization. However, in *CMR1*disruptant, the accumulation of *SCD1* and *THR1* transcripts was quite low compared with the wild type. The level of accumulation of *PKS1* transcript was almost the same as the wild type. On the other

hand, accumulation of those three melanin biosynthesis genes during appressorial melanization was same level between the disruptant and the wild type. These results indicate that *CMR1* is a novel type of transcriptional activator with Cys2His2 type zinc-finger and Zn(II)2Cys6 binuclear cluster motifs that regulates the expression of *SCD1* and *THR1* during mycelial melanization in *C. lagenarium*.

61. Transformation of *Pleurotus ostreatus* to phleomycin resistance. Beom-Gi Kim[1], and Yumi Magae[2]. [1]National Institute, Applied Microbiology, Suweon, Kyunggi-do, South Korea. [2]National Institute, Bioresources, Tsukuba, Ibaraki, Japan.

Pleurotus ostreatus (Fr.) Kummer, the oyster mushroom, is one of the most widely cultivated edible mushrooms. Transformation strategy is necessary for molecular studies of this fungus as well as for developing new breeding method of strain improvement. In aims of developing a stable integrative transformation system for *P. ostreatus*, two vectors containing phleomycin resistance selection marker (*ble* gene) and regulatory sequences of beta-tublin (-tub) gene of *Pleurotus sajor-caju* were constructed. First, isolated -tub gene of *P. sajor-caju* was sequenced. The gene(sized 3958 bp) consisted of 939 bp promoter, 10 introns and a transcription terminal sequence. Two vectors were constructed utilizing vector pGpht (this plasmid was a gift from Dr. J.G.H. Wessels). pPhKM1 contained b-tub promoter sequence, *ble* gene and *Schizophyllum commune* GPD terminator. pPhKM2 contained *ble* gene and the -tub regulatory sequences. Each vector was cotransformed into homokaryotic *P. ostreatus* ura mutant strain (MGL2042-8) with pTura3-2. After the colonies grown on minimal medium were transferred to phleomycin medium, transformants were selected. Transformation efficiency of pTura3-2 vector was ca 30 colonies per 1 micro g DNA while cotransformation efficiency was 10%. Southern blot analysis of the transformants indicated chromosome integration of vectors. Many and different intensities of hybridizing bands showed random and multiple site chromosome integrations. Following the success of cotransformation, transformation of dikaryotic *P. ostreatus* wild type strain was attempted using pPhKM1, pPhKM2 and pGpht.

62. Cloning and nucleotide sequence of the catalytic subunit of DNA polymerase-gamma of *Neurospora crassa*. Tak Ko1, Bonnie L. Seidel-Rogol[2], and Helmut Bertrand[1]. [1]Michigan State University, Microbiology, East Lansing, MI, USA. [2]SUNY at Plattsbergh, Bilogical Science, Plattsbergh, NY., USA.

Most of the proteins involved in mitochondrial gene replication and expression are encoded by nuclear genes. Included in this group of proteins is DNA polymerase-gamma (pol-G), which is part of the complex involved in the replication of mtDNA. Identification of the gene for this protein in strict aerobes like *Neurospora crassa* by mutations has been difficult, most likely because such events are lethal. We used the known amino-acid sequences of the polymerases from *Xenopus laevis*, and three yeast species to clone the pol-G gene from *N. crassa*. After two rounds of PCR with degenerate primers and using *N. crassa* genomic DNA as a template, an appropriately-sized PCR product was identified. The PCR product was cloned and sequenced to design specific primers. Screening of the Orbach/Sachs pMOCosX cosmid library by PCR with these primers and by hybridization with the PCR product revealed that the X25:10C cosmid contains the complete pol-G gene. Sequence analysis showed that pol-G has a 3918 nucleotide open reading frame encoding 1305 amino acids (146 kDa). RFLP mapping located the gene in

linkage group III between pro-1 and ad-2. Comparison of the nine available DNA polymerase-gamma sequences revealed several highly conserved sequence blocks, and that the polymerase domain is more highly conserved than the exonuclease domain. The *N. crassa* and *S. cerevisiae* polymerase-gamma polypeptides have long C-terminal extensions that are not found in the homologous proteins from other species.

63. Neurospora proteins that bind methylated DNA and DNA mutated by RIP. Gregory O. Kothe, Michael R. Rountree and Eric U. Selker, Institute of Molecular Biology, University of Oregon, Eugene, OR, USA.

Using gel-mobility-shift assays we have detected two factors in *Neurospora crassa* that bind methylated DNA sequences. A high-mobility factor was identified that is specific for methylated DNA. We refer to this factor as M-BP1 (Methyl Binding Protein 1). A low-mobility factor was identified that binds methylated DNA or DNA mutated by RIP. This factor binds most efficiently to DNA that is both methylated and contains RIP mutations. We refer to this factor as M/R-BP1 (Methyl/RIP Binding Protein 1). M/R-BP1 and M-BP1 may be involved in establishing and/or maintaining methylation patterns in Neurospora. It is also possible that these proteins function "downstream", exerting their effects after methylation has been set up (eg. repressing gene expression). To test these possibilities we are purifying M/R-BP1 and M-BP1, characterizing their properties, and cloning the genes that encode them. We will then generate and characterize mutants with defects in these genes.

64. Quality control of protein secretion in *Aspergillus niger*-isolation of the calnexin and UDP:glucose glycoprotein glucosyltransferase genes. Joanna Lambert[1], David B. Archer[2], David J. Jeenes[2], Elodie Morlon[2], and John F. Peberdy[1]. [1]University of Nottingham, Biological Sciences UP, Nottingham, Nottinghamshire, UK. [2]Institute of Food Res., Genetics and Microbiology, Norwich, Norfolk, UK

The ability of filamentous fungi to secrete high levels of glycosylated proteins has led to an interest in exploiting these organisms as hosts for the production of recombinant chemotherapeutic proteins. However, it is apparent that secreted yields of heterologous proteins are significantly lower than yields of native proteins (Peberdy, Trends in Biotechnology 12:50-57, 1994). Work is being carried out to express heterologous proteins in *Aspergillus niger* by identifying possible bottlenecks in the secretion process. An aspect of this involves the study of quality control in the glycosylation pathway of secreted proteins. Calnexin and calreticulin are lectins that function as molecular chaperones in the endoplasmic reticulum. These proteins recognise the terminal glucose residues on glycoproteins and prevent their secretion from the cell if the molecule is incorrectly processed. Together with UDP:glucose glycoprotein glucosyltransferase these chaperones form part of a novel mechanism for promoting folding, oligomeric assembly and quality control in the ER (Helenius *et al*., Trends in Cell Biology 7: 193-200, 1997). The calnexin gene has been identified in *A. niger*, and a full genomic clone sequenced which shows approximately 60% identity with other calnexin genes. The promoter region contains unfolded protein response elements that are seen in other chaperones, however data obtained does not support these as being functional. It has not been possible to isolate calreticulin from *A. niger*, and to date this protein has only been found in higher eucaryotes. The enzyme UDP:glucose glycoprotein glucosyltransferase has a key function in maintaining glycan

chains so unfolded proteins are recognisable by chaperones. This gene for this protein has been isolated from *A. niger* and a genomic clone is being sequenced.

65. Cellulase discovery and 18S rDNA studies of five chytrids. Lene Lange, Michael Skjøt, Martin Schülein, Paivi Kattila, and Sakari Kauppinen. Novo Nordisk A/S, Enzyme Research, Bagsvaerd, DK2880, Denmark.

In the last few years interesting cellulases have been described from anaerobic members of the Chytridiomycetes, e.g. from *Neocallimastix spp*, *Piromyces spp* and *Orpinomyces spp*. However, also the aerobic chytrids have been shown to produce interesting cellulases: recently, we have cloned a new cellulase belonging to the glycosyl hydrolase family 45 from the aerobic chytrid *Rhizophlyctis rosea*. In the present study we focus on the phylogenetic relations between the Chytridiomycetes and the other groups of true fungi as well as the phylogenetic relations between the four orders of the Chytridiomycetes (i.e. Blastocladiales, Chytridiales, Neocallimasticales and Spizellomycetales), including both holocarpic/eucarpic and aerobic/anaeobic species. From these studies, full 18S rDNA sequence data from five chytrids will be presented, enabling the construction of an improved phylogenetic tree of the Chytridiomycetes, rooted in the fungal system. Further, the full amino acid sequence of the family 45 cellulase cloned from *R. rosea* will be presented. Comparisons will be made to the 30 other newly cloned fungal family 45 cellulases, originating from all groups of the fungal system and representing a wide variety of ecological niches.

66. Analysis of functional domains in NMR protein of *Neurospora crassa*. Ta-Wei David Liu. The Ohio State University, Biochemistry, Columbus, Ohio, USA.

Nmr gene is the major negative regulatory gene in the nitrogen control circuit of *Neurospora crassa*, which, together with the positive regulatory gene, *NIT2*, governs the expression of many unlinked structural genes for nitrogen utilization. The NMR protein is required to establish nitrogen repression of multiple structural genes. However, NMR does not appear to possess DNA binding activity. Previous studies have shown that the NMR protein interacts with the positive-acting NIT2 protein via direct, specific protein-protein binding (Pan *et al*. Mol Microbiol 26:721, 1997; Xiao et al. Biochemistry 34:8861, 1995). Five highly conserved regions in NMR and the homologous proteins from *Aspergillus nidulans* and *Gibberella fujikuroii* were identified. One or more of these regions might play an essential role in the interaction between NMR and NIT2 protein. In the present study, these possible functionally important regions of the NMR protein were investigated by site-directed mutagenesis. We are examining the ability of the mutant NMR proteins with each of the conserved regions deleted to interact with *NIT2* and to function in nitrogen repression. The results of in vitro assays for protein-protein binding and in vivo functional assays for NMR activity will be discussed.

67. Potential role of plant signal(s) in pea pathogenicity (*PEP*) gene expression in *Nectria haematococca*. X. Liu[1], Y. Han[2], C. C. Wasmann[1], H. C. Kistler[2] and H. D. VanEtten[1]. [1]Department of Plant Pathology, Univ. of Arizona, Tucson, [2]Plant Molecular and Cellular Biology Program, Plant Pathology Department, Univ. of Florida, Gainesville.

Genes (*PDA*) for detoxifying the pea phytoalexin pisatin and other pea pathogenicity (*PEP*) genes are located on dispensable chromosomes in *N. haematococca*. Previously we had identified 5 transcripts (cDNA1 to cDNA5) in the cosmid 55-D-8, which was shown to be capable of complementing pathogenicity to nonpathogenic isolates, by screening a cDNA library constructed from mRNA derived from infected pea tissues. Complementation experiments indicate that cDNA1, cDNA2, and cDNA5 can contribute to pathogenicity independently. To search for potential signal(s) involved in *PEP* gene expression, we used RT-PCR approach to examine the production of 5 transcripts *in vitro* in mycelia subject to various treatments such as starvation, pisatin induction etc. Our preliminary results indicate that cDNA2 expression can only be detected under the induction of pisatin, suggesting that plant signal(s) may be required for expression of *PEP* genes. Currently, quantitative RT-PCR strategy is being employed to verify whether pisatin and or other plant signals play a role in regulation of the expression of *PEP* genes.

68. Pilot scale genome sequencing of *Aspergillus nidulans* and cDNA sequencing of *Aspergillus oryzae*. Masayuki Machida[1], Mari Nakagawa[1], Sumiko Kunihiro[1], Kumiko Takase[1], Makoto Yasukawa[2], and Mariko Manabe[3]. [1]National Institute of Bioscience and Human-Tech., Molecular Biology, Tsukuba, Ibaraki, Japan. [2]Fukushima Tech. Center, Kooriyama, Fukushima, Japan. [3] National. Food Research Institute, Tsukuba, Ibaraki, Japan.

In the course of world wide effort to complete the genome sequence of *Aspergillus nidulans*, we started the pilot scale sequencing of a part of the ordered cosmids library. We picked the cosmids locating in the middle part of chromosome VIII and neighboring to the cosmid which has been sequenced by Prade *et al*. The sequencing was done mainly by the primer walking method using the internal-labeling protocol and analyzed by Li-Cor model 4200L DNA sequencer. Since longer than 800 nucleotide sequence could be analyzed in a single run, approximately 160 reactions were expected to complete the sequence of both strands of cosmid's inserts. We examined the condition to adapt the internal-labeling protocol to cosmid sequencing and found that the successful long-read sequencing depended on higher concentration of IRD-labeled dATP, optimization of cosmid amount and the higher temperature for denaturation step. We have initiated the random cDNA sequencing of *Aspergillus oryzae* cDNA libraries prepared from the cells grown in a rich medium and in the starved condition. We are preparing the database of the above sequence data on our Web server, which will be soon available.

69. The *AVR1-MARA* Locus of *Magnaporthe grisea*. M. Alejandra Mandel, Uvini P. Gunawardena , Travis M. Harper, and Marc J. Orbach. Univ of Arizona, Plant Pathology, Tucson, AZ, USA.

AVR1-MARA is a stable avirulence gene of *Magnaporthe grisea* that elicits a resistant response in the rice cultivar Maratelli. To address the question of how this gene functions, we are using a map-based approach to clone it. We are also using mutagenic approaches to address its apparent genetic stability. We have reported the cloning of the virulent allele, *avr1-MARA*, and the mapping of the avirulent allele. The two alleles differ by the presence of two regions of 60 kb and 14 kb only in the avirulent locus. The avirulent locus is approximately 85-90 kb with major portions unclonable in *E. coli* (Mandel et al., 1997). To localize the avirulence gene within the locus, we have used transformation-mediated gene disruption methods to delete all, or part of the

locus. By this method, the gene has been localized to the region of the locus that contains the 60 kb *AVR*-associated sequences. Two approaches are being taken to isolate this region; one, a combination of Long Distance and Inverse PCR methods has resulted in the isolation of more than two thirds of this region. Sequence analysis of these segments has shown them to be unusual in *M. grisea*, with the DNA being 70% AT. Analyses of these sequences will be presented. The second approach to clone this region is Transformation-Associated-Recombination, an *in vivo* ligation method using *Saccharomyces cerevisiae* as a cloning host. Analyses of virulent mutants of *AVR1-MARA* and the distribution of the *AVR1-MARA* locus in populations of *M. grisea* will be presented. Mandel, M.A., V.W. Crouch, T.M. Harper, and M.J. Orbach. 1997. Physical mapping of the *Magnaporthe grisea AVR1-MARA* gene reveals the virulent allele contains two deletions. Mol. Plant-Microbe Interact. 10:1102-1105.

70. Antioxidant and metabolic functions of the alternative oxidase of *Histoplasma capsulatum*. Joan E. McEwen, and Clayton H. Johnson. McClellan VA Hosp. and Univ. of Arkansas Med., Medical Research, Little Rock, AR, USA.

Most fungi possess two mitochondrial respiratory pathways. The cytochrome pathway consists of electron carriers that ultimately reduce oxygen to water via the enzyme cytochrome oxidase. The alternative pathway, found in fungi, protists and plants but absent from mammals, consists of a single protein termed alternative oxidase, which reduces oxygen to water. Because alternative oxidase is absent in mammals, it will be an attractive target for development of antifungal drugs if it can be demonstrated to be important for virulence or survival of the pathogenic fungus during infection of a mammalian host. We are investigating the function of alternative oxidase in the pathogenic fungus *Histoplasma capsulatum*. Two lines of evidence suggest this enzyme performs an antioxidant function. 1) Both mRNA level and protein activity are elevated after exposure of *H. capsulatum* to hydrogen peroxide. 2) After expression of the *H. capsulatum* alternative oxidase cDNA in *S. cerevisiae*, antioxidant function was demonstrated by the "heat-induced cell death assay". The metabolic function of alternative oxidase involves its electron transport activity. We demonstrated that *H. capsulatum* yeast are able to grow, albeit slowly, when the cytochrome pathway is inhibited, and that this growth is abolished when alternative oxidase is also inhibited. This suggests that the alternative oxidase branch is able to support the bioenergetic needs of the organism. We hypothesize that this function is important during pathogenesis, when host antifungal efforts involving nitric oxide or other environmental stresses may inhibit the cytochrome pathway. Experiments on the effect of nitric oxide on *H. capsulatum* mitochondrial function and gene expression are underway.

71. Barrage formation in *Neurospora crassa* is independent of mating type and heterokaryon incompatibility. Cristina O. Micali, and Myron L. Smith. Carleton University, Biology, Ottawa, Ontario, Canada.

Barrages are evident in mating reactions between *N. crassa* strains (Griffiths and Rieck, 1981 Can. J. Bot. 59: 2610-2617) but have not been extensively studied. In other ascomycetes and many basidiomycetes, barrages are used as indicators of vegetative incompatibility and may, in some cases, be correlated to differences at *het* genes. We find that barrages in *N. crassa* are very similar in appearance to those described for *Cryphonectria parasitica*, *Podospora anserina* and *Sclerotinia sclerotiorum* and, as in other fungi, are most pronounced when strains are confronted

on medium low in nitrogen. Although variable in intensity, barrages in *N. crassa* can be divided into two broad categories; clear zone and dark line. The two types are not mutually exclusive and combinations of the two were observed. The appearance of a clear zone is correlated to a decrease or complete absence of perithecial production between strains with different mating types. The genetic control governing barrage formation seems to be complex. However, using well characterized laboratory strains, we show that barrage formation in *N. crassa* is independent of mating type and heterokaryon incompatibility genes *het-6* and *het-c*. Barrages may form between strains with common or different alleles at these loci, but do not form when the strains are confronted to themselves. Unlike many plant pathogenic ascomycetes, wild isolates of *N. crassa* failed to form barrages when confronted to each other or to tester laboratory strains. The reason for this is unknown but, pairings between some inbred F1 and F2 progeny of these wild-type strains produce barrages, which suggests that some level of inbreeding among strains may be necessary for barrage formation.

72. Tandemly repeated *het-6* incompatibility sequences in *Neurospora crassa*. Cristina O. Micali, Nadereh H. Mir-Rashed, Reza M. Dehghany, Raymond Tropiano, and Myron L. Smith. Carleton University, Biology, Ottawa, Ontario, Canada.

Heterokaryon incompatibility in the *het-6* region, involves two closely linked genes, *un-24*$^{+}$ and *het-6*. *un-24*$^{+}$ encodes the large subunit of ribonucleotide reductase and is about 14 kbp centromere distal to *het-6. het-6* putatively encodes a 680 amino acid protein. Stable heterokaryon formation between strains is prevented if they carry different alleles (PA or OR) at *un-24*$^{+}$and *het-6*. Here, we present evidence that the *het-6* sequence is tandemly repeated. The *het-6*^{OR} allele has been characterized and has incompatibility activity in transformation assays. We characterized a region of PA-background DNA which shares about 70% identity with, and segregates opposite *het-6*^{OR}. A majority of strains examined exhibit diagnostic fragments of either *het-6*^{OR} or *het-6*^{PA} based on a PCR assay. However, a small number of strains produce both the PA and OR fragments. In other experiments, we have attempted to PCR amplify *het-6*^{PA} using primers based on *het-6*^{OR} flanking sequences. Of three separate clones independently derived in this way, none have incompatibility activity and all are more similar in sequence to *het-6*^{OR} than to *het-6*^{PA}. These observations could be due to either gene duplication or cross-contamination during PCR amplifications. The former appears to be correct for the following reasons. First, using nested PCR primers we can amplify both OR and PA-like sequences separately from a single strain. Second, segregation analyses show that *het-6*^{OR} and *het-6*^{PA} probes hybridize to a non-identical set of two and five fragments, respectively, that co-segregate with other LGIIL RFLP markers. In view of these findings, tandemly repeated incompatibility factors around the *N. crassa het-6* locus may be analogous to MHC and HLA loci in other eukaryotes.

73. Regulation of *cat-1* during *Neurospora crassa* development and oxidative stress. Shaday Michan[1], Fernando Lledías[1], James D. Baldwin[2], Don O. Natvig[2], Rosa E. Navarro[1], Jesus Aguirre[1], and Wilhelm Hansberg[1]. [1]Universidad Nacional Autonoma de Mexico, IFC, Bioquimica, México, D.F, México. [2]University of New Mexico, Biology, New Mexico, Albuquerque, USA.

Neurospora crassa has 3 catalases. Cat-1 is the main activity during the whole asexual life cycle. It increases during germination and with each morphogenetic transition of conidiation. It is induced under stress conditions, such as heat, intense light and paraquat treatment. Cat-1 is specifically modified by singlet oxygen during germination, conidiation and under stress conditions. *cat-1* genomic and cDNA clones were fully sequenced. Sequence analysis revealed an open reading frame interrupted by two introns predicting a 719 amino-acid polypeptide (MW=79,139). Two strong and few weak transcription initiation sites were found by primer extension and the polyadenilation site was determined Accumulation of *cat-1*messenger was detected during germination, conidiation and under stress conditions. Alignment of *cat-1* shows 65% identity to the predicted peptide from AFCATGENE of *Aspergillus fumigatus*, 64% to CatB of *A. nidulans* and 56% to CatR of *A. niger*. Beside the central highly conserved region, these catalases show sequences homologies in the amino-and carboxyl-terminal regions. This and the precise conservation of intron I position indicates that these genes are homologs. A plasmid with *cat-1* interrupted by *pyr4* was used to transform a *pyr4*uridine auxotroph. Twelve transfomants wich lacked Cat-1 activity were isolated. One transformant, lacking Cat-1 antigen in a Western-blot assay and over-expressing Cat-3, is being characterised.

74. Phylogenetic analysis reveals past and present hybridization events between two North American species of the *Heterobasidion annosum* complex. Matteo Garbelotto, Ignacio Chapela, and William Otrosina. Department of Environmental Science, Policy, and Mangement, University of California, Berkeley, CA, Last author: Tree Root Biology Institute, USDA Forest Service, Athens, GA.

The *Heterobasidion annosum* complex includes several intersterility groups (ISGs) with varying degrees of genetic divergence amongst them. The North American S and P ISGs represent the two most divergent taxa in the complex, and should be regarded as different species. In spite of the partial interfertility (18%) between the two groups, the S and P ISGs have remained genetically distinct, and until recently hybrids had never been found in nature. The recent discovery of a large long-lived hybrid genotype in California prompted further research on mechanisms regulating fungal hybridization in nature. Isozyme analysis and PCR-generated markers have shown that, while each group is defined by the presence of many fixed alternate alleles, there appears to be some reciprocal gene introgression limited to areas where the two ISGs coexist. This study assesses whether the resulting paraphily for these groups is due to introgressive hybridization or to incomplete lineage extinction of ancestral polymorphisms. A phylogenetic analysis of two putative introgressed markers (introns in the large subunit of the mitochondrial ribosomal DNA) was performed for individuals in an area ranging from the state of Washington to Southern Mexico, including an outgroup of isolates from Europe. Nonconcordance between the accepted phylogeny of the complex and the phylogeny of the putative introgressed markers strongly supports the introgressive hybridization hypothesis or another mode of horizontal interspecific gene transfer. The phylogenies of both introns can be best explained by interspecific transfer occurring during specific and limited time periods, rather than continuosly. Alternative hypotheses are provided to define factors that may determine periods of interspecific gene flow in this fungal complex.

75. A special design of the *nit-3* gene promoter that facilitates NIT2 and NIT4 interaction. Xiaokui Mo, Peter Philippsen. The Ohio State University, Biochemistry, Columbus , Ohio, USA.

In *Neurospora crassa*, the synthesis of nitrate reductase (encoded by *nit-3* gene), an enzyme required for the utilization of inorganic nitrate, is activated by NIT2 and NIT4 proteins under the condition of nitrogen derepression and nitrate induction. NIT2, a major global regulatory protein, plays a crutial role in the nitrogen metabolism. NIT4 is a pathway specific factor. A NIT2-NIT4 protein-protein interaction is required for the optimal expression of nitrate reductase. An important goal is to investigate whether the *nit-3* gene promoter has a special design that facilitates the interaction between NIT2 and NIT4. The *nit-3* gene promoter region, either containing four GATA sequences for NIT2 binding or two symmetric octameric sequence elements for NIT4 binding, or both these NIT2 and NIT4 sites was used to replace the promoter of the *cys-14* gene. The *cys-14* gene encodes sulfate permease-II, facilitating the assimilation of sulfate in mycelia. Its promoter is highly regulated and required only one positive-acting factor (CYS3) to turn the gene on. Transformants containing *cys-14* gene, in which promoter has been modified, were grown under the condition of nitrogen repression or nitrate induction. Results of sulfate transport assays implied that the CYS14 expression was regulated by NIT2 and NIT4. Neither one alone could turn the gene from "off" to "on". acknowledgements: This work is supported by grant GM23367 from the National Institutes of Health.

76. Aspartic protease gene expression in *Fusarium venenatum* CC1-5. Seri Intan Mokhtar, M.G. Wiebe, G. D. Robson, and A. P. J. Trinci. University of Manchester, Microbiology, Manchester, England, UK.

Fusarium venenatum is used in the UK for the large-scale production of Quorn mycoprotein in continous flow fermentation and has recently been developed as a potential host for heterologous protein production.In *F. venenatum*, the major extracellular protease produced during exponential growth is an aspartic protease of about 40 Kda. Southern hybridization of the genomic DNA indicates that only one copy of the gene is present. A 1.2 Kb cDNA of the aspartic protease gene from *F. venenatum* was obtained by RT-PCR and was cloned and sequenced. The expression of the aspartic protease gene was investigated in glucose limited chemostast cultures using casein as an inducer. When casein was added to the culture filtrates in the present of ammonia, the protease activity of the culture filtrates was lower than when casein was added in the absence of ammonia but in the presence of nitrates. Dot blot analysis on the mRNA suggested that the aspartic protease gene was expressed 2 hours after being induced by casein. An antibody against the aspartic protease was raised and used in Western analysis to detect the presence of the aspartic protease in the culture filtrates.The aspartic protease can be detected in the culture filtrates 8 hours after casein was added into the culture.

77. The CCAAT binding AnCF-complex is essential for the formation of a DNase I sensitive site in *A. nidulans*. Frank M. Narendja, Meryl A. Davis and Michael J. Hynes Department of Genetics, University of Melbourne Parkville 3052, Australia.

In *A. nidulans* CCAAT sequences are found upstream of a number of genes and are recognised by AnCF, a complex consisting of three evolutionary conserved subunits HapB,HapC,Hap E (1). Many eukaryotic promoters are assembled into chromatin structures hypersensitive to DNase I prior to transcriptional activation. We have found that the Hap-complex is involved in the formation of a DNase I hypersensitive region in the promoter of the *amdS* gene in *A. nidulans* In a *hapE* deletion strain no DNase I hypersensitive site is formed. Likewise, a point mutation in the

CCAAT motif as well as a 530 bp deletion which removes most of the regulatory motifs of the *amdS* promoter including the CCAAT box results in complete loss of the DNase I hypersensitive region. This DNase I hypersensitive region can be restored by insertion of a 30 bp oligonucleotide carrying the CCAAT motif. DNase I hypersensitive regions have been found in the CCAAT containing promoters of the *niiA, fmdS* and *gdhA* genes and were also *hapE* - dependent. In all 4 promoters CCAAT boxes are located within the DNase I hypersensitive region. These data imply a critical role for the AnCF complex in establishing DNase I sensitive regions in *A. nidulans*. Recently a role for the NF-Y complex in chromatin rearrangement in vertebrates has also been proposed (2,3). This work was supported by the Austrian Science Foundation (J 1518-GEN) and the Australian Research Council. 1. Steidl, S., Papagiannopoulos, P., Litzka, O., Andrianopoulos A., Davis, M.A., Brakhge, A.A. and Hynes, M.J. *Mol. Cell Biol.* in press 2. Jin, S. and Scotto, K.W. (1998) *Mol. Cell Biol.* 18: 4377-4383. 3. Li, Q., Herrler, M., Landsberger, N., Kaludov, N., Ogryzko, V.V., Nakatani, Y. and Wolffe A.P. (1998) *EMBO J.* 17:6300-6315.

78. The *Aspergillus nidulans* METR sulphur regulator belongs to bZIP transcriptional factors. Renata Natorff, Marzena Sieñko, Jerzy Brzywczy, and Andrzej Paszewski. Institute of Biochemistry and Biophysics, Warsaw, Poland.A new class of tight methionine auxotrophic mutants, which can grow only on methionine as a sulphur source was obtained. Mutations in these strains are recessive and belong to the same locus, named *metR*. The *metR* gene has been localised in chromosome III between *argB* and *phenA* genes. Mutations in the *metR* gene are epistatic to mutations in the sulphur negative regulatory genes *scon*. They impair expression of some sulphur metabolism structural genes (i.e. sulphate permease, arylsulphatase, homocysteine synthase). The expression of the *metR*gene seems to be not regulated by sulphur source. We have cloned and characterised the genomic and cDNA copies of the *metR* gene. The deduced METR protein contains a leucine zipper and an adjacent basic region (bZIP), which together constitute a bipartite sequence-specific DNA-binding domain. This domain is highly similar to analogous domain of the *Neurospora crassa* positive-acting sulphur regulatory protein CYS3. However, no or very little homology is found in the remaining parts of the proteins. The *N. crassa cys-3* gene does not complement *metR* mutations. This work was supported by the KBN grant no 6 P04A 035 14

80. Cloning of a gene *pacC* homologue fron *Neurospora crassa*. Sérgio Ricardo Nozawa[1], Walter Maccheroni Jr.[2], Monica Stropa Ferreira[3], André Justino[1], Nilce Maria. Martinez-Rossi[4], and Antonio Rossi[1]. [1]University of São Paulo, Departament of Chemistry, Ribeirão Preto, São Paulo, Brazil. [2]University of São Paulo, Genética-ESALQ, Piracicaba, São Paulo, Brazil. [3]UNESP, Genética, Rio Claro, São Paulo, Brazil. [4]University of São Paulo, Genética, Ribeirão Preto, São Paulo, Brazil.

We have cloned a *N. crassa pacC* homologue, a gene responsible for the general control of ambient pH response in *Aspergillus nidulans*. Based on regions of homology found between the PacC protein sequences of *A. nidulans*, *A. niger* and *P. chrysogenum*, degenerate oligonucleotides were designed and used to amplify genomic fragments from *N. crassa* by PCR. The nucleotide sequence of a 137 bp amplification product was determined and shown to contain an open reading frame of 30 amino acids having ~93% identity to the *A. nidulans* PacC protein. The PCR product was used as a probe to screen a genomic library of *N. crassa* and three of the

recovered clones complemented the pacC14 strain of *A. nidulans*. The existence of an *A. nidulans pacC* homologue in *N. crassa* is puzzling because, according to models proposed in the literature, *A. nidulans* appears to control the transcription of acid and alkaline phosphatases in response to the stimulus generated by extracellular pH, whereas *N. crassa* synthesizes both enzymes irrespective of extracellular pH, thus controlling only their secretion into the external medium.

81. Analysis of TOXE - A unique protein involved in the regulation of HC-toxin biosynthetic genes in *Cochliobolus carbonum*. Kerry F. Pedley, Joong-Hoon Ahn, and Jonathan D. Walton. Michigan State University, DOE Plant Research Lab, East Lansing, MI, USA.

Cochliobolus carbonum, the filamentous fungus responsible for northern corn leaf blight, produces a host-selective toxin, HC-toxin, that confers increased virulence towards certain genotypes. We are investigating the biosynthesis of the HC-toxin by trying to isolate and characterize the enzymes and genes necessary for its production, secretion, and regulation. To date three linked genes, *HTS1, TOXA*, and *TOXC* have been identified and the products of these genes have demonstrated roles in the biosynthesis of HC-toxin. All three genes are unique to toxin-producing ($Tox2^+$) strains. Southern blot analysis has revealed that these genes are completely absent in toxin-non-producing ($Tox2^-$) strains. Recently we cloned another gene, *TOXE*, that is found only in $Tox2^+$ strains. When the *TOXE* gene is disrupted, the fungus does not produce HC-toxin and is only weakly pathogenic on plants. Sequence analysis of *TOXE* shows that its product contains a basic region characteristic of leucine zippers at its N-terminus and four ankyrin-like domains at the C-terminus. Based on this we hypothesize that the *TOXE* protein (TOXEp) might be a transcription factor involved in the regulation of the other genes involved in HC-toxin production. Northern blot analysis showed that the *TOXA* and *TOXC* messages are down regulated in strains with disrupted copies of *TOXE*. We are currently testing the hypothesis that TOXEp is directly involved in the transcriptional regulation of *TOXA* and *TOXC*.

82. A new compendium of Neurospora chromosomal loci. David D. Perkins[1], Alan Radford[2], and Matthew S. Sachs[3]. [1]Stanford University, Biological Sciences, Stanford, CA 94305-5020, USA. [2]University of Leeds, Biology, Leeds LS2 9JT, England. [3]Oregon Graduate Institute, Biochem.& Molec. Biol., Portland, OR 97291-14877,

"Chromosomal Loci of Neurospora crassa", the 1982 Neurospora compendium (Microbiological Reviews 46:426-570), has been revised and expanded. The new edition will be published in book form by Academic Press. Lists of loci mapped in each linkage group will be posted and the text sections containing information on individual loci will be available for inspection.

83. Isolation and functional characterisation of two sulphate permease genes from *Penicillium chrysogenum*. Enrica Pizzinini[1], Mart van de Kamp[2], Arnold J.M. Driessen[2], Wil N. Konings[2], and Geoffrey Turner[1]. [1]University of Sheffield, Molec Biol Biotechnol, Sheffield, South Yorkshire, UK. [2]University of Groningen, Molecular Microbiology, Groningen, The Netherlands.

In order to assess the influence of primary metabolic flux on penicillin production in *Penicillium chrysogenum*, we are investigating the sulphate assimilation pathway, which provides cysteine, one of the 3 precursor amino acids of penicillin. Since no sulphate permease gene had been isolated from a penicillin producing species, we designed degenerate oligonucleotides from known sulphate permeases, and sequenced PCR amplified fragments. Two types of fragment resembling sulphate permease were obtained, suggesting the presence of at least 2 genes, termed *sutA* and *sutB* (see poster by van de Kamp et al.). Full length clones of both *sutA* and *sutB* were obtained from a lambda library, and used to cotransform an *sB3* (sulphate permease) mutant of *Aspergillus nidulans*. Complementation was observed only with *sutB*. The degenerate primers were used to amplify sequences from *A. nidulans*, and a single permease-like fragment obtained, which resembled *sutB* more closely than *sutA*. An internal fragment of *sutB* was introduced into *P. chrysogenum* by co-transformation, and transformants were tested for growth on sulphate and methionine. A methionine-requiring transformant, resulting from homologous integration of the *sutB* fragment and disruption of *sutB*, was identified. Sulphate uptake was measured in wild-type, mutant, and transformed strains of *P. chrysogenum* and *A. nidulans* to determine the kinetics of sulphate uptake, and the effects of gene disruption and complementation. The combined data suggests that *sutB*, and its homologue in *A. nidulans*, *sB*, encode the major sulphate permease activities in these penicillin producers. We have yet to determine whether the leaky growth observed in *sutB* and *sB* mutants results from a second sulphate permease corresponding to *sutA*.

84. Analysis of protein traffic in defined secretion pathway mutants of *Aspergillus niger*.
Peter J. Punt[1], Anneke Drint-Kuijvenhoven[1], Vivi Joosten[1], Ingeborg A. van Gemeren[2], and Cees A.M.J.J. van den Hondel[1]. [1]TNO Nutrition and Food Research Institute, MGG, Zeist, Utrecht, The Netherlands. [2] Biotech. Appli. Center BV, Bussum, Noord Holland, The Netherlands.

A major part of our research is focused on the understanding of the molecular mechanisms underlying efficient protein-secretion by filamentous fungi. Two lines of research are followed to identify key points in the secretion pathway. In a first empirical research line the role of major ER-chaperone protein BiP on protein secretion was studied. A second research line was based on a systematic analysis of the fungal secretion pathway. Based on results obtained in *S. cerevisiae*, it was clear that the various transport steps of the secretion pathway are depending different stage-specific small GTPases.The *A. niger* genes encoding the most relevant of these GTPases were cloned. From one of these genes, *sarA*, of which the gene product is expected to play a role in one of the early transport steps from ER to Golgi, specific mutant alleles were generated. Introduction of the alleles into *A. niger* resulted specific conditional *A.niger sarA* mutants. Analysis of secretion characteristics of both BiP-overproducing and *sarA* mutant strains in transformants expressing various glucoamylase-gene-fusions showed that in both strains significant retention of fusion protein in the intracellular (membrane bound) fraction was observed. However, the yield of secreted recombinant protein was not affected. Results of this research will be presented and discussed.

85. Molecular characterization of the secretory pathway of *Aspergillus niger*.
Arthur FJ. Ram[1], Peter J. Punt[2], Roy C. Montijn[2], Cora MJ van Zeijl[2], and Cees AMJJ van den Hondel[2]. [1]State Leiden University, IMP, Leiden, Zuid Holland, The Netherlands. [2]TNO, MGG, Zeist, Utrecht, The Netherlands.

We have started research to analyse the molecular mechanism underlying protein secretion in *A. niger*. In the first instance we have isolated 9 distinct GTPase encoding genes from *A. niger* (*sarA*, *sagA-H*) corresponding to GTPases involved in most stages of the secretory pathway. Interestingly, several of these genes homologues are present in higher eukaryotes, but not in *S. cerevisiae*. These genes are used to generate a set of (conditional/deletion) mutants imposing defined blocks in the secretory pathway. To analyse transport and secretion of proteins we have developed a GFP-based secretion reporter system by fusing GFP to a carrier protein, glucoamylase (GLA). Expression of a *glaA::gfp* fusion construct resulted in fluorescence of the cell wall, probably representing secreted GLA::GFP fusion protein that is retained within the extracellular matrix. Periplasmic fluorescence was only observed in young mycelium. No periplasmic fluorescence is observed in older mycelia probably due to acidification of the medium, and/or increased protease activity. Targeting of the GLA::GFP fusion protein to the ER by fusing the ER retention signal (HDEL) to the fusion protein, resulted in intracellular, punctuated fluorescence, indicating retention of the fusion protein. The GFP-fusion proteins will be introduced into the various secretion mutants to validate and complement the results obtained with the analysis of secretion defects in our secretion mutants.

87. New vectors for gene diversification in Neurospora. John Paul Rasmussen[1], Frederick J. Bowring[1], Elie Kato[2], W. Dorsey Stuart[2], and David E.A. Catcheside[1]. [1]Flinders University, Biology, Adelaide, South Australia, Australia.

Recombination hotspots active in meiosis provide a means of diversifying pairs of DNA sequences differing at multiple sites. We have constructed plasmids permitting targeted transfection of heterologous genes located between *his-3* and the *cog* hotspot in Neurospora, this positioning enables enrichment of post-meiotic recombinants by selection of progeny that have experienced recombination at *his-3*.

88. Isolation and characterisation of a retrotransposon in the phytopathogenic fungus *Stagonospora nodorum*. Jennifer M. Rawson, Simon B. Cutler, and Christopher E. Caten. The University of Birmingham, Biological Sciences, Edgbaston, Birmingham, UK

A search for active transposable elements was undertaken in the phytopathogenic fungus *Stagonospora nodorum* (teleomorph: *Phaeosphaeria nodorum*) using a transposon trapping approach already used successfully in other fungal species (Daboussi and Langin, 1994, Genetica 93: 49-59). Spontaneous mutants of nine wild strains of *S. nodorum* defective in the nitrate reductase (*NIA1*) gene (Cutler *et al*., 1998, Current Genetics 34: 128-137) have been screened for DNA insertions by Southern hybridisation and PCR. A novel insertion of approximately 5kb was detected in one *NIA1* mutant of strain BS444. This insertion, flanked by some *NIA1* DNA, has been cloned and partially sequenced. Sequence comparison with the wild type *NIA1* allele has located the boundaries of the insertion, and suggests that the insertion event has caused an imperfect 11-13bp duplication at this site. Comparison of the two sequenced ends of the insertion has revealed a pair of perfect 169bp direct terminal repeats. Database searches using deduced amino acid sequences suggest some regions of the insertion have homology with published retroelements and reverse transcriptases. Southern hybridisation using the clone as a probe indicates that the insertion is present in 10-15 copies in the genome of BS444 and two related strains, but is absent from fourteen other strains screened.

89. Signal transduction in arbuscular mycorrhizas during pre-symbiosis. Natalia Requena, Petra Fueller and Philipp Franken. Max-Planck Institut für terrestrische Mikrobiologie. Karl-von-Frisch Strasse. 35043 Marburg, Germany.

Arbuscular mycorrhizal fungi are obligate biotrophs forming symbiosis with plant roots of more than 80% of all vascular plants. These fungi can only complete their life cycle after formation of the symbiosis. The life cycle begins with the germination of the spore in the soil and the formation of a pre-symbiotic mycelium, which explores the soil in search of the plant root. Only upon contact with the appropriate host root the fungus starts differentiating and forming the appressorium. In the absence of the plant the fungus retracts back the cytoplasm and arrests until the conditions are again optimal for re-germination. During this pre-symbiotic growth little it is known about the mechanisms or the signals controlling the growth and differentiation of the fungus. We have focussed our attention on this stage of the life cycle and on the genes that are regulated in response to a variety of signals either from the plant or from other soil microorganisms. We start to study these aspects by means of a molecular approach to monitor changes in the gene expression of the fungus *Glomus mosseae*(BEG12) in response to the rhizobacterium *Bacillus subtilis* NR1. The bacterium was found to induce specific increases in mycelial growth correlated with a regulation of the *GmFOX*2 expression, a highly conserved gene encoding a multifunctional protein of the peroxisomal beta-oxidation. We determined the gene structure and studied its expression in a time-course analysis in response to the bacterium. The results show that the fungus is able to respond to stimuli others than the plant changing its gene expression, although it might be sensing the bacteria through common signaling pathways.

90. Self-splicing activity of selected mitochondrial group I introns in *Podospora anserina* in vitro. Jill L. Salvo, Birgit Rodegheir, and John W. Carbone. Union College, Biology, Schenectady, NY, USA.

Self-splicing activity of four group I introns from the mitochondrial genome of the filamentous fungus *Podospora anserina* was assayed using in vitro RNA transcription analysis. Although the Podospora mitochondrial genome contains up to 30 group I introns, only two have been tested for self-splicing activity (LSU, intron 1 and cytochrome oxidase subunit-1, intron 3). Introns from cytochrome b, NADH dehydrogenase subunit-3, ATPase 6 and cytochrome oxidase subunit-1 (intron 5) were selected, and preliminary evidence suggests that all are capable of at least some RNA catalyzed reactions in vitro. The specific nature of these reactions, including use of cryptic splice sites, and the relationships between intron subgroup, location and sequence of the intronic ORF and possible tertiary structure will be discussed.

91. Expression of antisense *creA* RNA in *A. nidulans* causes partial derepression of *creA*-controlled genes. Anne Santerre Henriksen[1], L. Fernando Bautista[1], Morten Hentzer[2], Alexei Aleksenko[1], and Jens Nielsen[1]. [1] DTU, Biotechnology, Lyngby, 2800, Denmark. [2]DTU, Mikrobiology, Lyngby, 2800, Denmark.

The alpha-amylase gene from *A. oryzae* is subject to CREA-mediated carbon catabolite repression both in the host fungus and when expressed in a transgenic *A. nidulans*. An expression cassette containing a portion of the *A. nidulans creA* gene in the reverse orientation with respect to the *gpdh* promoter was introduced into the alpha-amylase-producing *A. nidulans*. This resulted

in a several-fold increase in the enzyme production. The level of alpha-amylase mRNA in the antisense transformants was elevated. The presence of both sense- and antisense-*creA*-transcript was detected. The kinetics of enzyme accumulation in control and antisense-derepressed strains was studied under well controlled fermentation conditions.

92. CPCR1, a transcription factor of the RFX family, shows specificity for cephalosporin C biosynthesis genes from *Acremonium chrysogenum*.Esther Schmitt and Ulrich Kück Lehrstuhl für Allgemeine Botanik, Ruhr-Universität Bochum D-44780 Bochum, Germany.

The biosynthesis of the ß-lactam antibiotic cephalosporin C in *Acremonium chrysogenum* is regulated by a variety of internal and external factors. These parameters effect the cephalosporin C-production e.g. on the basis of transcription initiation of the corresponding genes. Therefore the promoter regions of the cephalosporin C biosynthesis genes are of interest when the molecular regulation of this secondary metabolite is investigated. The two cephalosporin C biosynthesis genes *pcbAB* and *pcbC* share a divergent promoter region of 1.2 kb carrying several putative protein binding sites. A CCAAT-box about 350 bp upstream of the transcription start sites of the pcbC gene was used in a ONE-HYBRID screen to isolate a cDNA from *A. chrysogenum* encoding a transcription factor. Sequence analysis led to the identification of an open reading frame, which contains 830 amino acids. The gene was named *cpcR1* for cephalosporin C regulator 1. The CPCR1 protein belongs to a conserved family of DNA binding proteins, the RFX proteins. To our knowledge this is the first description of a member of this protein family from a filamentous fungus. One-hybrid experiments as well as gel retardation assays with mutated binding sites revealed that CPCR1 interacts specifically with an imperfect palindromic sequence in the pcbC promoter, which overlaps the CCAAT-Box. Using one- and two-hybrid systems it was shown that CPCR1 interacts with itself through the C-terminal part including the dimerization domain and binds DNA only as a homodimer.

93. Isolation of an autonomously replicating DNA sequence from *Aspergillus nidulans.*Seung-Hwan Jang, and Kwang-Yeop Jahng. Chonbuk National University, Biological Sciences, Chonju, Chonbuk, South Korea.

Using the yeast *Saccharomyces cerevisiae* and the integrative vector system, we have isolated and characterized an autonomously replicating sequence (ARS) from *Aspergillus nidulans*. The DNA fragment, designated ANR1, is 5.0 kb in size, and to be maintained in free from the chromosome in *S. cerevisiae*. The recombinant plasmid YIplac211-ANR1, consists of sequences derived from the 3.79 kb yeast integrative vector YIplac211 and ANR1, showed a 10^4-fold enhancement of transformation efficiency over that found for YIplac211, and easily recovered from the transformed yeast. Genetic analysis of transformants showed that YIplac211-ANR1 could be cured over 96% when cultured over 20 generation in complete medium suggesting that this sequence should be mitotically unstable. In Aspergillus nidulans, recombinant plasmid pILJ16-4.5 which carries the 4.5 kb EcoRI fragment of ANR1 showed a 170-fold enhancement of transformation efficiency compared to that of integrative vector pILJ16. Recombinant plasmid pILJ16-4.5 is mitotically unstable, being lost from 67% of asexual progeny of transformants. Southern analysis of transformant DNA showed pILJ16-4.5 to be maintained in free form. Sequence analysis had confirmed that the ANR1 fragment originated from mitochondiral DNA of *A. nidulans*.

94. Transformation of a mycoherbicide by electroporation: methodology and potential application. Amir Sharon, Micah Robinson, and Rudy Maor. Tel Aviv University, Plant Sciences, Tel Aviv , IL, Israel.

Colletotrichum gloeosporioides f. sp. *aeschynomene* causes anthracnose disease of *Aeschynomene virginica*. The fungus has been used for the production of the mycoherbicide College, which is used to control *Aeschynomene virginica* in rice and soybean fields in certain regions in the USA. Genetic engineering of the fungus towards enhanced virulence, broader host range, or pesticide resistance may provide isolates with superior biocontrol properties that will have a wider application range. A procedure for transformation of *C. gloeosporioides* f. sp. *aeschynomene* by elecroporation of germinated conidia has been developed. The new procedure provides a method for high and stable expression of heterologous genes in the fungus as well as for targeting of genes into homologous sites in the fungal genome. The transformants obtained are highly stable in vitro as well as in planta. These methods will be used to generate environmentally-safe strains with improved biocontrol properties. The methodology is general and can be used to develop similar transformation protocols in additional species including fungi with economical and agricultural importance.

95. Heterologous protein expression vectors and molecular breeding of basidiomycetous fungal strains with high lignin- and xylan-degrading activities. Kazuo K.S. Shishido[1], Ken-ichiro K.O. Ogawa[1], Shinya S.M. Matsuda[1], Madoka M.K. Kikuchi[1], Takashi T.Y. Yamazaki[1], Susumu S.K. Kajiwara[1], Akira A.T. Tsukamoto[2], and Jun J.S. Sugiura[2]. [1]Tokyo Institute of Technology, Life Science, Yokohama, Kanagawa, Japan. [2]Oji Paper Co. Ltd., Adv.Technol.Research Lab., Shinonome, Tokyo, Japan.

We have constructed two chromosome-integrating vectors pLC1 and pLC2. The former carries the *Lentinus edodes ras* gene promoter and *priA* gene terminator, and the latter does the basal promoter and terminator of *priA* gene. Both vectors are very useful for the expression of foreign genes in various basidiomycetous fungi. The manganese (II) peroxidase (MnP) cDNA (designated *mnp*c) derived from *Pleurotus ostreatus* was fused between the promoter and terminator of the vectors. These plasmids were introduced into protoplasts of monokaryotic *Coprinus cinereus trp1* strain with the *C. cinereus TRP1*-containing plasmid pCc1001 and into those of monokaryotic *Coriolus hirsutus arg1* strain with the *C. hirsutus ARG1*-containing plasmid, obtaining *C. cinereus* Trp^+ transformants and *C. hirsutus* Arg^+ transformants, all of which show high lignin-decolorization and -degradation activities. Southern-blot analysis revealed that the transformants all possess *mnp*c sequence (5 to 10 copies) on their chromosomes. *Bacillus subtilis*endo (beta-1,4-) D-xylanase structural gene (*xyn*) was fused after the signal sequence of *P. ostreatus mnp*c after removing its own signal sequence. The resulting modified gene (*xyn'*) was fused between the promoter and terminator of pLC1 and pLC2. Through introduction of these recombinant plasmids into the *C. cinereus trp1* genome together with pCc1001, Trp^+ transformants, showing higher xylan-degradation activities, were obtained.

96. Mating-type associated incompatibility in *Neurospora crassa*. Patrick K.T. Shiu. University of British Columbia, Botany, Vancouver, BC, Canada.

The mating-type locus in the haploid filamentous fungus, *Neurospora crassa*, controls mating and sexual development. The fusion of reproductive structures of opposite mating-type, A and a, is required to initiate sexual reproduction. However, the fusion of hyphae of opposite mating-type during vegetative growth results in growth inhibition and cell death, a process which is mediated by the *tol* locus. Mutations in *tol* are recessive and suppress mating-type associated heterokaryon incompatibility. In this study, we describe the cloning and characterization of *tol*. The *tol* gene encodes a putative 1011-amino acid polypeptide with a coiled-coil domain and a leucine-rich repeat. Repeat-induced point mutations in *tol* result in mutants that are wild-type during vegetative growth and sexual reproduction, but which allow opposite mating-type individuals to form a vigorous heterokaryon. Transcript analyses show that *tol* mRNA is present during vegetative growth but absent during a cross. These data suggest that *tol* transcription is repressed in order to allow the co-existence of opposite mating-type nuclei during the sexual reproductive phase. *tol* is expressed in a *mat A, mat a*, A/a partial diploid and in a mating-type deletion strain, indicating that MAT A-1 and MAT a-1 are not absolutely required for transcription or repression of *tol*. These data suggest that TOL may rather interact with MAT A-1 and/or MAT a-1 (or downstream products) to form a death-triggering complex. Studies on recently isolated TOL-interacting proteins (tip; isolated by yeast 2-hybrid system) will shed lights on the mechanism of mating-type mediated vegetative incompatibility.

97. Promoter-Tagged Restriction Enzyme Mediated Insertion (PT-REMI) mutagenesis in *Aspergillus niger*. Jeffrey R. Shuster, and Mariah Bindel Connelley. Novo Nordisk Biotech, Inc., Molecular Genomics, Davis, CA, USA.

Promoter-tagged restriction enzyme mediated insertion (PT-REMI) DNA mutagenesis was performed in the fungus, *Aspergillus niger*, using a plasmid containing a strong transcriptional promoter. Two DNA-tagged mutants were analyzed in detail. A white-spored mutant was shown to contain a plasmid insertion that disrupted a gene showing strong identity to the polyketide synthase *wA* gene of *A. nidulans*. A morphological mutant was shown to contain a plasmid insertion in the 5'-upstream region of a gene showing strong identity to cytochrome C oxidase subunit V, *COX5*. The insertion of the plasmid resulted in enhanced expression for the *COX5* RNA demonstrating that a combination of REMI with a promoter can be used to activate gene transcription.

98. Induced expression of a novel *Aspergillus fumigatus* putative drug efflux gene in response to itraconazole.John W. Slaven[1], Michael J. Anderson[1], Dominique Sanglard[2], Graham K. Dixon[3], Jacques Bille[2], Ian S. Roberts[4], and David W. Denning[1]. [1]University of Manchester, Medicine, Manchester, Gr Manchester, UK. [2]CHUV, Microbiology, Lausanne, Switzerland. [3] Zeneca Pharmaceuticals, Cancer and Infection, Macclesfield, Cheshire, UK. [4]University of Manchester, Biological Sciences, Manchester, Gr Manchester, UK

Two agents are licensed for the treatment of *A. fumigatus* infection, amphotericin B and itraconazole. Resistance to itraconazole has been detected *in vitro* and has been validated *in vivo*. Studies of *Saccharomyces cerevisiae* and *Candida* species have shown that one mechanism of azole resistance is drug efflux by ATP-binding cassette (ABC) transporters. An *A. fumigatus* genomic library was screened with a probe from the *C. albicans* ABC transporter gene, *CDR1*. This screening revealed a novel gene, *ADR1*, which has a high level of identity to other fungal

multi-drug resistance (MDR) genes. The encoded protein contains conserved amino acid residues within recognised MDR motifs. As well as hybridising to the gene, an *ADR1* probe hybridised to other sequences in the genome at high stringency. Dot blot analysis showed that *ADR1* mRNA is expressed at over 5-fold higher levels in a resistant isolate (AF72) which is unable to accumulate itraconazole. However, expression was only up-regulated when AF72 was grown in the presence of itraconazole. The regulation of this gene was also studied in response to heat shock, oxidative stress and other anti-fungal drugs, including terbinafine. Adr1 is a novel ABC transporter, possibly involved in the efflux of itraconazole from *A. fumigatus*. Complementation studies in yeast will be undertaken to establish the protein's substrate range.

99. Isolation of extragenic suppressor mutations of the *palI30* mutation of *Aspergillus nidulans*. Daphne Smith[1], Bruce Dawson[2], Valerie Alexander[2], and Steven H. Denison[1]. [1] Mississippi College, Dept of Biological Sci, Clinton, MS, USA. [2] Brandon High School, Brandon, MS, USA

The product of the *palI* gene of *Aspergillus nidulans* is a component of the ambient pH signal transduction pathway. To ensure production of alkaline-specific extracellular enzymes in alkaline environments and acid-specific extracellular enzymes in acid environments, this pathway modifies the *pacC*-encoded transcription factor in response to alkaline pH. Strains with mutations in the *palI* gene mimic growth in acidic conditions, showing (at pH 6.5) increased production of extracellular acid phosphatase, decreased production of alkaline phosphatase and increased sensitivity to molybdate in the growth medium. The *palI* gene encodes a protein with four putative transmembrane domains, and may therefore function as the pH sensor in the signal transduction pathway. In order to identify new components of this signalling pathway, we have isolated extragenic suppressor mutations of the *palI30* mutation. Following 4-NQO mutagenesis of spores, colonies were selected initially for growth on 17 mM sodium molybdate and secondarily for patterns of acid and alkaline phosphatase production more like wild type. Suppressor mutations have been identified in the *pacC* and *pacX* genes. Sequence analysis of these *pacC* mutations has identified amino acids which might be important in maintaining the closed form of PacC (L. Rainbow, J. Tilburn and H. N. Arst, Jr., unpublished). Mutations in the *pacX* gene are also able to suppress mutations in other *pal* genes and certain mutations in *pacC* (E. Bignell, J. Tilburn and H. N. Arst, Jr., unpublished).

100. Glycosylation of proteins in *Aspergillus niger*: isolation of the N-acetylglucosamine-phosphate-tranferase gene (GPT) Tine Kring Sorensen, Paul Dyer, Ulrike Laube, and John Peberdy. University of Nottingham, Biological Sciences, Nottingham, Nottinghamshire, UK.

Filamentous fungi secrete a range of enzymes, the majority of which are hydrolytic and play an important role in fungal nutrition. Some fungi are capable of secreting high levels of proteins and have consequently attracted attention as potential producers of heterologous proteins, but so far yields obtained have been very low. Not only are improved yields required, but also the proteins should be highly authentic, thereby preserving functionality and avoiding antigenic reactions. Many proteins secreted by fungi are glycosylated. Most information on glycosylation has come from studies on mammalian and yeast systems, but only little is known about the process in filamentous fungi. It is therefore desirable to better understand the events of protein secretion in fungi, thus making it possible to control the process and thereby tailor the glycans produced on

secreted proteins. Studies are in progress to investigate the biochemical and molecular controls of glycosylation in *Aspergillus niger*. One aspect of this involves the ER-located enzyme UDP-*N*-acetyl glucosamine:dolichyl phosphate *N*-acetyl glucosamine-1-phosphate transferase (GPT), catalyzing the first step of the N-glycosylation pathway: the synthesis of *N*-acetylglucosamine-dolichol phosphate. The GPT-gene has now been cloned from a genomic library of *A.niger* by screening with a PCR derived homologous fragment (470 bp) isolated from genomic DNA. The ORF was 1.4 kb with 2 introns (58, 91 bp). The sequence from *A.niger* showed about 46% identity with the GPT gene cloned from yeasts and 35-37% identity with rodents and human. Northern blotting, using a homologous probe, produced a 2.4 kb mRNA transcript. Expression studies are in progress. Acknowledgement of funding sources: The Danish Research Academy and The Danish Research Council.

101. AnCF, the CCAAT binding complex of *Aspergillus nidulans* contains products of the *hapB*, *hapC* and *hapE* genes and is required for activation by the pathway specific regulatory gene, *amdR*.Stefan Steidl[1], Peter Papagiannopoulos[2], Olivier Litzka[1], Alex Andrianopoulos[2], Meryl A. Davis[2], Axel A. Brakhage[1] and Michael J. Hynes[2]. [1]Institut fuer Mikrobiologie und Genetik, TU Darmstadt, Germany [2]Department for Genetics, University of Melbourne, Australia.

CCAAT binding factors positively regulating the expression of the *amdS* gene (encoding acetamidase) and two penicillin biosynthesis genes (*ipnA* and *aatA*) have been previously described in *Aspergillus nidulans*. The factors were called AnCF and PENR1 respectively. Deletion of the *hapC* gene, encoding a protein with significant similarity to Hap3p of *Saccharomyces cerevisiae*, eliminated both AnCF and PENR1 binding activities. We now report the isolation of the genes *hapB* and *hapE* which encode proteins with central regions of high similarity to Hap2p and Hap5p of *S. cerevisiae* and to the CBF-B and CBF-C proteins of mammals. The HapB, HapC and HapE proteins have been shown to be necessary and sufficient for the formation of a CCAAT binding complex in vitro. Strains with deletions of each of the *hapB, hapC* and *hapE* genes have identical phenotypes of slow growth, poor conidiation and reduced expression of *amdS*. Furthermore, induction of *amdS* by omega amino acids, which is mediated by the AmdR pathway specific activator, is abolished in the hap deletion mutants as is growth on gamma-aminobutyric acid (GABA) as a sole nitrogen or carbon source. AmdR and AnCF bind to overlapping sites in the promoters of the *amdS* and *gatA* genes. It is known that AnCF can bind independently of AmdR. We suggest that AnCF binding is required for AmdR binding in vivo.

102. Characterization of a *Neurospora crassa* strain that escapes senescence associated with the over-replication of a mitochondrial retroplasmid.Charles B. Stevenson, A. Nicole. Fox, Erica B. Larson, and John C. Kennell. Southern Methodist University, Biological Sciences, Dallas, TX, USA.

Variant forms of the Mauriceville and Varkud mitochondrial retroplasmids cause growth impairment and senescence in *Neurospora spp.* Senescence is generally associated with deletions or rearrangements of the mitochondrial (mt) genome that result from the integration of variant plasmids; however, certain variants of the Mauriceville retroplasmid can impair growth without integrating into mtDNA. Senescence associated with the MS4416 variant retroplasmid is highly

predictable and correlates with an increase in the plasmid copy number and alterations in the expression of specific mitochondrial gene products. Here, we report the isolation and characterization of a mutant strain that escapes senescence. This long-lived derivative strain shows vigorous and indefinite growth while tolerating high levels of the variant plasmid and *in vivo* labeling studies indicate the strain has a wild-type mitochondrial protein expression profile. Inheritance studies show that the long-lived trait is controlled by nuclear genes, whereas heterokaryon analysis reveals unanticipated senescent phenotypes which involve the integration of the variant plasmid into the mtDNA. Our findings suggest specific gene products or pathways exist in Neurospora that help alleviate mitochondrial dysfunction and control suppression associated with defective mtDNAs.

103. Gene rearrangement and surface variation in *Pneumocystis carinii*. James R. Stringer, Joshua Schaffzin, Scott Keely, Susan Sunkin, and Melanie Cushion. University of Cincinnati, Microbiology, Cincinnati, OHIO, USA.

P. carinii have on their surface a major surface glycoprotein (MSG). MSG is a protein family encoded by about 100 heterogeneous genes, which are located at the ends of each of the fifteen chromosomes that comprise the genome. Organisms within a population can have different MSG isoforms on their surface. This antigenic variability appears to be accomplished by regulation of MSG gene expression. The first hint at the mechanism of MSG gene regulation came from the discovery that a 400 basepair sequence (called the UCS) is on the 5' end of essentially every MSG mRNA, regardless of the MSG isoform encoded. The UCS was found to reside at the end of a single chromosome, which suggested that the UCS locus serves as an MSG expression-site. Studies on the structure of the expression-site locus showed that it can be occupied by different MSG genes in different organisms in a population. The number of different MSG genes residing at the expression-site in a population seems to vary, but more than 25 different MSGs were at this locus in the population that was studied at this level. Residence at the expression site seems to be both necessary and sufficient for expression of a specific MSG gene because all of the MSG genes that were at the expression site were represented by mRNAs, and mRNAs from MSG genes not linked to the UCS were not detected. Restriction of expression of the MSG family to the gene attached to the UCS implies that each organism has but one MSG isoform on its surface. The presence of multiple MSG genes at the UCS locus in a single population shows that gene rearrangements involving UCS and MSG genes occur. The mechanism and frequency of these rearrangements is under investigation.

104. Characterization of DNA de novo methylation signals by using short synthetic oligonucleotides in vegetative cells of *Neurospora crassa*. Hisashi Tamaru, and Eric U. Selker. University of Oregon, Inst. of Mol Biol., Eugene, OR, USA.

DNA is modified by cytosine methylation in many eukaryotes including Neurospora. Our goal is to understand how certain cytosines are targeted for methylation. Most DNA sequences that are subject to de novo methylation in *N. crassa* have undergone RIP (repeat-induced point mutation) and are therefore relatively rich in A+T and are enriched for TpA dinucleotides. To further understand the nature of DNA *de novo* methylation signals we quantified the capacity of short oligonucleotides to trigger methylation. We constructed a *his-3* targeting vector that carries a 100 bp zeta-eta fragment surrounded by a lightly mutated allele of the *am* gene (amRIP4). The

mosaic amRIP4::zeta-eta construct does not establish methylation by itself at the *his-3* locus but provides a sensitive background to test many short (ca. 25 bp) fragments for their capacity to trigger methylation. We show that various fragments consisting only of A and T can trigger methylation but to different extents. Poly A:T did not trigger significant methylation. Sequences rich in TpA dinucleotides triggered methylation but were less potent than some sequences with fewer TpA dinucleotides. Thus, recognition of *de novo* methylation signals in *N. crassa* does not simply involve counting the main product of RIP, TpA.

105. HMG CoA reductase has a role in both primary and secondary metabolism in *Penicillium paxilli*. Emily Telfer, and Barry Scott. Massey University, Molecular BioSciences, PalmerstonNorth, Manawatu, New Zealand.

3-Hydroxy-3-methylglutaryl Coenzyme A reductase (HMGR) catalyses the conversion of HMG to mevalonic acid, the first step in isoprenoid biosynthesis. Mevalonic acid is a key intermediate for the synthesis of a range of primary and secondary metabolites. In the filamentous fungus *Penicillium paxilli* mevalonic acid is a key precursor for the biosynthesis of the secondary metabolite paxilline; an indole diterpenoid that is produced in large quantities in submerged cultures at stationary phase. The aim of this research is to determine whether *hmg* expression alters with the onset of paxilline biosynthesis. Using a probe generated by PCR the *P. paxilli hmg* was isolated from a lambda GEM11 genomic library and sequenced. Sequence analysis identified several characteristic promoter motifs associated with a constitutively expressed primary metabolite gene. The putative transcription site for this gene was determined by RT-PCR using a set of nested primers. RT-PCR analysis of *hmg* expression during the growth cycle of a submerged culture of *P. paxilli* showed a dramatic increase in expression of this gene compared to that of Beta-tubulin (*tub-2*). Furthermore, this pattern of expression mimiced that of two key paxilline biosynthetic genes, *paxR* and *paxG*, which have recently been cloned from *P. paxilli* (see Young *et al*. this meeting). This result would suggest that there is a regulatory mechanism within the cell for coordinate induction of primary (*hmg*) and secondary (*pax*) metabolite genes.

106. The creC carbon catabolite repression gene of *Aspergillus nidulans* encodes a protein containing WD40 repeats. Richard B. Todd, Robin A. Lockington, and Joan M. Kelly. University of Adelaide, Genetics, Adelaide, SA, AUSTRALIA.

Many microbial genes are subject to carbon catabolite repression (CCR), the repression in the presence of a preferred carbon source (e.g. glucose, sucrose) of the genes required for growth in the presence of less favourable carbon sources (e.g. acetamide, proline, quinate). In *Aspergillus nidulans* mutations which relieve CCR were previously selected as suppressors of the *areA217* loss-of-function mutation for growth on sucrose plus acetamide. One class of recessive mutations map to *creC*, which is tightly linked to *glnA* on linkage group II. *creC* mutants display pleiotropic effects for growth on a range of carbon sources including decreased growth on some carbon sources such as quinate, and partially derepressed expression in repressing conditions of some enzymes such as alcohol dehydrogenase and acetamidase. We have exploited the proximity to *glnA* to clone by complementation the *creC* gene. Sequence analysis revealed that *creC* encodes a 630 amino acid polypeptide which contains a proline-rich region and WD40 repeats. CreC shows a high level of similarity with proteins of unknown function in

Schizosaccharomyces pombe, mouse and human, but there is no close homologue in the *Saccharomyces cerevisiae* genome sequence. Regions of *creC* required for function have been localized by C-terminal deletion analysis and the determination of the sequence changes in *creC* mutant alleles.

107. *SNF1* and carbon utilization in *Cochliobolus carbonum*. Nyerhovwo J. Tonukari, John S. Scott-Craig, and Jonathan D. Walton. Michigan State University, DOE Plant Research Lab, East Lansing, MI, USA.

Cochliobolus carbonum, an ascomycetous pathogen of maize, secretes a variety of extracellular enzymes, such as pectinases, xylanases, glucanases and proteases, that can degrade plant cell wall polymers. The production of most cell wall degrading enzymes is substrate-induced and can be repressed by preferred carbon sources such as glucose. In *Saccharomyces cerevisiae* the Snf1 (sucrose non-fermenting) kinase is required for transcription of glucose-repressed genes when glucose is limiting. The *SNF1* gene is broadly conserved in eukaryotes, where it is involved in stress responses. Degenerate oligonucleotide primers based on Snf1 homologs from *S.cerevisiae*, *Arabidopsis* and rat were used to amplify a 400-bp fragment from *C. carbonum* genomic DNA which was then used to isolate cDNA and genomic copies of the gene. A specific Snf1 mutant was created by transformation-mediated gene replacement via homologous recombination. There was significant reduction in the growth of the mutant on solid media when xylan, pectin, cellulose and corn cell walls were used as carbon sources. The growth of the mutant was similar to that of the wild type when glucose is the carbon source. The total xylanase, glucanase and pectinase activities in culture filtrates of the mutant using corn cell walls as carbon source were reduced by approximately 60, 40 and 30%, respectively.

108. Developmental regulation of two forms of arginase from *Neurospora crassa.* Gloria E. Turner, and Richard L. Weiss. UCLA, Chemistry & Biochemistry, Los Angeles, CA, USA.

The existence of multiple arginases in *N. crassa* poses several interesting questions regarding function, regulation and evolutionary significance of the two forms. Multiple arginases are found in all vertebrates but this is the first characterization from a non-vertebrate. The function and regulation of liver arginase, an essential component of the urea cycle, is well understood; however the physiological role of the ubiquitous non-hepatic arginases is not known. In vertebrates the two major forms are products of separate genes, whereas in *N. crassa* the two major forms are differentially expressed from a single gene. A 1.4-kb transcript is synthesized in minimal medium which is then translated into a 36-kDa protein; however, in the presence of arginine, an additional 1.7-kb transcript is made. This mRNA has a 41-kDa protein as its translation product. This expression is controlled by the inducer arginine via mechanisms that are presently being investigated. In *Xenopus* it has been shown that the extrahepatic arginases are highly expressed during the transition from tadpole to frog. Given this precedent for arginase developmental expression, we examined the levels of the two major arginase transcripts and protein in conidia and germinating conidia in the wild-type strain, 74-R23-1A. Both forms appear to differ slightly in their developmental pattern with the arginine specific transcript just detected in conidia and the smaller transcript appearing at 2 hours. We have determined that arginase expression is elevated early in germination and decreases with time. This developmental

profile is similar to several amino acid biosynthetic genes. This was an unexpected result for an inducible catabolic enzyme.

109. Isolation, characterisation and regulation of two sulphate transporter encoding genes from *Penicillium chrysogenum*.Mart van de Kamp, Theo A. Schuurs, Arnold Vos, Arnold J.M. Driessen, and Wil N. Konings. Department of Molecular Microbiology, Biological Centre, University of Groningen, The Netherlands.

Using a PCR-based approach, we have isolated two *Penicillium chrysogenum* genes, *sutA* and *sutB*, that encode sulphate transporters (see Poster abstract by Pizzinini *et al*.). Full-length genomic clones were obtained and sequenced. The presence of introns was determined from a comparison of genomic and cDNA sequences. Hydropathy profile analysis indicated that the SutA and SutB proteins contain 10-12 membrane-spanning regions. The gene products are members of the SulP superfamily of secondary sulphate transporters. We have studied the regulation of the *sutA* and *sutB* genes using Northern analyses and sulphate uptake experiments. In mycelium of *P. chrysogenum* grown under penicillin-producing conditions, the expression of *sutA* is much weaker than expression of *sutB*. The expression of both *sutA* and *sutB* is regulated by the sulphur content of the medium. Growth in the presence of excess sulphate results in repression of both genes and negligible sulphate uptake, whereas growth under sulphate starvation condition induces *sutA* and *sutB* expression which is reflected in high sulphate uptake rates.

110. Inter-nuclear gene silencing in *Phytophthora infestans*.Pieter van West[1], Sophien Kamoun[1,3], John W. van 't Klooster[1], Neil A.R. Gow[2], and Francine Govers. WAU, Phytopathology, Wageningen, The Netherlands. [2]Univ of Aberdeen, Molecular Biology, Aberdeen, Scotland. [3] Present address: Department of Plant Pathology OSU, Wooster OH, USA.

Transformation of the diploid oomycete *Phytophthora infestans* with antisense, sense and promoter-less constructs of the coding sequence of the elicitin gene *inf1* resulted in transcriptional silencing of both the transgenes and the endogenous gene. To investigate the mechanism of gene silencing we took advantage of the fact that *P. infestans* has coenocytic mycelia and that mycelial cells may contain multiple nuclei that can differ genetically, resulting in heterokaryotic strains. It appeared that: (i) transcriptional gene silencing is dominant in multinucleated cells, (ii) the silenced state can be transmitted from nucleus to nucleus in heterokaryotic strains, and (iii) gene silencing is maintained in a non-transformed nucleus after nuclear separation (van West et al., 1999). In addition, we showed that upon fusion of a silenced non-transgenic strain with a strain containing wildtype nuclei, the silenced state could be transmitted again to the wild type nuclei. Transcriptional gene silencing in *P. infestans* apparently involves inter-nuclear transfer of signals from silenced (transgenic and non-transgenic) nuclei to wild type nuclei, leading to stable gene silencing in the wild type nuclei. These findings support a model reminiscent of paramutation and involving a trans-acting factor that is capable of transferring a silencing signal between nuclei.

111. Transposon mediated mutagenesis in *Magnaporthe grisea*.François Villalba[1], Marc-Henri Lebrun[1], Aurélie Hua-Van[2], Marie-Josée Daboussi[2], and Marie-Claire Grosjean-

Cournoyer[1]. [1]Rhône-Poulenc Agro, Biotechnology, Lyon, Lyon, FRANCE. [2]Université Paris-Sud, I. G. M., Orsay, Orsay, FRANCE.

Transposable elements (TEs) have been characterized in a broad range of organisms, from prokaryotes to higher eukaryotes. Based on their mode of propagation, they have been divided into two major classes (Finnegan, 1989). Class I elements transpose by reverse transcription of an RNA intermediate. Class II elements transpose by a DNA-DNA mechanism. TEs can generate mutations by moving from one genomic position to another through their insertion into a gene or its promotor. Daboussi and coworkers (1992) were the first to successfully use the nitrate reductase gene (*niaD*) as a transposon trap in filamentous ascomycetes. In this system, *niaD* mutants can be isolated by a direct selection for chlorate resistance. The class II element *Impala*, belonging to the Tc1-mariner superfamily, was identified as a *Fusarium oxysporum* mobile element using this strategy (Langin et al., 1995). This transposon is 1280 nucleotides long and contains two 27 pb inverted terminal repeats (ITRs) flanking an open reading frame encoding a putative 340 amino acid transposase. TEs can be usefull as genetic tools for gene tagging. Integration of the transposon into promoter or coding region can lead to a new phenotype allowing identification of unknown genes after isolating the genomic regions flanking the element. As *Impala* is an active transposon in *Fusarium oxysporum*, we decided to assay if it can be mobilized in an heterologous host such as *Magnaporthe grisea* in order to establish a transposon mediated mutagenesis system. Results concerning vectors used and *Impala* transposition will be presented. This work is supported by grants from a biotechnology contract between Rhône-Poulenc Agro and the MNSRE. Finnegan, 1989. Trends Genet. 5 : 103-107. Daboussi et al., 1992. Mol. Gen. Genet. 232 : 12-16. Langin et al., 1995. Mol. Gen. Genet. 246 : 19-28.

112. Characterization of an unprecedented group of genetic elements: linear, non-LTR retroplasmids of *Fusarium oxysporum*.Tobais Ch. Walther, Shannon L. Ross, and John C. Kennell. Southern Methodist University, Biological Sciences, Dallas, TX, USA.

Telomeres of eukaryotic chromosomes are maintained by enzymatic reactions that involve reverse transcriptase. It is widely speculated that the ribonucleoprotein complexes that maintain telomeres, telomerases, were derived from invasive genetic elements that encode reverse transcriptase; however, no retroelement counterpart has been identified that has structural similarities to telomeres or carries out reverse transcription in a manner that is mechanistically analogous to the reaction catalyzed by telomerases. We describe the complete sequence and primary structure of two related mitochondrial plasmids of *Fusarium oxysporum*, pFOXC2 and pFOXC3, which have features expected of genetic elements that are related to the precursors of telomerase. The plasmids have unique genomic structures which include a hairpin at one terminus and a telomere-like repeat of a 5 bp sequence at the other terminus and encode an active reverse transcriptase which shows specificity for the plasmid transcript. The plasmid replication cycle involves a novel strategy for copying terminal sequences via transcription of the hairpin structure and subsequent reverse transcription of the greater-than unit-length RNA. Our studies indicate that the *Fusarium plasmids* comprise a new group of genetic elements that replicate via reverse transcription: linear, non-LTR retroplasmids. The finding that the 3' termini of the plasmids have rudimentary features of eukaryotic telomeres suggests they may hold clues concerning the origin of telomerase and the evolution of linear DNA elements.

113. Non-zymogenic Chitin Synthase 3 (WdChs3p) localizes at the cytoplasm of *Wangiella dermatitidis.* Zheng Wang, and Paul J. Szaniszlo. University of Texas at Austin, Microbiology, Austin, TX, USA.

Because the class III chitin synthase (WdChs3p) of *Wangiella dermatitidis*, a dematiaceous (melanized) fungus that is associated with phaeohyphomycosis, has preliminarily been found to be a virulence factor, it was necessary to characterize this enzyme further and to investigate its localization and regulation. Heterologous expression of the WdCHS3 cDNA in S. cerevisiae demonstrated that WdChs3p was a non-zymogen and its membrane bound structure was essential to maintain enzyme function. The WdCHS3 gene with six tandem repeats of the myc epitope sequence incorporated at its 5' end was integrated at the WdCHS3 locus of the genome of *W. dermatitidis* by homologous recombination. Western analysis using monoclonal anti-myc antibody demonstrated that the WdChs3-myc protein level increased with increasing temperature of culture, corresponding to the fact that the WdCHS3 transcription is temperature and stress dependent. Production of WdChs3p-myc was significantly enhanced starting from 6 hr after cells were shifted from 25 C to 37 C, implying that translation of WdChs3p was also temporally regulated. Indirect immunofluorescence showed a cytoplasmic localization for WdChs3-myc in all three predominant vegetative morphologies (yeasts, multicellular forms and hyphae) of *W. dermatitidis* grown at 37 C, suggesting that WdChs3p may play a role in maintaining the cell wall integrity at temperatures of infection. Although introduction of WdCHS3 with its own promoter into the polyketide synthase locus in the wdchs3D disruption strain restored lost chitin synthase activity, a truncated WdChs3p with the deletion of its first 55 amino acids was not functional. This is in contrast to yeast chitin synthases in which N-terminal sequences are not important.

114. Arginine-specific translational attenuation mediated by the *Neurospora crassa arg-2* arginine attenuator peptide appears independent of the charging status of tRNA. Zhong Wang, Anthony Gaba, and Matthew S. Sachs. Oregon Graduate Institute, Biochemistry and Molecular Bio, Portland, OR, USA.

The arginine attenuation peptide (AAP) is encoded by an upstream open reading frame (uORF) in the 5'-leader of the *N. crassa arg-2* mRNA. It is critical for Arg-specific translational regulation of the mRNA in vivo and in vitro in *N. crassa*. In vitro, Neurospora ribosomes that have translated the AAP stall when [Arg] is high. Here we show that the *N. crassa* AAP and a related AAP encoded by a uORF in the 5'-leader of the homologous *Saccharomyces cerevisiae* mRNA, *CPA1*, exert Arg-specific, negative translational regulation in *N. crassa*, *S. cerevisiae* and wheat germ cell-free translation reactions. AAP-containing mRNAs were used to demonstrate that the extent of translational regulation by [Arg] was proportional to [Arg] at concentrations between 150 and 5,000 micromolar. Yet, in the *S. cerevisiae* and wheat germ systems, each of the arginyl-tRNAs examined appeared fully charged even at much lower Arg concentrations (10 micromolar). These experiments indicate that the level of charged Arg-tRNAs is not significant for AAP-mediated, Arg-specific translational control. (Supported by NIH GM47498.)

115. Differential gene transcription in galled and healthy tissues of pine infected with *Cronartium quercuum* f. sp. *fusiforme*. Jaimie M. Warren, and Sarah F. Covert. The University of Georgia, Department of Genetics, Athens, GA, USA.

Cronartium quercuum f. sp. *fusiforme* causes the formation of woody galls on pine (Pinus spp.). To better understand the biology of this host-pathogen interaction, we used differential display to identify 22 transcripts which are differentially transcribed in healthy and galled tissues of slash pine (*Pinus elliotti*) infected with *C. q. fusiforme* Several of these are homologous to previously cloned genes. Transcripts present in galls, but absent or transcribed at lower levels in healthy tissues include clones homologous to a fungal carboxypeptidase, a plant heat shock protein, a fungal cyclophilin, an NADPH cytochrome reductase, and a fungal cytochrome c oxidase polypeptide. Transcripts present in healthy tissues but absent or transcribed at lower levels in galls include clones homologous to a plant receptor-like kinase and a pine lipid transfer protein. The latter homologies are particularly interesting because these types of protein are associated with suppression of cell division and antimicrobial properties in plants, respectively. Ongoing work focuses on determining the genome of origin of each clone and confirming the differential transcription patterns of all 22 cDNAs in slash pine. The transcription patterns of these fragments will also be determined in healthy and infected loblolly pine (*Pinus taeda*) and lodgepole pine (*Pinus contorta*). This analysis will determine if the differential transcription of these cDNAs is consistent in three different host species infected by *C. q. fusiforme* .

116. Analysis of the structure and function of isocitrate lyase from *Aspergillus nidulans*. Kanchana Weeradechapon, K Linda. Britton, Sarah J. Langridge, Svetlana E. Sedelnikova, Geoffrey Turner and David W. Rice. University of Sheffield, Krebs Institute, Sheffield, South Yorkshire, UK.

Isocitrate lyase is essential for growth of microorganisms on 2C compounds and fatty acids, and is located in the glyoxysomes in *Aspergillus nidulans*. The structure of isocitrate lyase from *A. nidulans* has been solved at 2.8 using X-ray crystallography. The secondary structure of this tetrameric enzyme is arranged into two main domains, one of which forms a peripheral head domain to the enzyme molecule, and corresponds to an internal sequence of approximately 100 amino acids which is not found in prokaryotic ICLs. To understand the structure/function relationships of this enzyme including the molecular factors which control the differential targetting of the enzyme to glyoxysome in eukaryote and cytoplasm in prokaryote, a null mutant *A. nidulans* has been constructed by transformational deletion of the isocitrate lyase (*acuD*) gene for use as an expression host for *acuD* mutants generated *in vitro*. Mutations made to date include deletion of the internal additional amino acid sequence, and the disordered C-terminal region which may be involved in protein targetting. Mutated *acuD* was inserted into the null mutant by cotransformation. The results of the structure analysis of this enzyme and of the programme of mutagenesis designed to test proposals on the enzyme mechanism and intracellular location will be presented.

117. Growth disassociated production of heterologous protein by *F. venenatum*. Marilyn G. Wiebe[1], Geoffrey D. Robson[1], and Jeff Shuster[2]. [1]University of Manchester, School of Biol. Science, Manchester, Gtr. Manchester, UK. [2] Novo Nordisk Biotech. Inc, 1445 Drew Avenue, Davis, California, USA.

Most recombinant proteins generated in filamentous fungi are produced in fed-batch cultures, in which specific growth rate decreases progressively with time. Because of this, such cultures are more suited to the production of growth rate disassociated than growth rate associated products. *Fusarium venenatum* A3/5 has been transformed (JeRS 325) to produce *Aspergillus niger* glucoamylase (GAM) under the control of the *Fusarium oxysporum* trypsin-like protease promoter. No glucoamylase was produced during exponential growth of *F. venenatum* JeRS 325 in batch culture, and in glucose-limited chemostat cultures glucoamylase was produced in a growth rate disassociated manner with GAM concentration decreasing with increase in dilution rate and with the specific production rate of GAM remaining approximately constant over the dilution rate range of 0.05 $h^{-1}$to 0.19 h^{-1}. Specific production rates of 5.8 mg and 4.0 mg GAM [g biomass]$^{-1}$ h^{-1} were observed in glucose-limited chemostat cultures in the presence and absence of 1 g mycological peptone L^{-1}.

118. Cloning of two delta9 desaturase genes from *Mortierella alpina*. Prasert Wongwathanarat, Donald A. MacKenzie, Andrew T. Carter, and David B. Archer. Institute of Food Research, Genetics and Microbiology, Norwich, UK.

The long chain polyunsaturated fatty acids are of both nutritional and pharmarcological importance to man because they can prevent and be used to treat a wide range of human diseases and disorders by serving as precursors for hormones such as prostaglandins and leukotrienes. They also have an important role in enhancing infant brain development. Recently, it has been shown that the oleaginous soil fungus, *Mortierella alpina*, which belongs to the order Mucorales has the potential to synthesize long-chain polyunsaturated fatty acids such as arachidonic acid (C20:4,n6) on a commercial scale. This fungus also produces smaller amounts of oleic acid (C18:1, n9), linoleic acid (C18:2, n6), gamma-linolenic acid (C18:3, n6) and eicosapentaenoic acid (C20:5, n3). Physiological approaches to increase the yield of arachidonic acid or to change the fatty acid composition have been successful with this fungus in fermentors and conditions have been optimized at commercial scale. Strain improvement has also been acheived by standard mutagenesis. Alternatively, genetic modification of this fungus is another approach to increase the yield of arachidonic acid or to change the fatty acid composition. It has been shown that synthesis of arachidonic acid requires a series of desaturase genes and an elongase gene. The delta9 desaturase is the first enzyme involved in this pathway. To isolate and clone the delta9 desaturase gene encoding this enzyme, PCR using degenerate primers with homology to other known delta9 desaturase genes from several fungi was performed. Interestingly, it was found that there were two delta9 genes from this fungus and another delta9-like desaturase gene which had only 50% amino acid identity to other delta9 genes. Identification of this gene is underway. Northern and RT-PCR analysis of the two delta9 desaturase genes suggested that only one of these genes was expressed in *M. alpina*.

119. Using DNA-Tagged mutagenesis to improve heterologous protein production in *Aspergillus oryzae*. Debbie S. Yaver, Howard Brody, Michael Lamsa, Rebecca Munds, Stephen H. Brown, Suzie M. Otani, and James A. Johnstone. Novo Nordisk Biotech, 1445 Drew Avenue, Davis, CA, USA.

In order to identify *Aspergillus oryzae* genes that are important for heterologous protein production a large library of DNA-tagged mutants was generated and screened for production of

a model heterologous protein. Using restriction enzyme-mediated integration (REMI) two libraries of 27,000 and 28,000 transformants were made in an *A.oryzae* strain producing a *Thermomyces lanuginosus* lipase using *pyrG* as the tagging DNA and either *Bam*HI or *Eco*RI enzyme, respectively. The libraries were screened robotically for lipase production, and mutants with either decreases or increases in lipase production were isolated. The *pyrG* tagging vector along with the genomic DNA flanking the integration event has been rescued from one of the yield improved mutants (DEBY10.3), and the nucleotide sequence of the flaking DNA shares identity to the *Aspergillus nidulans palB* gene. As expected for a *palB* minus strain, the DEBY10.3 mutant is unable to grow on pH 8.0 minimal medium plates. Three lines of experimental evidence demonstrate that the increase in lipase yield in DEBY10.3 is linked to the *palB* minus phenotype generated by the integration of the tag into the *palB*gene. These results will be presented.

120. A color-selectable and site-specific integrative system for gene expression studies in the dematiaceous fungus *Wangiella (Exophiala) dermatitidis.* Xiang-cang Ye, Bin Feng, and Paul J. Szaniszlo. University of Texas at Austin, Microbiology, Austin, TX, USA.

Many dimorphic, darkly pigmented fungi of the form-family Dematiaceae of the Fungi Imperfecti are pathogens of humans. As an initial attempt to explore potential virulence factors in the model dematiaceous fungus *Wangiella dermatitidis*, we established a gene overexpression system with properties of site-specific integrative transformation and color identification. Using a partial polyketide synthase gene (WdPKS1) as the targeting sequence and transformation by electroporation, we found that from 30 to 52% of resulting transformants contained exogenous markers site-specifically integrated into the target locus. This disrupted melanin precursor synthesis and produced albino colonies immediately distinguishable from the black parental strain and from nonspecific transformants. In addition, a heterologous glucoamylase promoter, glaA, was examined under various conditions for overexpression of the lacZ reporter gene and the WdCDC42-1 gene of *W. dermatitidis*. With this promoter, enhanced beta-galactosidase activity required an elevated temperature of 37C and an agar medium containing maltose as a sole carbon source. Our results showed that this unique integrative gene expression system allows the production of isogenic transformant strains for gene overexpression analysis in *W. dermatitidis* and most likely other black fungi.

121. An asparaginase gene which is under ammonia and oxygen repression. Darryl D. Yorkey, Patricia M. Shaffer. University of San Diego, Chemistry, San Diego, CA, USA.

Aspergillus nidulans has two asparaginase genes, *apnA* (Chrom. II) and *ahrA* (Chrom. VIII). Enzymatic studies demonstrated that the expression of the *ahrA* encoded enzyme is under both ammonia and oxygen repression which is regulated by the positively-acting protein product of the *areA* gene [Shaffer et al. (1988) *Mol. gen. Genet.* **212**, 337-341]. Neither of these genes were able to be cloned by complementation of null mutants. Homology cloning based on two homologous regions present in 13 known asparaginases produced two PCR products which, upon sequencing, yielded the conserved regions. These two are being used to probe the cosmid libraries assigned to Chomosomes II and VIII. The final analysis of this gene is still in progress. The significance of this research is two fold: it provides data on an environmentally regulated gene and the availability of another source of asparaginase used as a cure for childhood acute

lymphoblastic leukemia. This research is funded by a USD Faculty Research Grant and by a grant from the Lee Leichtag Family Foundation

122. The hyper-osmotic stress response pathway of *Neurospora crassa* is the target of phenylpyrrole fungicides. Yan Zhang, Randy Lamm, Christian Pillonel, Jin-Rong Xu, and Stephen Lam, Novartis Crop Protection, Inc., Research Triangle Park, NC 27709.

Osmotic sensitive mutants of *Neurospora crassa* are sensitive to high osmolarity, therefore unable to grow on medium containing 4% NaCl. They are also resistant to dicarboximide and aromatic hydrocarbon fungicides. In this study, osmotic-2 (*os-2*) and osmotic-5 mutants were found to be resistant to phenylpyrrole fungicides. The *Neurospora crassa* pMOcosX library was used in sib-selection to isolate the gene(s) complementing the osmotic sensitive phenotype of *os-2*. A cosmid was identified and mapped to the position of *os-2* on LG IVR. A cDNA clone which encodes a homolog of yeast *HOG1* MAP kinase was used to screen the same cosmid library and the same cosmid was isolated. A subclone of the cosmid containing solely the *HOG1* homolog could functionally complement the osmotic sensitive phenotype of os-2, suggesting the *os-2+* locus encodes a homolog of yeast *HOG1*. The gene would be referred to as the *os-2* gene. Transformants of *os-2* mutants with the *os-2* gene became phenylpyrrole fungicide sensitive, indicating that the hyper-osmotic stress response pathway of *Neurospora crassa* is the target of phenylpyrrole fungicides. Three *os-2* alleles were sequenced by PCR amplification and sequencing of overlapping DNA fragments and mutations in the *os-2* gene have been identified. Gene knock-out and cytological studies are currently underway.

123. The *bmp1* kinase gene is essential for fungal pathogenicity in *Botrytis cinerea*.Li Zheng, Mathew Campbell, Stephen Lam and Jin-Rong Xu, Novartis AgBiotech Research Institute, Research Triangle Park, NC27709.

The PMK1 mitogen-activated protein (MAP) kinase pathway has been recently shown to be important for appressorium formation in the rice blast fungus *Magnaporthe grisea*. Here we tested whether the same MAP kinase is essential for plant invasion processes in the gray mold fungus *Botrytis cinerea* which has different plant infection mechanism . We used a PCR-based approach to isolate MAP kinase homologues from *B. cinerea*. The BMP1 (Botrytis MAP kinase required for Plant infection) MAP kinase is highly homologous to the Magnaporthe PMK1. BMP1 is a single copy gene. The *bmp1* gene replacement mutants have no defect in fungal growth on artificial media. One of the *bmp1* knock-out mutants is reduced in conidiation. All *bmp1* mutants tested lost their pathogenicity on carnation flowers and tomato leaves. When the wild type BMP1 allele was reintroduced into one of the *bmp1* knock-out mutants, all BMP1+ transformants were fully pathogenic as the wild type strains. Further studies with SEM indicated that conidia from *bmp1* mutants could germinate normally on plant surfaces. However, these germinating conidia failed to penetrate through the cuticle and macerate plant surface tissues. *bmp1* mutants also appeared to be defective in infection through wounding. These results indicated that the BMP1 MAP kinase is essential for plant infection in *B. cinerea*. This PMK1/BMP1 pathway may be widely concerved in other plant pathogenic fungi for regulating plant infection processes such as appressorium formation and cell wall degrading enzyme production

124. Localization and expression profiling of genes expressed during appressorium formation on chromosome 7 in *Magnaporthe grisea*. Heng Zhu, Woobong Choi, Ralph A. Dean. Clemson University, Plant Pathology , Clemson, SC, USA.

Magnaporthe grisea is an important model system for the study various aspects of plant-fungal interactions, including the mechanisms regulating induction and formation of the appressorium. In previous studies, a minimum tile containing 41 BAC clones of chromosome 7 was constructed. Hybridization of cDNA from appressorium formation stage to *Hind*III digested minimum BAC tile identified a 128 *Hind*III fragments. These fragments identified 466 cDNA clones from an appressorium stage cDNA library using a two-dimensional probe pooling strategy. The cDNA clones were sequenced and mapped to a 100kb resolution on chromosome 7. 310 of 466 were found to be unique cDNA sequences. Only four had been previously identified in *M. grisea*. The DNA from the unique cDNA clones was double-stamped onto nylon filters, and hybridized to cDNA probes made from 4 developmental stages - appressoria, mycelia, conidia, and germing conidia. Based on expression profiles, cDNA clones were assigned to different developmetal stages. A model assigning putative functions to these newly discovered genes associated with appressorium formation will be presented. **Sexual and Asexual Differentiation Abstracts**

Sexual and Asexual Differentiation Abstracts

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125. *MCH1*, a gene encoding Sch9 homolog kinase of *Magnaporthe grisea*. Kiichi Adachi[1], Martin Urban[2], and John E. Hamer [1]. [1]Purdue University, Biological Sciences, West Lafayette, IN, USA. [2]Monsanto Europe, Belgium.

In the rice blast fungus *Magnaporthe grisea* cAMP signaling pathways seem to control a variety of processes such as vegetative growth, asexual and sexual development and appressorium formation. Molecular genetic analysis of genes encoding adenylate cyclase (*MAC1*), PKA regulatory subunit (*SUM1*) and PKA catalytic subunit (*CPKA*) suggests that cAMP signaling pathways diverge at the level of PKA regulation. Two lines of evidence support this hypothesis. First, *mac1* knockout mutants exhibit pleiotropic defects in growth, conidiation, mating and appressorium formation, whereas *cpkA* knockout mutants have a specific defect only in appressorium penetration. Second, the defects of *mac1* knockout mutants are suppressed by the point mutation in *SUM1*. In order to identify cAMP effectors other than CpkA, we carried out PCR screening using degenerate primers designed to identify conserved subdomains in PKA catalytic subunits. However, we failed to obtain PCR products similar to PKA catalytic subunits other than CpkA. Under less stringent conditions, we identified a PCR product with high homology to the Sch9 kinase of *Saccharomyces cerevisiae*. Sch9 kinase has overlapping functions with PKA catalytic subunits in *S. cerevisiae* but is distinct from typical PKA catalytic subunits. Using the PCR product as a hybridization probe, genomic and cDNA clones of *M. grisea* were isolated and sequenced. *MCH1* (*Magnaporthe* Sch9 homolog 1) encodes 714-amino

acid protein with 47% identity to Sch9 kinase. Results of gene knockout experiments with *MCH1* are in progress and will be presented.

126. Identification of a homeobox gene in the Euascomycete *Podospora anserina*.Sylvie Arnaise, and Robert Debuchy. Universte Paris XI, IGM, Orsay, Essonne, France.

The homeobox-containing genes are widely represented among eukaryotic organisms, nevertheless until now none has been identify in the filamentous ascomycetes. Here we report the discovery of a homeobox gene in the filamentous ascomycete *Podospora anserina*. Assuming conservation of the homeodomain motifs in eukaryotic proteins we performed PCR experiments using degenerate primers corresponding to homeodomain motifs and *P. anserina* cDNA. This gave an amplification product of an expected size of 120 bp. This fragment was used as a probe to screen a *P. anserina* library and allowed us to isolate a 3.9 kb *Pst*I fragment which contains a putative gene with two introns and coding for a protein of 354 aa. This protein displays significant similarities with other homeodomain proteins suggesting that the 3.9 kb fragment contains a homeobox gene. Isolation of the corresponding cDNA indicated that the putative homeobox gene is transcribed and confirmed the position of the introns. To investigate the function of this gene two kinds of mutants were constructed. First the resident gene was deleted by substituting it with a recombinant gene in which the entire coding region was replaced by the bacterial hygromycin resistance gene. Second, to overexpress the gene, we replaced its 5'UTR by the higly expressed glyceraldehyde-3-phosphate dehydrogenase promoter of *P. anserina*. The phenotypes of these two kinds of mutant strains are being investigated and will be presented.

127. Interaction between and transactivation by mating type polypeptides of *Neurospora crassa.* Tom C. Badgett and Chuck Staben. University of Kentucky, Biological Sciences, Lexington, KY, USA.

The polypeptides encoded by the mating type idiomorphs of *Neurospora crassa* control diverse aspects of the fungal life cycle. Biochemical characterization of the MAT a-1, MAT A-1, and MAT A-3 reveal new activities that correlate with important biological activities of the polypeptides. All three polypeptides have domains capable of activating transcription in a yeast reporter system. The transcriptional activation domains of both MAT a-1 and MAT A-1 are not critical for either mating or vegetative incompatibility activities in *Neurospora*. Two hybrid assays establish the ability of MAT a-1 to interact with MAT A-1. Mutations that interfere with this interaction correlate with mutations that eliminate vegetative incompatibility, but not mating, in Neurospora. These results suggest the hypothesis the interaction of MAT a-1 with MAT A-1 stimulates vegetative incompatibility. Two-hybrid interaction screens of Neurospora cDNA libraries with MAT a-1 have identified other polypeptides likely to be important in the various activities associated with the mating type locus.

128. The fluffy gene of *Neurospora crassa* regulates macroconidiation and con gene expression.Lori Bailey Shrode, and Daniel J. Ebbole. Texas A&M University, Plant Pathology & Microbiology, College Station, Texas, USA.

Strains of an otherwise wild type background expressing a *cpc-1-fl* fusion gene were induced to conidiate in minimal medium. Expression of the *fl* fusion gene in the *acon-2* and *acon-3* mutant backgrounds demonstrated that these genes are required for development. However, overexpression of *fl* combined with nitrogen starvation was sufficient to induce conidiophore morphogenesis in the *acon-2* mutant strain. The morphogenesis induced by *fl* overexpression closely resembles typical conidiophore development, however, some conidiation-induced genes were not expressed. *fl* expression was always found to be sufficient for expression of *eas*. However, high level expression of *con-6* and *con-10* was only observed in the wild type strain background and then only with growth conditions that normally induce conidiation and *con-6* and *con-10* expression. Thus, elevated *fl* expression is sufficient to induce conidiophore morphogenesis and *eas* expression, but is not sufficient to induce *con-6* and *con-10* expression.

130. Neurospora mating pheromone precursor genes. Piotr Bobrowicz[1], Rebecca Lowe[2], Amy Miller[1], Wei-Chiang Shen[1], Pam Kazmierczak[1], Lori Bailey Shrode[1], Daniel J. Ebbole[1], Deborah Bell-Pedersen[2], and Neal K. Van Alfen[1]. [1]Texas A&M; University, Plant Pathol. & Microbiol, College Station, Texas, USA. [2]Texas A&M; University, Biology, College Station, Texas, USA.

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

131. A Tc1-like Transposable Element in *Coccidioides immitis*.Michael A. Bolaris[1], Tao Peng[2], Kris I. Orsborn[2], John N. Galgiani[2], and Marc J. Orbach [1].[1]University of Arizona, Plant Pathology, Tucson, Arizona, USA. [2]VA Medical Center, Infectious Diseases, Tucson, Arizona, USA.

We report the isolation, characterization and distribution of a Tc1-like transposable element in *Coccidioides immitis*, a dimorphic fungal pathogen of mammals known as the Valley Fever fungus. During the analysis of a Proline Rich Antigen (PRA) gene which is being used for vaccine development, a Tc1 transposable element was discovered upstream of the PRA open reading frame (ORF). A complete copy of this element has been cloned from the Silveira strain of *C. immitis* and designated Tcim1 for **t**ransposon ***C. im****mitis* **1**. The transposon is approximately 1.8 kb in size, and contains a single open reading frame with homology to the Tc1 class of transposases. The transposase ORF is interrupted by two introns. Inverted terminal repeats of 29 bp are present at the ends of this transposon, a characteristic of this class of element. DNA hybridization analyses have shown variation in the copy number among strains of

C. immitis, ranging from no copies to eight copies. Comparisons of Tcim1 banding patterns between Arizona and California isolates will be presented. Preliminary results suggest that there may be different intensities of hybridization among these strains and this may represent sequence divergence as a result of geographic isolation. This is the first transoposon isolated from this human pathogen and may be a useful tool for typing strains and for the study of *C. immitis* populations. Very little research has been conducted with field isolates of *C. immitis*, with most strains used in research being clinical isolates. We are using a PCR-based method to identify *C. immitis* soil isolates, to compare their Tcim1 profiles to those of clinical isolates.

132. Cloning and characterisation of an *Aspergillus nidulans abaA* homologue in the dimorphic fungus *Penicillium marneffei*. Anthony R. Borneman, Michael J. Hynes, and Alex Andrianopoulos. University of Melbourne, Gentics, Parkville, Victoria, Australia.

Penicillium marneffei like many other pathogenic species of fungi is dimorphic. At 25 C, *P. marneffei* exhibits filamentous growth morphology, reproducing through the production of asexual spores borne on conidiophores. At 37C a yeast-like growth form replaces the filamentous morphology with single cells reproducing via fission. This form is pathogenic, and is associated with many cases of human and animal infection. Here we describe the cloning and characterisation of a *P. marneffei* gene which encodes an ATTS DNA binding domain protein - *PmabaA*. In *Aspergillus nidulans*, *AbaA* (an ATTS protein) regulates development of sporogenous cells during conidiation. In *Saccharomyces cerevisiae*, Tec1p, an *AbaA* homologue regulates pseudohyphal development. This implies a conserved role for ATTS proteins in regulating fungal development systems. A *P. marneffei* homologue may also have a regulatory role during development in this species, perhaps during dimorphic growth. *PmabaA* was therefore cloned via low stringency hybridisation to the *A. nidulans abaA* gene. A high level of homology between the two is apparent at the protein level, with complete conservation of the DNA binding motif. Amino acid conservation is limited at the C-termini of the proteins and may suggest functional divergence in the *P. marneffei* homologue however it is able to complement the *abaA* mutation in *A. nidulans* indicating functional conservation. A transformation protocol for this fungus was developed and enabled the creation of a *PmabaA* mutant by targeted deletion. The aconidial mutant phenotype at 25C indicates that *PmabaA*, like its *A. nidulans* homologue plays a crucial role in regulating cellular morphogenesis. The cloned *abaA* genes from both *P. marneffei* and *A. nidulans* complement the mutant phenotype, indicating functional interchangeability between the two proteins *in vivo*.

133. Two fungal galectins: their expression, localization and secretion during fruitbody development. Robert P. Boulianne[1], Ursula Kües[1], Benjamin C. Lu[2], and Markus Aebi[2]. [1]ETH, Institut für Mikrobiologi, Zurich, Zurich, Switzerland. [2]University of Guelph, Molecular Biology and Gen, Guelph, Ontario, Canada.

Fruitbody development in *Coprinus cinereus* requires a transition from a linear radiating pattern of hyphal growth to the development of multiple hyphal:hyphal interactions that comprise the fruitbody. These initial stages of fruitbody morphogenesis occur during knot, initial and primordial stages of development. We isolated two fungal galectins, a pair of low molecular weight lectins that specifically bind beta-galactose residues and their derivatives. We show that these proteins are essentially fruitbody specific proteins and their expression is differentially

regulated with respect to light, nutrition and the presence of compatible *A* mating type genes. The dimeric galectins are believed to mediate hyphal:hyphal interactions, since they are present within the cell walls and the extracellular matrix. While it is clear that galectins are secreted, they lack typical secretion signal sequences and are not glycosylated, even though potential N-linked glycosylation sites are present. In order to test whether galectins are secreted independently of the classical secretory pathway, we examined the secretion of galectins when expressed (heterologously) in *Saccharomyces cerevisiae*. The *Coprinus* galectins were secreted to the cell wall in yeast. In mutants blocked in the transport of proteins from the ER to the Golgi (sec18) the galectins were still found extracellularly. These results show for the first time that a filamentous fungal protein can be secreted independently of the classical secretory pathway.

134. Elucidating the role of linoleic acid in Aspergillus development. Ana M. Calvo-Byrd[1], Lori L. Hinze[1], Harold W. Gardner[2], and Nancy P. Keller[1]. [1]Texas A&M; University, Plant Pathology and Micro, College Station, Texas, USA. [2] USDA-ARS, NCAUR, Peoria, Illinois, USA.

Development of conidia and overwintering bodies (cleistothecia and sclerotia) of *Aspergillus nidulans, Aspergillus flavus* and *Aspergillus parasiticus* is affected by linoleic acid and light. Specific morphological effects of linoleic acid include inducing precocious and increased asexual spore development in *A. flavus* and *A. parasiticus* strains and altered sclerotial production in some *A. flavus* strains where sclerotial production decreased in the light but increased in the dark. In *A. nidulans*, the asexual to sexual spore ratio increased with increasing amounts of linoleic acid. Spore development was also induced in all three spp. by hydroperoxylinoleic acids, linoleic acid seed-derivatives produced during fungal colonization. The sporogenic effects of these linoleic compounds on *A. nidulans* are similar to the sporogenic effects of *A. nidulans* psi factor, an endogenous mixture of hydroxylinoleic acid moieties. Light significantly increased asexual production in all three spp. The sporogenic effects of light, psi factor, linoleic acid and linoleic acid derivatives on *A. nidulans* required an intact *veA* gene. To better study the function of linoleic acid in Aspergillus development, the gene required for the formation of linoleic acid, *odeA*, that encodes a delta-12 desaturase, has been cloned and disrupted in *A. nidulans veA+* and *veA1* strains. A better understanding of the molecular mechanisms governing *Aspergillus* lipid metabolism could contribute to the design of control strategies to reduce the survival and spread of seed-colonizing aspergilli.

135. Analysis of white collar 2 mutants reveals a central component of the Neurospora circadian clock and distinct mechanisms regulating frequency in light and dark. Mike A. Collett, Jay C. Dunlap, and Jennifer J. Loros. Dartmouth Medical School, Dept of Biochemistry, Hanover, NH, USA.

White collar 2 (wc-2), encoding a PAS domain containing, Zn-finger transcription factor, is essential for almost all blue light responses in *Neurospora*. It was predicted that *wc-2* would be a component of the *Neurospora* circadian clock, acting by increasing levels of the central clock component, *frequency* (Crosthwaite et al., Science 276: 763-769, 1997). To test this we examined the effect on the clock of several *wc-2* alleles thought to be partially functional. It was expected that if *wc-2* was a clock component the partially functional *wc-2* alleles might yield an altered period length as well as lowered levels of *frq* transcript and protein in constant darkness.

Indeed, compared to wild type, strains containing one of these partially functional alleles, *wc-2* (ER24), displayed increased period length, reduced temperature compensation and reduced levels of *frq* mRNA and FRQ protein in constant darkness. We also examined photoinduction of *frq* transcript, which in a wild-type strain is robustly induced in response to light, in these mutant strains. In either constant light, or after a light pulse, strain *wc-2* (ER24) displayed *frq* mRNA and protein levels virtually identical to wild type. However, presumptive loss of function alleles of *wc-2* had reduced levels of *frq* mRNA under all conditions tested. These data suggest that *wc-2*'s function in *frq* regulation is essential and limiting in constant darkness. However, although *wc-2* is required for full *frq* mRNA induction by light, it is not limiting in the light signal transduction pathway leading to *frq* transcription.

136. Vegetative coexpression of the mating-type genes determining nuclear identity is lethal in *Podospora anserina*. Evelyne Coppin and Robert Debuchy. Université Paris Sud - CNRS URA 2225, Batiment400, Orsay, 91405, FRANCE.

The *mat+* and *mat-* mating types control a recognition process between cells during fertilization and between nuclei after fertilization, when reproductive male and female nuclei which have divided in a common cytoplasm form a mat+/mat- pair within the ascogenous hyphae. Four genes encoding transcriptional factors have been characterized, *FPR1* in the *mat+* locus, *FMR1*, *SMR1* and *SMR2* in the alternative *mat-* locus. Fertilization is controlled by *FPR1*and *FMR1* while internuclear recognition inside the fruiting body is controlled by *FPR1* which confers the mat+ nuclear identity and *FMR1* and *SMR2*which confer the mat- nuclear identity.*SMR1* would be necessary to restore growth of ascogenous hyphae after the nuclei have paired. By using the RT-PCR method, mature transcripts of the four *mat* genes were detected in the fertilized female organs whereas only *FPR1* and *FMR1* transcripts were detected in vegetative mycelium. Increased or induced vegetative expression of the four *mat* genes by replacing their natural promoter by the constitutive *Aspergillus nidulans gpd* promoter has no vegetative effect when the recombined gene is alone in the wild-type strain. However, *mat+* ascospores carrying both a *gpd::FMR1* fusion and a constitutively transcribed *SMR2* transgene are unable to germinate. Ascospore lethality is suppressed by introduction of the *gpd::SMR1* fusion. This suggests that the vegetative coexpression of the three nuclear identity genes triggers the recognition mechanism at an inappropriate time and thus interferes with some essential growth process and this confirms the role of *SMR1* in growth restoration following internuclear recognition.

137. Addressing clonality in *Magnaporthe grisea* using vegetative compatibility. James C. Correll[1], Tyler L. Harp[1], Fleet N. Lee[1], and Robert S. Zeigler[2]. [1]University of Arkansas, Plant Pathology, Fayetteville, AR, USA. [2]Kansas State University, Plant Pathology, Manhattan, KS, USA.

Spontaneous nitrate nonutilizing (nit) and sulfate non-utilizing (sul) mutants were recovered from *P. grisea* and used to characterize a collection of archived and contemporary rice-infecting field isolates into vegetative compatibility groups (VCGs). Pairing of certain phenotypically distinct nit mutants yielded robust heterokaryons as did certain nit x sul pairings. However, some cross-feeding was evident in some nit x sulpairings indicating that caution should be exercised in the interpretation of such reactions. Four distinct VCGs were identified among archival and contemporary rice-infecting isolates of *P. grisea* collected from Arkansas, Texas, Louisiana, and

California. Each VCG corresponded with a distinct MGR586 group (or lineage) among the contemporary isolates in all but one case; VCG US-004 contained contemporary isolates collected from Arkansas belonging to MGR586 group D and contemporary isolates recovered from California in 1997 which belonged to group H. VCG US-004 also contained two isolates recovered from Texas and Arkansas in the mid 1970's which belonged to MGR586 group H. The data indicate that distinct barriers to vegetative compatibility are present in *P. grisea* and that certain VCGs may represent genetically distinct populations in Arkansas. However, the impact vegetative compatibility barriers have on horizontal gene transfer in *P. grisea* is unknown. A preliminary genetic analysis of vegetative compatibility with the sexually compatible strains Guy11 and 2539 of *P. grisea* indicated that multiple vegetative incompatibility (vic) loci were present. The use of VCGs may be very helpful in characterizing the population structure of this important pathogen worldwide.

138. An efficient genetic system for analysis of *Gibberella circinata.* Sarah F. Covert, Angela Briley, and M. Margaret Wallace. University of Georgia, Forest Resources, Athens, GA, USA.

Genetic analysis of *Gibberella circinata*, the causative agent of pitch canker disease of pines, has been thwarted by a low frequency of mating success in the laboratory. We describe two findings which should facilitate genetic analysis of this fungus and related species. First, we determined that previously described degenerate primers can be used to amplify a portion of the MAT-2 mating type gene from *G. circinata*. This led to cloning and sequencing of a fragment of the MAT-2 gene, which in turn made it possible to distinguish between *G. circinata* isolates of opposite mating types either by hybridization or PCR amplification. Second, we discovered that out of the 18 *G. circinata* isolates in our collection, the one female fertile isolate expressed its fertility at 20°C, but not at 25°C, the temperature typically used for crossing the closely related *Gibberella fujikuroi* mating populations. It is evident, therefore, that when sexual reproduction in other closely related species is initially being investigated, the crosses should be established at a variety of temperatures. Once we learned that female fertility in this *G. circinata* isolate was expressed at 20°C, a high frequency of mating success was achieved. Eleven out of seventeen isolates crossed successfully with this isolate. PCR analysis indicated that five of the six non-mating isolates were of the same mating type as the female fertile isolate, and thus expected to be incompatible with it.

139. cAMP-dependent neutral trehalases catalyze intracellular trehalose breakdown during spore germination in *Aspergillus nidulans* and *Neurospora crassa.* Christophe d'Enfert [1], Beatriz M. Bonini[2], Pio D.A. Zapella[3], Thierry Fontaine[4], Marie-Kim Chaveroche[1], Aline M. da Silva[3], and Hector F. Terenzi[2]. [1]Institut Pasteur, Physiologie Cellulaire, Paris, , France. [2]Universidade de Sao Paulo, Departamento de Biologia, Ribeirao Preto, SP, Brazil. [3]Universidade de Sao Paulo, Departamento de Bioquimic, Sao Paulo, SP, Brazil. [4]Institut Pasteur, Laboratoire Aspergillus, Paris, France.

A cAMP-activable Ca^{2+} -dependent neutral trehalase was identified in germinating conidia of *Aspergillus nidulans* and *Neurospora crassa*. Using a PCR approach, *Aspergillus nidulans* and *Neurospora crassa* genes encoding homologues of the neutral trehalases found in several yeasts were cloned and sequenced. Disruption of the *AntreB* gene encoding *A. nidulans* neutral trehalase revealed that it is responsible for intracellular trehalose mobilization at the onset of

conidial germination and that this phenomenon is partially involved in the transient accumulation of glycerol in the germinating conidia. While trehalose mobilization is not essential for the completion of spore germination and filamentous growth in *A. nidulans*, it is required to achieve wild-type germination rates under carbon limitation suggesting that intracellular trehalose can partially contribute the energy requirements of spore germination. Furthermore, it was shown that trehalose accumulation in *A. nidulans* can protect germinating conidia against an otherwise lethal heat shock. Since transcription of the treB genes is not increased following a heat-shock but induced upon heat-shock recovery, it is proposed that, in filamentous fungi, mobilization of trehalose during the return to appropriate growth is promoted by transcriptional and post-translational regulatory mechanisms, in particular cAMP-dependent protein kinase-mediated phosphorylation.

140. A molecular dissection of signaling cascades regulating mating and virulence in *Cryptococcus neoformans*. Robert C. Davidson, and Joseph Heitman. Duke University, Genetics, Durham, NC, USA.

Recent findings reveal that a pheromone induced mating pathway operates in the human fungal pathogen *Cryptococcus neoformans*; similar to the mating pathway characterized in *Saccharomyces cerevisiae*. Mating in *S. cerevisiae* is mediated by pheromones recognized by membrane bound receptors which trigger a MAP kinase cascade, culminating in transcription of mating response genes. We have cloned a gene, *CPK1*, which encodes a homolog of the *FUS3/KSS1* MAP kinases in the pheromone response pathway of *S. cerevisiae*. In contrast to several other homologs of the mating response pathway found in *C. neoformans*, which are mating type specific, the *CPK1* gene is present in both *MATa* and *MATalpha* cells. This indicates that the gene is not located within the *MATalpha* locus that contains the *MFalpha1* and *MFalpha2* pheromone genes, and the *STE11* and *STE12* homologs. Furthermore, this observation suggests that at least this element of the signaling cascade is shared by the two mating types. Disruption of the *CPK1* gene by homologous recombination reveals that the *CPK1* MAP kinase is required for mating in both mating types and is required for *MATa* cells to autorespond to the *MFalpha1* pheromone. This finding suggests that *MATa* cells may contain divergent homologs of *STE11* and *STE12*involved in mating. In addition a *MATalpha cpk1* mutant strain is defective in haploid fruiting. The *cpk1* defect in haploid fruiting is suppressed by overexpression of *CnSTE12*, indicating that *CPK1* functions upstream of *STE12* in this cascade. Furthermore, conserved MAP kinases play an essential role as virulence factors in pathogenesis of the rice blast fungus *Magnaporthe grisea* and the human pathogen *Candida albicans*, and *CPK1* may play a similar role in *C. neoformans*.

141. Nuclear positioning model and internuclear recognition in *Podospora anserina*. Robert Debuchy. Université Paris-Sud, IGM Bâtiment 400, Orsay, France, France.

In heterothallic Euascomycetes, fertilization is followed by mitotic divisions of parental nuclei, resulting in a plurinucleate stage. Nuclei of opposite mating type then recognize one another and form dikaryons which undergo karyogamy and meiosis within ascogenous hypha. Genetic analysis indicated that internuclear recognition (IR) in *P. anserina* is controlled by mating-type genes. These genes encode transcription factors which have nucleus-limited expression. This allows nuclei with different mating type to express a pattern of specific proteins directly involved

in IR. As the molecular nature of these proteins is unknown, the exact mechanism of internuclear recognition remains elusive. Schuurs *et al*. (1998) have proposed that internuclear distance affects gene expression through a pheromone/receptor system in *Schizophyllum commune*. A similar system is proposed to control IR in *P. anserina*. Each nucleus controls the expression and localization of specific pheromones and receptors for the opposite mating-type pheromone in a portion of the wall and membrane respectively. When two compatible nuclei are close enough to superimpose their specific domains, this permits the binding of pheromones to the responsive receptors and generates a spatial cue.This cue triggers the formation of the ascogenous hypha and the migration of the two compatible nuclei inside the budding ascogenous hypha. Genetic data suggest that the genes controling IR could also control degradation systems which help the nuclei to preserve their domain from contamination by compatible pheromones. Schuurs TA, Dalstra HJP, Scheer JMJ and Wessels JGH. 1998. Fung. Genet. Biol. 23: 150-161.

142. Interfertility among self-sterile strains of *Glomerella graminicola*. Meizhu Du[1], Juan Wang[2], Robert Hanau[2], and Lisa Vaillancourt[1]. [1]University of Kentucky, Plant Pathology, Lexington, KY, USA. [2]Purdue University, Botany and Plant Patholog, West Lafayette, IN, USA.

Although the ascomycete *Glomerella graminicola* was originally described as homothallic, self-sterile but cross-fertile strains are common. Furthermore there appear to be multiple mating types among these cross-fertile isolates. The mechanism of this unusual mating system is unknown, but it is proposed that it involves complementary mutations in the developmental pathway for self-fertility. Analysis of random spore and tetrad progeny from a cross of two self-sterile isolates of *G. graminicola* indicated that sexual compatibility between these strains is controlled by two loci. One of the loci appeared to confer self-nonself recognition, a characteristic reminiscent of the mating type idiomorphs that regulate interfertility in heterothallic ascomycetes such as *Neurospora crassa* and *Cochliobolus heterostrophus* A degenerate PCR approach designed to amplify a DNA fragment which is highly conserved among ascomycete mating type idiomorphs was performed using the two *G. graminicola* isolates. The conserved DNA fragment was present in both of the compatible self-sterile isolates of *G. graminicola* , suggesting that mating type idiomorphs are not primarily responsible for cross-fertility between these strains. Experiments are currently underway to determine whether the conserved sequence is at the same locus in both strains, and also to evaluate the distribution of this sequence among self-sterile and self-fertile strains of *G. graminicola*

143. Molecular links between morphogenesis and pathogenicity in *Ustilago maydis*. Flora Banuett. Department of Biochemistry and Biophyscs. University of California. San Francisco, CA 94143.

Ustilago maydis, the causal agent of corn smut disease, exhibits three basic cell morphologies during its life cycle: 1) a unicellular haploid form, 2) a filamentous dikaryotic form, and 3) a round cell type, the teliospore. The teliospore, which exhibits no vegetative growth, undergoes meiosis and gives rise to the unicellular haploid form. The unicellular form is cigar-shaped, grows by budding, and is not pathogenic. Haploids of different mating type fuse to give rise to the filamentous dikaryon.This form is pathogenic and is characterized by cylindrical cells separated by septa. It undergoes a discrete developmental pathway in the plant resulting in formation of round teliospores, which are surrounded by a specialized cell wall resistant to

adverse environmental conditions. Genetic programming by the mating type loci (a and b), cell-cell signalling, plant signalling, and environmental conditions govern these morphological transitions. I am interested in the molecular mechanisms that govern morphology of the different forms in *U. maydis* and how the machinery responsible for maintenance of shape affects pathogenicity. In order to identify the machinery responsible for morphology, I have taken a multiprongued approach: isolation of morphological mutants and a candidate gene approach. A mutation that alters cell shape and the developmental program in the plant has led to the identification of a gene with similarity to samB of *A. nidulans*. This and other results will be described.

144. Biogeographical relationships and microevolutionary patterns in the *Tricholoma matsutake* species complex. Matteo Garbelotto and Ignacio H. Chapela. Department of Environmental Science, Policy and Management, University of California, Berkeley, CA, USA.

Matsutakes, a taxonomically challenging complex of related Tricholoma species, are intensively harvested in the Northern Hemisphere as valuable non-timber forest products. Ribosomal DNA sequencing and Amplified Fragment Length Polymorphism (AFLP) analyses were employed to search for a clarification of (a) species delimitation, (b) biogeographical relationships, and (c) evolutionary patterns within this group. Twenty-five taxa, traditionally regarded as very close relatives, fell into one of two sister clades, one including the commercial matsutakes, and one including species from the *zelleri-focale-robustum* complex. We find that species complexes in both clades appear to have their origin in an angiosperm-related ectomycorrhizal symbiosis, and that tracking of hosts in evolving forests in North America and Eurasia led secondarily to a conifer association. Commercial matsutakes are all part of a recently derived, conifer-associated lineage. Sequencing and AFLP data indicate that this lineage contains natural groupings which are not consistent with either accepted taxonomic arrangements nor with the market's price-structure: Eurasian matsutakes are vicariantly related to Eastern North American and Mexican fungi but surprisingly not to populations from the North American Pacific North West. The North American continent, by including all three commercial taxa, is the likely region of speciation for the complex. This study elucidates evolutionary relationships among different matsutakes species, and suggests a model for their present-day distribution and population structure. We interpret the new understanding of the systematics in this narrow group as an expression of a dynamic geographic mosaic of ectomycorrhizal forests in North America, where Eocene climatic changes led to a confrontation, and then separation, of interacting fungal populations in angiosperm and coniferous hosts.

145. Sexual development of *Aspergillus nidulans* tryptophan auxotrophic strains. Sabine E. Eckert and Gerhard Braus. Univ. of Goettingen, Molecular Microbiology, Goettingen, Niedersachsen, Germany.

A study on the interplay between sexual development and tryptophan biosynthesis in the ascomycete *Aspergillus nidulans* was initiated. To elucidate the connection, strains were examined which are unable to regulate their tryptophan biosynthesis and are dependent on external supply. Therefore, the auxotrophic mutants *trpA, trpB, trpC* and *trpD* were tested for growth and differentiation. These strains which carry mutations in the structural genes for the biosynthetic enzymes of each step in the formation of tryptophan from chorismate, are unable to

form fruitbodies on medium containing low tryptophan concentrations. We show that cleistothecia formation can be restored by high tryptophan supplementation. Cleistothecia formation of the *trpA* and *trpD* strains can also be restored by high concentrations of either the tryptophan precursors anthranilate and indole or auxin. Fertilty of ascospores of the strains can only be restored to about one hundredth of the wild-type fertility. Oversupplementation with tryptophan inhibits cleistothecia formation, but not conidiation. Tryptophan supplementation was found to result in auxin production of *A. nidulans. trpC* transcript levels and enzyme activities remain stable during development whereas transcript levels and enzyme activities of another biosynthetic gene as control are reduced. We conclude an extensive connection between a balanced tryptophan biosynthesis and sexual development in *Aspergillus nidulans* .

146. Identification of mating regulated cDNAs in *Phytophthora infestans.* Anna-Liisa Fabritius, and Howard S. Judelson. University of California, Plant Pathology, Riverside, California, USA.

Sexual development in the heterothallic oomycete, *Phytophthora infestans*, is a complex process that involves the differentiation of male and female gametangia, meiosis, fertilization, and formation of a diploid sexual spore called the oospore. To study molecular events underlying this development, we have compared gene expression between mating and vegetative cultures. The suppression subtractive hybridization technique was used to obtain cDNAs that showed differential gene expression. Subtracted cDNA libraries of 7500 to 8000 clones were constructed from mating and non-mating cDNAs, and screening for differential gene expression is underway. In preliminary screening, three genes were identified that are up-regulated and two which are down-regulated during mating as suggested by Northern blot analysis; additional clones are currently being characterized. Also, two cDNAs were cloned that were expressed in either A1 or A2 mating type strains used in this study but were not present in the genomic DNA. The origin of these cDNAs may be extrachromosomal and is currently being investigated. Sequencing of differentially expressed cDNAs suggested that one of the down-regulated cDNAs is very similar to sorbitol dehydrogenases ($P <= 1.4 \times 10^{-15}$). All other cDNA clones represent novel genes since no significant matches were found in databases. To study the structure and function of the corresponding genes, genomic clones have been isolated from the bacterial artificial chromosome (BAC) library and subcloned

147. The cyclin homologue *PclA*: A new regulatory protein for asexual development of *Aspergillus nidulans*. Niklas Schier, Ralf Liese and Reinhard Fischer. Max-Planck-Institut für terrestrische Mikrobiologie, Karl-von-Frisch-Str., D-35043 Marburg, Germany.

Cyclins comprise a family of proteins, which interact with cyclin-dependent kinases (CDK), the key regulators of the eukaryotic cell cycle. Here, we show that a yeast Pcl1 homologue, named *PclA*, is involved in the regulation of asexual development of the filamentous fungus *Aspergillus nidulans*. A new developmental mutant derived from insertional mutagenesis was genetically and phenotypically characterized. The mutant produces multiple layers of phialides and thus resembles the abaA mutant. It fails to produce chains of conidiospores. Vegetative growth and sexual development is not affected in the mutant. The mutation was mapped to linkage group VIII and the mutant phenotype was complemented by transformation with a chromosome VIII specific cosmid library of A. nidulans. A 2.1 kb EcoRI-fragment complemented the mutation in

trans and hybridized to a 2.3 kb transcript in RNA extracted from vegetative and developmental cells. The ORF of the cDNA encodes a putative 420 aa protein, which is 34 % identical to the yeast cyclins Pcl1 and Pcl2 (279 aa and 308 aa in length). An N-terminally truncated PclA derivative (271 aa) was sufficient for rescuing the mutant phenotype. Determination of the *pclA1* mutation revealed a Protein kinase C phosphorylation site to be essential for the biological function of PclA. These results suggest a cyclin-dependent phosphorylation cascade triggering the late stages of asexual development of *A. nidulans*.

148. Determining fungal phylogenies by the use of microsatellite markers. Matthew C. Fisher, and John W. Taylor. UC Berkeley, Plant Biology, Berkeley, CA, USA.

Uncovering the correct phylogeny of closely related fungal species requires data from multiple gene genealogies. However, this requires considerable investment in sequencing or SSCP strategies because polymorphisms are rare and taxon sampling needs to be as inclusive as possible. An alternative to using sequencing strategies is the use of short tandem repeats (STRs, or microsatellites) as phylogenetic markers. These markers are highly polymorphic, theoretically enabling the determination of recently evolved lineages, and easily scored. However, a concern in their use in phylogenetic reconstruction is the potential for constraints on allele sizes to occur, resulting in homoplastic distributions of alleles. Here, we report the development of a panel of microsatellites from the pathogenic fungus *Coccidioides immitis* and analyze the mutational processes that lead to the patterns of genetic diversity seen in this species complex.

149. Population level genetic differences observed between geographically distant populations of *Ustilago maydis*. James R. Garton. University of Minnesota, Plant Biology, St. Paul , MN, USA.

Genetic analysis of *Ustilago maydis* populations shows differences in the structure of these populations. Analysis was performed with RFLP probes generated by a partial restriction digest of *U. maydis*. Fungal isolates from Minnesota, Illinois, Nebraska, North Carolina, Ohio, and Uruguay were analyzed with these probes. Data gathered in this analysis shows that populations separated by greater geographical distances were also less similar to each other genetically. This preliminary data suggests that gene flow does not occur at a high level in this pathogenic species.

150. Small GTPases and cytoskeletal organization at the mating of Schizophyllum.Markus Gorfer, Marjukka Uuskallio, and Marjatta Raudaskoski. Department of Biosciences, University of Helsinki, Helsinki, Finland.

Homobasidiomycetes, such as *Schizophyllum commune,* respond to potential mating partners by recognizing specific pheromones secreted by cells of opposite mating-type. Recently it has been shown that the pheromones are recognized by serpentine 7TM receptors probably linked to a heterotrimeric G-protein. The perception of the pheromone signal leads to reciprocal exchange and migration of nuclei between the mates, which implies hyphal fusions, septal break-down and reorganization of cytoskeletal components. In animal and yeast cells small GTPases, such as the products of the rho-, ras- and cdc42-gene families are known to affect the cytoskeletal organization. We have used the polymerase chain reaction to isolate members of these gene families from *S. commune*. The cloned PCR- products were further used for screening the cDNA

library prepared from 8 h mating between compatible strains, at which time the expression of the pheromone and receptor encoding genes is high. This procedure has led us to isolate cDNAs of a rho and ras gene from *S. commune* highly homologous with *Saccharomyces cerevisiae RH03* and *Neurospora crassa ras2* genes, respectively. The Northern hybridization revealed an increase in the *rho3* but not in the *ras2* expression at the mating in comparison with that in the nonmated homokaryons. A genomic clone of *rho3* was isolated and mutagenized at amino acids corresponding to the mutations G12V and T17N in p21 to create an constitutively activated GTP-bound and a dominant negative GPD-bound form of *rho3*, respectively. Effects of expression of these mutant *rho3*-alleles in a wild-type background should allow the elucidation of RHO3-function in signal transduction and cytoskeletal organization at the intercellular nuclear migration during mating of compatible homokaryons of *S. commune*.

151.Characterization of two *swo* mutants involved in polarity establishment and maintenance in *Aspergillus nidulans*. Debra L. Haas and Michelle Momany, University of Georgia, Athens, GA, 30602 .

The germination of asexual propagules of *Aspergillus nidulans* employs two distinct growth modes: isotropic growth and polarized growth. When spores of *A. nidulans* initiate germination, they first grow isotropically adding cell wall material uniformly in every direction. Later they switch to polarized growth, with new wall components added to the tips of emerging germ tubes. This polarized growth continues via apical extension and results in hyphae and ultimately a vegetative colony. We previously screened for mutants defective in the germination process and identified 8 non-allelic mutants, *swo*A-H. (*swo* for swollen phenotype) Here we describe the initial characterization of 2 *swo* mutants, *swo*D and *swo*F. The kinetics of germination and septation of *swo*D and *swo*F mutants, as well as the assignment of linkage groups and initial cloning of the *swo*D and *swo*F genes are presented.

152. The *nsdD* gene encoding a putative GATA type transcription factor regulates positively the sexual development of *Aspergillus nidulans*. Kap-Hoon Han, and Dong-Min Han. Wonkwang University, Division of Life Science, Iksan, Chonbuk, South Korea.

The *nsdD* (*n*ever in *s*exual *d*evelopment) gene encodes a putative GATA type transcription factor that has a type IVb zinc finger DNA binding domain at the C-terminus. Null mutants that the total *nsdD* ORF was deleted showed the typical NsdD phenotype. However, being cultured for a week or more after inoculation onto the center of the plate, a few of cleistothecia bearing mature ascospores could be found in null mutant strains. On the other hand, the *nsdD19* mutant strain which contains a nonsense mutation within the *nsdD* coding region never developed cleistothecia at all under the same conditions, suggesting a dominant negative effect of the truncated NSDD proteins. When NSDD was over-expressed under the *niiA* promoter with nitrate, the number of cleistothecium was dramatically increased, implying a positive regulatory role of NSDD on sexual development. Northern blot analysis using total RNAs from a wild-type strain grown in minimal media revealed that the *nsdD* gene was expressed throughout the life cycle. However, its expression was repressed under the certain conditions, such as in the presence of high concentrations of salts, under which sexual development was inhibited. It was also found that the phenotypes of reduced or delayed sexual development in *veA1* or *nsdB5* could be suppressed when multiple copies of the *nsdD* gene are present, or the gene is over-expressed.

153. Reactive oxygen species and development in *N. crassa.* Wilhelm Hansberg, Fernando Lledías, Adelaida Díaz, and Pablo Rangel. Universidad Nacional Autónoma de México, Biochemistry, México, D.F., Mexico.

Since we have demonstrated specific enzyme oxidation under cell differentiation and stress conditions, we asked if antioxidant enzymes like catalases were also vulnerable to in vivo alteration by reactive oxygen species. Cat-1 is present in the whole vegetative life-cycle of *N. crassa.* Its specific activity increases step wise with each morphogenetic transition of the conidiation process. Cat-1 was modified in these transitions and under stress conditions. Purified Cat-1 was oxidized through a sequential reaction of the four monomers with singlet oxygen, giving rise to five active catalase conformers with increasingly acidic isoelectric points, similar to the ones observed in vivo. Cat-1 was modified with a pure source of singlet oxygen and modification was hindered by reducing agents or singlet oxygen scavengers. Cat-1 modification was traced to the heme. Heme molecular mass and asymmetry increased with modification. Bacterial, fungal, plant, and animal catalases were susceptible to modification by singlet oxygen. Reaction with singlet oxygen could explain in part the variety of catalases in several organisms and modifications detected in some catalases. Modification of catalases during development and under stress could indicate in vivo generation of singlet oxygen. The oxidation of Cat-1 was used to monitor generation of singlet oxygen during germination of conidia. Oxidation of total protein increased within 10 min of germination and was correlated with light intensity. Oxidized Cat-1 appeared during germination in relation to light intensity and correlated inversely with the amount of carotenes with eleven or more conjugated double bonds. Our results indicate that, as soon as conidia are hydrated, Cat-1 was oxidized, oxidized enzyme disappeared and Cat-1 was synthesized de novo; changes were more apparent in the carotene mutant strains than in the wild type. Acknowledge: supported in part by grants IN206097 and IN208994 from DGAPA, Universidad Nacional Autónoma de México and grant 2246P-N9508 from Consejo Nacional de Ciencia y Tecnología, México.

154. Mating type genes of *Heterobasidion annosum*, S-type. Nils O S. Högberg, and Tom Bruns. UC Berkeley, Plant & Microbial Biology, Berkeley, CA, USA.

Heterobasidion annosum (Fr.) Bref. is a heterothallic bipolar basidiomycete fungus with a large number of alleles at the mating type locus. It forms a species complex with several intersterility groups. The S-group of *H. annosum* is a pathogen of spruce and fir. The mating type locus in this species include homeodomain regions that could be amplified with primers based on homology with sequences from other basidiomycete mating types. The primer sequences were biased towards species in the genera of Coprinus and Schizophyllum. Nevertheless, the sequences obtained from *H. annosum* are very different from other basidiomycetes and more related to homeodomain sequences from various animal phyla. They co-segregate with the mating type. The results will be used for studies of the evolutionary history of the *H. annosum* species complex. The work is funded by a grant from the STINT-foundation.

155. Filamentous growth in a dimorphic fungus: Characterization of genes associated with the switch. Wei Hong[1], David G. Smith[1], Long Wang[2], Kevin Smith[1], and Michael H. Perlin[1]. [1]University of Louisville, Department of Biology, Louisville, KY, USA. [2]University of California, Neurosciences, San Francisco, CA, USA.

Many fungal pathogens utilize a switch between a yeast-like form and a filamentous form as an integral part of their overall strategy of disease production. *Microbotryum violaceum* is a fungal plant pathogen that infects over 200 host species in the carnation family (Caryophyllaceae), producing its spores in the anthers of infected plants. However, individual isolates are host-limited and can only infect one or a few host species. One important aspect of the infection process is that haploid yeast-like cells of opposite mating-type must conjugate and then, receiving a signal (normally host-derived) must differentiate to grow as dikaryotic hyphae. In addition to the host-provided signal, the transition from yeast-like to filamentous forms is dependent on other factors, including lower temperature (14^{o} C) and reduced nutrient availability. The overall goal of the current work was to identify and characterize genes associated with the switch to filamentous growth in this fungal pathogen. It is likely that genes expressed upon mating and during the early stages of hyphal development are involved in controlling the switch and also in pathogenicity. The use of RNA differential display (RDD) in our laboratory successfully identified 3 genes whose expression is limited to the mating and/or hyphal stages; in addition, homologs of a high-affinity methylammonium permease were isolated. Besides the "normal" formation of dikaryotic hyphae, which requires a host-provided signal, we have also isolated filamentous diploid mutants, filamentous haploid mutants, and filamentous aneuploid strains. Several of these mutants produce what appear to be true hyphae, but which lack DAPI-stainable nuclear material throughout the hyphal extension. Genes expressed in mated and/or these additional filamentous forms but not in haploid yeast-like cells (and vice versa) are being identifed by RDD and expression of the genes already recovered will be examined in these "pseudohyphal" strains, as well. These experiments will enhance understanding of the process of dimorphism as it intersects that of disease production.

156. Regulation of dikaryon-expressed genes by FRT1 in *Schizophyllum commune*. Stephen J. Horton, Gail E. Palmer, and William J. Smith. Union College, Biological Sciences, Schenectady, NY, USA.

The gene *FRT1* has previously been shown to induce homokaryotic fruiting in transformation recipients of the basidiomycete *Schizophyllum commune*. In this study, we demonstrate by gene disruption experiments that *FRT1* is dispensable for dikaryotic fruiting. Non-fruiting homokaryotic *FRT1* disruptant strains exhibited enhanced aerial growth of mycelia compared to wild-type. Introduction of a functional *FRT1* allele into the disruptant restored the wild-type colony morphology. Transcript abundance of the dikaryon-expressed *SC1* and *SC4* hydrophobin genes, and the *SC7* gene were greatly elevated in homokaryotic *FRT1* disruptant strains. Growth of the disruptant strains under continuous light was found to inhibit the elevation of *SC1* and *SC4* transcript levels, but not of *SC7* mRNA. These data suggest that the role of *FRT1* in vegetatively-growing homokaryons is to act as a negative regulator of dikaryon-expressed genes.

157. Construction of an equalized cDNA library from *Colletotrichum lagenarium* and its application to isolation of differentially expressed genes. Atsuko Inagaki[1], Yoshitaka Takano[1], Yasuyuki Kubo[2], Kazuyuki Mise[1], and Iwao Furusawa[1]. [1]Kyoto University, Agriculture, Kyoto, Kyoto, Japan. [2]Kyoto Prefectural Univ., Agriculture, Kyoto, Kyoto, Japan.

For efficient isolation of differentially expressed genes of a phytopathogenic fungus *Colletotrichum lagenarium*, differential screening system by using an equalized (normalized)

cDNA library is established. To isolate differentially expressed genes involved in infection process of conidia, mRNA from conidia undergoing appressorium differentiation was used for cDNA synthesis. The equalization of cDNA was performed twice by using kinetic method and the products were cloned into a plasmid vector. We evaluated the equalized cDNA library by colony hybridization with nine probes prepared from *PKS1, SCD1, THR1, TUB1, CMR1* cDNA clones, and 4 randomly selected clones. The result showed a reduction in 'abundance variation' from 900-fold in the original library to 14-fold in the equalized cDNA library, indicating that the cDNAs were successfully equalized. Next, we performed differential screening of 1900cDNA clones in the equalized cDNA library. We identified 11 independent cDNA clones designated *CAD1* through *CAD11*genes that were expressed in conidia differentiating appressoria but not in vegetative mycelia. The expression of *CAD1, CAD2, CAD3,* and *CAD4* was investigated in more detail. The transcripts of *CAD1* and *CAD2* hardly accumulated in preincubated conidia whereas those of *CAD3* and *CAD4* accumulated highly and slightly, respectively. The accumulation of the four *CAD* transcripts increased after the start of appressorium differentiation process. In our wild type strain of *C. lagenarium*, developments of conidia could be controlled by incubation conditions of conidia. Conidia incubated in water at 32C germinated and elongated germ tubes without appressorium differentiation. Conidia in 0.1% yeast extract solution at 32C elongated vegetative hyphae. The four *CAD* transcripts accumulated in conidia elongating germ tubes as same as in conidia differentiating appressoria. On the other hand, the four *CAD* transcripts remarkably reduced in conidia elongating vegetative hyphae.

158. Phylogenetic study of *Histoplasma capsulatum*. Takao Kasuga[1], Gina Koenig[1], Thomas J. White[1], and John W. Taylor[2]. [1]Roche Molecular Systems, Clinical Micology, Alameda, CA, USA. [2]UC Berkeley, Plant & Microbial Biology, Berkeley, California, USA.

An ascomycetous fungus *Histoplasma capsulatum* is the etiologic agent of histoplasmosis. The fungus has been isolated from all over the world, and several distinct varieties are known which show difference in virulence and host specificity. It is not clear whether *H. capsulatum* varieties form genetically isolated subspecies or the fungus has a more homogeneous panmictic population structure and difference in pathogenicity among varieties is due to difference in hosts. We have applied phylogenetic analysis on 64 geographically diverse *Histoplasma capsulatum* isolates representing the three varieties *capsulatum, duboisii* and *farciminosum* using partial DNA sequences of four protein coding genes. Parsimony and distance analysis of the separate genes were generally congruent and analysis of the combined data identified six clades: (1) class 1 North American *H. c.* var. *capsulatum,* (2) class 2 North American *H. c.* var. *capsulatum,* (3) Panamanian *H. c.* var. *capsulatum,* (4) South American *H. c.* var. *capsulatum* group A, (5) South American *H. c.* var. *capsulatum* group B, and (6) *H. c.* var. *duboisii*. *H. c.* var. *farciminosum* was found within the South American *H. c.* var. *capsulatum* group A clade. With the exception of the South American *H. c.* var. *capsulatum* group A clade, genetic distances within clades were an order of magnitude lower than those between clades and each clade was supported by a number of shared, derived nucleotide substitutions, leading to the conclusion that each clade was genetically isolated from the others. Under a phylogenetic species concept based on possession of multiple shared derived characters as well as concordance of four gene genealogies, *H. capsulatum* could be considered to harbor six species instead of three varieties.

159. A G protein alpha subunit, GNA-3, mediates conidiation in *Neurospora crassa* by regulating adenylyl cyclase. Ann Marlena Kays, Patricia S. Rowley, Rudeina Baasiri, and Katherine A. Borkovich. University of Texas Medical School - Houston, Microbiology, Houston, TX, USA.

Extracellular signals mediated by G protein coupled receptors are transmitted to heterotrimeric guanine nucleotide binding proteins to generate a cellular response. Our lab previously identified and characterized two G protein alpha subunits, *gna-1* and *gna-2.* A third G alpha subunit, *gna-3,* cloned in *Neurospora crassa* is most identical at the peptide sequence level to CPG2 (90.7%) and MAGA (86.5%) from *Cryphonectria parasitica* and *Magnaporthe grisea,* respectively. A deletion of *gna-3* has been marked by resistance to the bacterial hygromycin B phosphotransferase (*gna-3::hph*). Western analysis indicates that GNA-1 and GNA-2 are present at wild-type levels in the *gna-3* mutant. The sexual cycle of the *gna-3* mutant is defective in homozygous crosses and produces a large proportion of inviable ascospores. During vegetative growth on solid medium, the *gna-3* mutant exhibits denser conidiation and stunted aerial hyphae. The addition of exogenous cAMP to solid cultures and standing liquid cultures restores the strain to wild-type morphology. Grown in submerged cultures, the *gna-3* mutant conidiates abundantly; this phenotype is suppressed by exogenous peptone, but not cAMP. Intracellular cAMP levels of the *gna-3* mutant are reduced 65% in submerged culture and 90% during vegetative and sexual growth on solid surfaces, relative to wild type. Adenylyl cyclase activity in *gna-3* mutants is reduced 70%; however, the decreased activity results from a loss of active enzyme and not from a loss of GNA-3 stimulation of adenylyl cyclase. Phenotypic and biochemical results suggest that GNA-3 mediates conidiation in *Neurospora crassa* by regulating the amount of active adenylyl cyclase enzyme.

160. The role of a Myb-like regulator of fungal development. Ellen M. Kellner, Tom H. Adams. Cereon Genomics, LLC, Microbial Discovery, Cambridge, MA, USA.

Conidiation of the filamentous ascomycete *Aspergillus nidulans* follows a developmental program of cell differentiation which culminates in the production of spore-bearing structures called conidiophores. This developmental program serves as model system for the study of cellular differentiation and inhibition of proliferative growth pathways. The *flbD* gene product has been proposed to function in an initiation pathway which leads to development. Strains which harbor a deletion of the *flbD* locus exhibit a fluffy phenotype associated with delayed conidiation and uncontrolled proliferative growth. In order to further understand the pathways in which *flbD* functions, a screen for mutations which suppress the phenotype of a *flbD* null mutant were sought. Twenty such mutations were isolated which restore wild-type timing of conidiation to a strain harboring a *flbD* deletion. These mutations map to two linkage groups designated *sfdA* and *sfdB.* Strains harboring mutations in *sfdA* or *sfdB* have been shown to exhibit a reduced growth phenotype on agar plates and unlike wild-type strains, are capable of forming conidiophores in submerged culture. The N-terminal region of FlbD is very similar to the DNA binding regions of the Myb family of transcription factors including the protooncogene c-myb. We show here that FlbD activates transcription from a yeast reporter gene containing consensus c-myb binding sites as upstream activating sequences. Partial funding for this work is provided by NIH National Research Service Award Number 1 F32 GM20072-01 to E.M.K.

161. Structural and functional characterization of the *veA* gene required for sexual development in *Aspergillus nidulans*. Hee-Seo Kim1, Kyung-Jin Kim[1], Kwang-Yeop Jahng[1], Keon-Sang Chae[1], Dong Min Han[2], and Suhn-Kee Chae[3]. [1]Chonbuk National University, Faculty of Biol. Sci., Chonju, Chonbuk, South Korea. [2]Wonkwang University, Div. Life Science, Iksan, Chonbuk, Korea. [3]Paichai University, Div. Life Science, Taejon, Taejon, Korea.

The *veA* gene has been isolated by complementation of the *veA1* mutation. The nucleotide sequences of the cDNA as well as its genomic DNA were determined, showing that there is one open reading frame (ORF) possibly coding for a 573 amino acid polypeptide in the gene interrupted by two introns. No similarities in the amino acid sequence as well as in the nucleotide sequence were found to anyone currently available in GenBank and SWISS-Prot, respectively. It had four putative PEST regions that contain large amounts of proline (P), glutamic acid (E), serine (S), and threonine (T), and a nuclear localization signal, PKRARAC. The nucleotide sequence of the *veA1* mutant gene was also determined and was differed by one nucleotide from a wild type one. The mutant ORF seemed to have the 37th methionine codon of the wild type one as a new initiation codon. The expression of the gene started from just after 14 hours competence, peaked at 8 hr, and thereafter decreased, suggesting that it act at the early developmental stage. A null mutant of the gene constructed by replacing a part of its ORF with an *argB* gene never entered sexual development even under conditions where sexual development preferentially occurs. The induced expression of the gene in a *veA1* mutant resulted in the formation of sexual structures even in a submerged culture where wild type strains never form the sexual structures. These results indicated that the gene is an activator for sexual development, although it also can be a negative regulator for asexual development.

162. Two novel genes highly expressed in sexual tissues of *Neurospora crassa*. Hyojeong Kim and Mary Anne. Nelson. Univ. of New Mexico, Biology, Albuquerque, New Mexico, USA.

Two novel and highly expressed genes have been isolated from starved and sexual tissues of the filamentous fungus, *Neurospora crassa*. These genes were those most frequently expressed in the starved mycelial and perithecial cDNA libraries of Neurospora Genome Project (Nelson et al., Fungal Genetics and Biology 21, 348-363, 1997). The most abundantly expressed of the two genes, NP2A8, has no easily identifiable ORF. The mRNA is mostly non-coding with multiple stop codons and no region of good codon bias. NP2A8 mRNA also has an unusual predicted secondary structure. The gene encodes a 10 kDa putative protein as determined by in vitro transcription and translation, and it appears to be essential. An ORF with good codon bias has been identified in the second most abundantly expressed gene, NM2H2. It encodes a 27 kDa putative protein that contains a possible transmembrane helix and a possible signaling peptide cleavage site. The putative protein also contains a novel 16 tandem repeat of 13-14 amino acid residues. We are currently using repeat-induced point mutation (RIP) to analyze the functions of these two genes in the development of *N. crassa*.

163. Assessment of clone size in *Rhizopogon vinicolor* based on PCR amplification of microsatellite loci from mycorrhizal roots. Annette M. Kretzer[1], Randolph Molina[2], and Joseph W. Spatafora[1]. [1]Oregon State University, Botany & Plant Pathology, Corvallis, Oregon, USA. [2]USDA Forest Service, Corvallis, Oregon, USA.

We have identified ten microsatellite loci in the basidiomycete false-truffle *Rhizopogon vinicolor* T20787 (Boletales, Hymenomycetes). Four loci with the sequence motifs $(GTG)_7$, $(GTG)_{11}$, $(TGG)_6(CGG)_5$ and $(TCG)_4N_{24}(TCG)_6$ were chosen and screened for length polymorphisms across individuals. Heterozygosity was estimated to range between 0.65 and 0.81. Based on the observed allele frequencies, chances to encounter the same allele combinations at all four loci at random are below 0.0009.We used these four polymorphic loci to assess the size of *R. vinicolor* clones within two plots located on Mary's Peak (Oregon Coast Range) and in the H.J. Andrews Experimental Forest (Oregon Cascades). Both sites are within mixed forests that are roughly 75-100 years old. We have chosen to sample mycorrhizae of *R. vinicolor* instead of sporocarps for the following reasons: (i) *R. vinicolor* is unique in forming tuberculate mycorrhizae that have a distinctive morphology and are fairly easy to sample due to their large size. (ii) The hypogeous sporocarps formed by *R. vinicolor* are no less cryptic than the mycorrhizae, but are less frequently encountered and are more seasonal in their production. Results are currently available from the Mary's Peak site and indicate the presence of one large clone (at least 15 m across) in addition to several potentially smaller ones.An analogous project is under way examining clone sizes in *Suillus lakei*, which is closely related to *R. vinicolor* but forms epigeous mushroom-like sporocarps. Results from the *S. lakei* clone study will be available at the time of the meeting. The long-term goal is to address differences in population dynamics between *R. vinicolor* and *S. lakei* as a function of their different reproductive life histories.

164. Microscopic analysis of *spo11* and *msh5* mutants of *Coprinus cinereus*. Sandra T. Merino, Martina Celerin, and Miriam E. Zolan. Indiana University, Biology, Bloomington, Indiana, USA.

We are using the fungus *Coprinus cinereus* to study genes necessary for meiosis and DNA repair. Because of its naturally synchronous meiosis, *C. cinereus* is an excellent organism to use in studying the relationships between meiotic chromosome behavior and other aspects of DNA metabolism. Mutants defective in meiosis have been identified using restriction enzyme-mediated integration (REMI) transformation. In our analysis, REMI mutants of two genes were characterized; one is a *mutS* homolog, *msh5,* and the other is a homolog of the *Saccharomyces cerevisiae SPO11,* which encodes a meiotic recombination-specific type II topoisomerase. Fluorescence *in situ* hybridization (FISH) has been used to examine pairing between homologs at 3 and 6 h post-karyogamy. The *msh5* mutant (R1660) shows pairing at nearly wild-type levels, while the *spo11*mutant reveals less pairing than is seen for wild-type strains. Electron microscopic examination of silver-stained chromosomes revealed that R1660 forms full-length synaptonemal complexes (SCs) by K+3, and the chromatin appears to be as condensed as in wild-type. At K+6, the SCs remain, but the chromosomes are less condensed than those seen at K+3 and chromatin loops are apparently highly extended. Analysis of the *spo11* mutant shows variable axial core formation and no visible synapsis at 6 hours after karyogamy. Immunolocalization of tubulin and DAPI stained DNA analysis indicate neither the *msh5* mutant nor the *spo11* mutant complete meiosis.

165. HMG domain proteins involved in mating and sexual development of *Coprinus cinereus*. Michael J. Milner. University of Oxford, Plant Sciences, Oxford, Oxon, UK.

Transcription factors possessing HMG domains play an important role in mating recognition and sexual development in both ascomycete and basidiomycete fungi. In the ascomycetes *Schizosaccharomyces pombe, Neurospora crassa* and *Podospora anserina*, genes encoding HMG domain proteins are found within the mating type loci themselves. In *S. pombe* and the basidiomycete *Ustilago maydis*, an HMG domain protein has also been implicated in regulating gene transcription in response to pheromone stimulation. Genes that are known to have pheromone responsive promoters in *C. cinereus* are the *B* mating type genes that encode the pheromones and pheromone receptors. Analysis of the promoter sequences identifies several copies of the motif ACAATGG which resembles the so- called pheromone response element in the promoters of pheromone responsive genes of both *S. pombe* and *U. maydis*. We have used degenerate primer PCR to isolate two genes encoding HMG domain proteins from *Coprinus cinereus*. *hmg1* and *hmg2* encode HMG domains with strong homology to those of the pheromone response factors of *S. pombe* (Ste11) and *U. maydis* (Prf1). A third gene encoding an HMG domain protein, *pcc1*, has been described by Murata, Fujii, Zolan and Kamada (*Genetics* 149,1753, 1998) and has a domain that more closely resembles that of the ascomycete mating type genes *Mat-Mc, mta1*, and *FPR1*. We will describe experiments designed to show the roles of these different proteins in mating and sexual development of *C. cinereus*.

166. The homothallic ascomycete *Glomerella cingulata* has at least one putative mating-type idiomorph. Agnieszka Mudge and Matt Templeton, Plant Defence Group, The Horticulture & Food Research Institute of New Zealand, Mt. Albert Research Centre, Private Bag 92-169, Auckland, New Zealand.

In most ascomycetes there are two mating types determined by two alleles at a single regulatory locus called MAT. For sexual reproduction to take place successfully the two mating types must be heterozygous at this locus. The alleles of the MAT locus lack sequence homology and hence have been termed "idiomorphs". MAT loci have been studied in only a handful of ascomycetes, but some common features include the presence of conserved DNA binding motifs, such as the HMG (High Mobility Group) box protein, most of which are also found in transcriptional regulators of other eukaryotes. These genes are found in single copy per genome, are constitutively expressed and control fertility. Other functions attributed to these genes include pheromone production, control of fruiting bodies and ascospore production, and self/non-self recognition at both cellular and nuclear levels. A single copy 300 bp HMG box from the homothallic ascomycete *Glomerella cingulata* has been amplified using published degenerate primers. An approximately 15 Kb genomic sequence containing the HMG box is currently being characterised.

167. Differential expression of catalase genes during stress and development in *Aspergillus nidulans.*Rosa E. G. Navarro, Laura W. Kawasaki, and Jesus L. Aguirre. Instituto de Fisiologia Celular-UNAM, Genetica Molecular, Mexico, D.F., Mexico.

Catalases are H_2O_2-detoxifying enzymes present in most living organisms that use H_2O_2 as a unique substrate. We study catalase gene expression as part of a general project aimed at demonstrating a causal relationship between oxidative stress and development (Hansberg and Aguirre, 1990). Two catalase genes, designated *catA* (Navarro et al., 1996) and *catB* (Kawasaki et al.,1997) have been characterized in *A. nidulans.* More recently, a third catalase (CatC) has

been detected using *catA/catB* double mutants. Why are different enzymes required to carry out the same reaction?. Part of the answer is related to the fact that these catalases are differentially expressed both, in time and space, throughout the life cycle. The *catA* gene encodes catalase A, which is specifically localized to asexual and sexual spores, whereas catalase B is induced during stationary phase of growth, conidiation and oxidative stress. Part of catalases A and B have been immunolocalized to the cell wall of conidia and hyphae, respectively. Mutants affected in *catA* or *catB* are sensitive to H_2O_2 at different stages of *A. nidulans* life cycle. How is this differential regulation accomplished?. We have found that the *catA* mRNA accumulates in response to different types of stress. However, translation of the mRNA is coupled to the spore formation, in a process mediated by the *catA* mRNA 5' UTR region. On the other hand, the *catB* gene is regulated at the transcriptional level. A 580-bp region of the *catB* promoter necessary for regulation during stationary phase and oxidative stress has been defined. The study of *catA*, *catB* and eventually *catC*, will allow us to understand an important part of the antioxidant response and the mechanisms of gene regulation that operate during development. Partially supported by grant No. IN206097 from PAPIIT-UNAM, Mexico.

168. Cytological and molecular characterization of developmental mutants from *Sordaria macrospora.* Minou Nowrousian, Sandra Masloff, Stefanie Poeggeler, and Ulrich Kueck. Ruhr-Universitaet Bochum, Allgemeine Botanik, 44780 Bochum, NRW, Germany.

During sexual development, mycelial cells from most filamentous fungi differentiate into typical fruiting bodies. In our effort to elucidate this process of multicellular development, we chose the homothallic ascomycete *Sordaria macrospora* as a model organism and isolated several sterile mutants with defects in fruiting body formation. Using an indexed cosmid library of *S. macrospora* [1], we have been able to identify DNA sequences capable of restoring fertility in the mutant strains. Here we present the isolation and characterization of two sterile mutants, mutant pro1 which only forms protoperithecia, and mutant per5 with defects in fruiting body maturation. Mutant pro1 is complemented by the *sdr1* gene (*sexual development regulator 1*) which encodes a protein with significant homology to the DNA binding domain from C6 zinc finger transcription factors. Therefore, SDR1 might be a transcriptional regulator of sexual development in *S. macrospora*. Using Southern hybridization and PCR, we identified homologous sequences in other homothallic as well as heterothallic ascomycetes. Mutant per5 is complemented by the *acl1* gene which codes for a subunit of ATP citrate lyase (ACL). The protein was shown to be localized in the cytosol by immunological *in situ* detection of the epitope tagged ACL1 polypeptide [2]. ACL is involved in the formation of acetyl-CoA for fatty acid and sterol biosynthesis, and the highest enzymatic activity is observed during the transition from vegetative growth to the formation of reproductive structures. The ascomycetes *Aspergillus nidulans* and *Podospora anserina* display a similar pattern of ACL activity, and we present a model explaining the involvement of ACL within differentiation processes in filamentous fungi. [1] J. Microbiol. Meth. (1997) 29: 49-61 [2] Mol. Cell. Biol. (1999) 19: 450-460

169. Acquisition and analysis of an EST database of barley mildew: Signal transduction pathways. Richard P. Oliver[1], Julia Kinane[1], Lene Bindslev [1], Sussie Dalvin [1], Soeren w. Rasmussen [1], Jacques Rouster [1], Henrietta Giese[2], Nick J. Talbot[3], and Michael Kershaw[3]. [1]Carlsberg Laboratory, Physiology, Valby 2500, Copenhagen, Denmark. [2]Risoe National

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In order to provide fundamental information about its pathogenicity, we have set out to sequence as many as possible of the barley mildew genes expressed throughout the interaction. Thus far, we have utilized two cDNA libraries, both of which are purely fungal. The first analysed is a germinated spore library (Justesen, Somerville & Giese, 1996) referred to as the CR3 library. The second is from "resting conidia", i.e. spores taken directly from sporulating leaves. Redundancy has been surprisingly low, especially for the CR3 library. There are significant differences in the identity of the genes found in the two libraries. Overall 50-60% of the clones have a clear similarity to known genes. Major catabolic activities appear to involve proteins, (a selection of) amino acids, lipid and glycogen breakdown. Major anabolism involves DNA, RNA, protein, lipid, sugar, a non-overlapping selection of amino acids and vitamins. A particular focus of interest is signal transduction. A protein kinase A catalytic subunit gene was found. This suggested a direct involvement of cAMP in the early stages of development. cAMP was found to inhibit germination and appressorium development on epidermal strips and artificial media. Subsequently, fragments of the regulatory subunit and adenylyl cyclase have been cloned by PCR. Amongst a wide range of potential activators tried, the most clear effect was with cholera toxin which stimulated germination on all surfaces tried. Cholera toxin may interact with one or more of the adenosyl-ribosylation factors which have been cloned in the library. Thus the components of paradigmatic signal transduction pathways have been identified. Their likely roles in pathogenicity will be discussed.

170. Mapping of intersterility genes in *Heterobasidion annosum*. Åke Olson, and Jan Stenlid. Swedish University of Agricultural Sciences, Forest Mycology, Uppsala, Uppland, Sweden.

Today there are three known genetic systems that control mating and somatic integrity among fungi: (i) somatic incompatibility (vegetative compatibility), which restrict free exchange of nuclei and cytoplasm between mycelia, (ii) mating type that allows individuals with different alleles at one or more mating type locus to mate (iii) intersterility, that define limits of biological species and allow mating between individuals with identical intersterility (IS) alleles at one or more of the intersterility loci. Very little is known about the system that restrict mating within IS groups. The best characterised IS system is in *Heterobasidion annosum* where Chase and Ullrich in 1990 showed that mating between and within IS groups is controlled by at least five genes, S, P, V1, V2 and V3 each with a + or a - allele. Mating requires at least one common + allele for both individuals at one or more of the five genes. Individuals with combinations of only +/- and -/- cannot mate. Our aim is to map and clone intresterility genes. Here we present results towards a linkage map of the *H. annosum* genome and the mapping of the IS genes S, P and V2. We have isolated 105 single spore progeny from a cross between the homokaryotic strains TC-32-1 and TC-122-12, with the genotypes S^-, P^+, $V1^*$, $V2^+$, and $V3^+$ reps. S^+,P^-, $V1^-$, $V2^-$, and $V3^+$ (*= not defined). About 100 polymorphic AFLP markers has been identified in the parental strains using five primer combinations. Analysis of the single spore isolates are in progress.

171. Alleic diversity at the *het-c* locus in *Neurospora tetrasperma* poses an evolutionary dilemma.Amy J. Powell[1], Gregory S. Saenz[1], John G. Stam[1], David J. Jacobson[2], and Donald O.

Natvig[1]. [1]University of New Mexico, Department of Biology, Albuquerque, NM, USA. [2]Stanford University, Biological Sciences, Stanford, CA, USA.

Heterokaryon (het) incompatibility governs self/non-self recognition in the vegetative phase of filamentous fungi. To form stable heterokaryons, strains must be identical at all het loci. Eleven such loci, including mating-type, have been identified in *Neurospora crassa*. The pseudohomothallic species *N. tetrasperma* occurs naturally as a mating-type heterokaryon. Although adapted for selfing, *N. tetrasperma* possesses mechanisms for outcrossing that could combine incompatible het alleles into a single heterokaryon. Therefore, *N. tetrasperma* must in some way avoid het incompatibility. Mating-type het incompatibility is genetically suppressed in *N. tetrasperma*. In principle, incompatibility conferred by other loci could result either from suppression or from maintenance of het locus homoallelism through persistent selfing. Our survey of *het-c* sequences from diverse *N. tetrasperma* strains demonstrated (1) that individual wild-type strains are invariably homoallelic for *het-c*, but (2) closely-related strains within a population can possess functionally different *het-c* alleles. Three allele groups (*het-c*^{OR}, *het-c*^{PA}, and a variant of *het-c*^{PA}) are present in *N. tetrasperma* and other species. Our results provide convincing evidence for both predominant selfing, which maintains homoallelism, and occasional outcrossing in nature. This suggests an evolutionary dilemma: Selection has maintained *het-c* variation in *N. tetrasperma* populations to provide vegetative individuals with self/non-self recognition. Conversely, crossing between strains with different *het-c* alleles should lead to decreased fitness through formation of heterokaryons with incompatible alleles, a prediction consistent with heterokaryon instability observed in laboratory outcrosses. Assuming *het-c* function is conserved in *N. tetrasperma*, it appears that outcrossing will create transient life cycle disruptions that must be resolved by additional matings to restore homoallelism.

172. Heterokaryons of *Phytophthora infestans* revealed by AFLP and RFLP markers.
Andrew I. Purvis, David S. Shaw, and Susan J. Assinder.University of Wales, Biological Sciences, Bangor, Gwynedd, UK.

Populations of *P. infestans* in the UK and other countries are highly diverse; A1 and A2 mating types can be found at a single site. From an evolutionary viewpoint, the role of sexual mating and oospore formation is not clearly understood. Heterokaryosis and parasexuality have long been suspected as contributing to variation but this needs to be confirmed using reliable molecular markers. To identify these, a comparison was made of the usefulness of Restriction Fragment Length Polymorphisms (RFLPs) and Amplified Fragment Length Polymorphisms (AFLPs) for assessing variability. Isolates from around the UK were screened using the multi-locus fingerprinting probe, RG57. A subset of isolates, some with the same and others with different RFLP genotypes, were fingerprinted with two pairs of AFLP primers. UPGMA trees were constructed using the RFLP and AFLP data independently. On both the RFLP and AFLP trees, isolates of mitochondrial haplotype IIa (as opposed to type Ia) clustered on a unique branch. On the AFLP tree (but not the RFLP tree) isolates of A2 mating type clustered, indicating an extra level of resolution. To investigate the parasexual cycle, heterokaryons were selected by pairing isolates with complementary sensitive and resistant mutations to streptomycin, chloramphenicol or metalaxyl (A1 x A1 or A2 x A2) on rye agar plates as vegetative hyphae or mixtures of zoospores. The resultant colony was transferred to appropriate double-drugged rye agar. Double-drug resistant growths and their single-zoospore derivatives are

being analysed for their RFLP/AFLP fingerprint patterns to determine whether they are heterokaryons of parental nuclei or somatic recombinants.

173. Incomplete chromosome pairing is correlated with a recombination block on the mating-type chromosome of *Neurospora tetrasperma*. Namboori B. Raju, and David J. Jacobson. Stanford University, Biological Sciences, Stanford, California, USA.

In *N. tetrasperma*, each ascospore encloses nuclei of opposite mating type. The dual-mating-type ascospores are the direct result of segregation of mating types at the first division of meiosis and overlapping spindles at the second and third divisions (Dev. Genet. 15: 104). Earlier genetic studies clearly show that there is little or no recombination between various markers on the mating-type chromosome (linkage group I), whereas markers on the remaining six chromosomes recombine as expected (Genetics 54: 293; Genetics 143: 789). We have examined meiotic chromosome pairing in various laboratory and wild-collected *N. tetrasperma* strains to see whether the recombination block is correlated with any cytologically detectable chromosome pairing anomalies. Three- to four-day-old perithecia were stained with the DNA-specific fluorochrome acriflavin and the young, developing asci were observed under a fluorescence microscope (Mycologia 78: 901). All but the longest chromosome showed intimate homologous pairing along the entire length. In contrast, the longest chromosome, which bears the mating type locus, usually showed a long unpaired segment in the middle, with normal pairing at both ends. In the sibling species *N. crassa*, all seven chromosomes, including the mating-type chromosome, show complete homologous pairing. We have not yet established a cause and effect relationship between the recombination block and incomplete pairing on the longest chromosome in *N. tetrasperma*. However, our observations are consistent with the notion that the recombination block between mating type and centromere (possibly due to impaired pairing) is a significant correlate of normal production of self-fertile ascospores in this pseudohomothallic species.

174. Programmed ascospore death in the homothallic ascomycete *Coniochaeta tetraspora*. Namboori B. Raju, and David D. Perkins. Stanford University, Biological Sciences, Stanford, California, USA.

Immature asci of *Coniochaeta tetraspora* originally contain eight uninucleate ascospores. Two ascospore pairs of each ascus mature and two degenerate. Arrangement of the two ascospore types in individual linear asci is what would be expected if death is controlled by a chromosomal factor segregating at the second meiotic division in about 50% of asci. Cultures originating from single ascospores or from single uninucleate conidia are self-fertile, producing eight-spored asci with four degenerating ascospores, generation after generation. These observations in *C. tetraspora* suggest that differentiation of nuclei into two types occurs *de novo* by epigenetic modification of a specific chromosomal locus prior to proliferation of ascogenous hyphae from the ascogonium, and that modified and unmodified nuclei behave as though they were of opposite mating type. In *C. tetraspora*, ascospores of one epigenotype die. A similar model might explain ascospore differentiation in *Sclerotinia trifoliorum* and *Chromocrea spinulosa*, in which all eight ascospores survive but four differ in size and in self-fertility. Sexual-phase-specific modification might also occur in ascogenous hyphae of homothallic species such as *Neurospora africana* and *Sordaria macrospora*, in which all eight ascospores are viable and

morphologically identical. Nuclei might then be temporarily differentiated into two types that interact during crozier formation and karyogamy as though they were of opposite mating type.

175. Progress in identifying the mating type determinant(s) of *Phytophthora infestans* by positional cloning. Thomas A. Randall. University of California, Riverside, Plant Pathology, Riverside, CA, USA.

Phytophthora infestans, a heterothallic oomycete, has two mating types, A1 and A2. A current model suggests that the A1 type is heterozygous for alternate mating type alleles and the A2 type is a homozygote. The mating type locus segregates in a non-Mendelian fashion in the vast majority of crosses, appearing to be linked to a system of balanced lethals. A RAPD marker (B1) that co-segregates with the mating type locus (<0.5 cM) was previously identified. A collection of RFLP, RAPD, and cDNA clones that are physically linked to B1 are being mapped relative to mating type in several crosses to localize the mating type determinant. To help in positional cloning, a bacterial artificial chromosome (BAC) library of an A1 strain of *P. infestans* was constructed in a derivative of pBELOBACII containing a selectable marker for *P. infestans* transformation. The library contains 16,128 ordered clones with an average insert of 75 kb, representing a 4.6-fold coverage of the genome. This library was probed with the B1 marker and eight positive BACs were identified with inserts ranging from 43-135 kb. Several of these BACs were determined to be from the A1 homolog. DNA markers linked to mating type are being mapped on these BACs to further localize the mating type locus. BACs have been successfully transformed into *P. infestans* and we are using selected BACs to transform an A2 strain of P. infestans to help identify the mating type determinant by complementaion.

176. High levels of gene flow and heterozygote excess characterize *Rhizoctonia solani* AG-1 IA (*Thanatephorus cucumeris*) from Texas. U. Liane Rosewich[1], Rodney Earl Pettway[1], Bruce A. McDonald[2], and H. Corby Kistler[1]. [1]University of Florida, Plant Pathology, Gainesville, Florida, USA. [2]ETH, Plant Sciences, Zuerich, Switzerland.

Seven single-copy nuclear RFLP probes differentiated 36 multilocus RFLP genotypes (MRGs) among 182 isolates of *Rhizoctonia solani* AG-1 IA, collected from six commercial rice fields in Texas. Population subdivision was assessed using F_{ST}, unbiased genetic distance (D) and analysis of molecular variance (AMOVA). Values near zero for clone-corrected data indicated a lack of population structure. Tests for Hardy-Weinberg equilibrium (HWE) among the 36 MRGs revealed that three out of seven loci significantly differed from HWE. Subsequent analysis demonstrated that departures from HWE were due to an excess of heterozygotes (and hence heterozygosity). Moreover, all loci displayed negative F_{IS}, with values between -0.186 and -0.551. These data suggest that the population has experienced population bottlenecks (leading to excess heterozygosity) and/or that sexual reproduction is rare (leading to heterozygote excess). We propose that a combination of these two population genetic processes could explain these data. Historic and epidemiological observations support our hypothesis.

177. Peroxisomes and mitochondria : sexual development and retrograde regulation in *Podospora anserina*. Gwenaël Ruprich-Robert, Denise Zickler, Véronique Berteaux-Lecellier, Arlette Panvier-Adoutte, and Marguerite Picard.Université Paris-Sud, IGM, Orsay, Essonne, FRANCE.

The roles that organelles may play in developmental processes have been yet poorly investigated. We have previously shown that mutants of the *Podospora anserina car1* gene, which encodes the peroxisomal membrane protein PEX2, are devoid of peroxisomes and exhibit pleiotropic defects. Unable to grow on oleic acid as sole carbon source, they are also unable to switch from the mitotic to the meiotic stages after fertilization : in homozygous crosses, crozier cells proliferate into new croziers instead of differentiating into meiotic ascal cells. Interestingly, in wild-type crosses, a massive proliferation of peroxisomes is observed in growing ascus cells (Cell, 1995, 81, 1043-1051). We have recently observed that mutants of the *cit1* gene, which encodes the mitochondrial citrate synthase, also show a sexual defect : they are mainly blocked in a specific step of the first meiotic prophase, the diffuse stage preceeding diplotene. Most of the *cit1*mutants bear a nonsense or frameshift mutation. Noteworthingly, these mutations have been identified as suppressors of the metabolic defect of the *car1* (*pex 2*) mutants. Our results may be explained through a retrograde regulation mechanism which involves a cross-talk between mitochondria and peroxisomes (via nuclear genes), as it has been observed in yeast (Can. J. Bot, 1995, suppl. 1, S205-S207) but not yet described in a filamentous fungus. Experiments are in progress to test this hypothesis and the two following ideas. First, a cytosolic (instead of peroxisomal) H2O2 production would have a mitogenic effect in the *car1*(*pex 2*) croziers. Second, a heterochronic peroxisomal activation (due to retrograde regulation) would be responsible of the meiotic cit1 phenotype.

178. Molecular analysis of laccase III of *Aspergillus nidulans*. Mario Scherer and Reinhard Fischer. Max-Planck-Institut für terrestrische Mikrobiologie, Karl-von-Frisch-Str., D-35043 Marburg, Germany.

Laccase is a copper containing polyphenol oxidase widely distributed among plants and fungi. Fungal laccases are involved in such divers processes as lignin degradation, morphogenesis and pathogenic interactions. Laccase I (*yA*) of *Aspergillus nidulans* is involved in pigment synthesis of asexual spores. In contrast, laccase II was described as differentially expressed during sexual spore development. During an approach to identify the laccase II gene of *A. nidulans* a partial laccase sequence appeared in the EST sequencing project (University of Oklahoma). We subsequently have cloned and sequenced the whole gene. The deduced amino acid sequence was not laccase II and thus the novel gene was named *lac3*. *lac3* codes for a protein of 595 aa with highest homology to laccase I of *A. nidulans* (36% identical aa). Laccase III was immuno-cytochemically localized in the hyphal cell wall. Expression of the enzyme starts at the onset of conidiospore germination and highest concentrations of the protein were detected at the hyphal tip suggesting a crucial function for cell extension. Western blot analysis showed that laccase III is present throughout the vegetative and asexual stage of the fungus, whereas it could not be detected during late sexual development. Knock out and overexpression experiments are in progress to prove the role of laccase III in hyphal tip extension.

179. *Magnaporthe grisea* mating pheromone precursor genes. Wei-Chiang Shen, Piotr Bobrowicz, and Daniel J. Ebbole.Texas A&M; University, Plant Pathology. & Microbiology, College Station, Texas, USA

Magnaporthe grisea, the rice blast fungus, is a heterothallic ascomycete and as such individuals of opposite mating type must meet in order to undergo sexual reproduction. The first step in the

mating process is for mating partners to recognize each other via mating pheromones. In *Saccharomyces cerevisiae* and *Ustilago maydis*, mating factors induce a pheromone response pathway and chemotropically attract cells of the opposite mating type to grow toward the pheromone source. In an effort to understand the pheromone response in a filamentous ascomycete, we have identified two putative pheromone precursor genes expressed in *Mat1-1* and *Mat1-2* strains of *M. grisea*. *Mat1-1* strains express a pheromone precursor gene predicted to contain a farnesylated and carboxy-methylated C-terminal cysteine residue. *Mat1-2* strains express a pheromone precursor gene that is related in gene organization to the alpha-factor pheromone of yeast. The sequence of the predicted pheromone bears strong sequence similarity those encoded by the putative pheromone genes of *Neurospora* and *Cryphonectria*. Expression analysis and characterization of the mating behavior of the *Mat1-1* pheromone precursor mutant will be presented. This work was supported by a grant from the USDA.

180. Self-fertility in *Phytophthora infestans*. Christine D. Smart. Cornell University, Plant Pathology, Ithaca, NY, USA

Oospores were produced by some isolates of *Phytophthora infestans* growing on a medium containing V-8 juice, but not in all isolates. The number of oospores produced by these isolates in this fashion was much smaller than the number produced in an outcross (with the opposite mating type). Single zoospore isolates (n=128) derived from any of the field strains did not segregate for ability to produce or not produce oospores on a medium containing V-8 juice. Thus, this phenotype was not caused by heterokaryosis or mixtures of strains. The ability to produce oospores was maintained through repeated subculturing, and after passage through host tissue. Some of the oospores were germinated and progeny were observed to segregate for alleles at the *glucose-6-phosphate isomerase - 1* locus. Thus, we conclude that this type of oospore production involved meiosis, and was not ameiotic apomixis. Analysis of a larger group of strains indicated that 96% (n=47) of A2 mating type strains and 6% (n=69) of A1 strains were self-fertile on V8 agar. The ecological implications of the different types of oospores are currently being investigated.

181. In *Microbotryum violaceum* there are two genes that encode methylammonium permeases associated with filamentous growth in yeast. David G. Smith, Wei Hong, and Michael H. Perlin. University of Louisville, Department of Biology, Louisville, KY, USA.

M. violaceum is a dimorphic fungus that is found as yeast-like haploid sporidia and as filamentous dikaryotic hyphae. For this plant pathogenic basidiomycete, the switch to filamentous growth normally only occurs after mating, and this only under conditions of nutrient starvation. In this study, homologues of signal transduction proteins involved in filamentous growth of yeast were sought. We had particular interest in the genes encoding methylammonium permeases in *Saccharomyces cerevisiae*. In *S. cerevisiae* there exists a family of proteins designated MEP1, MEP2, and MEP3. Only MEP2 (a high affinity ammonium transporter) was shown to regulate pseudohyphal growth under nitrogen starvation. Degenerate PCR primers were designed based on highly conserved hydrophobic amino acid sequences in the transmembrane regions. One set of primers amplified both an 800 bp and a 500 bp product. Sequence analysis of these cloned PCR products revealed that both products were highly homologous to MEP2. This is the first report to our knowledge of a methylammonium permease from a filamentous fungus.

When used as probes these fragments hybridized at different positions in Southern hybridization analysis. This analysis suggested that these were the only ammonium permease genes present in this organism. Based on the fact that MEP2 has such low expression levels in cells grown on nutrient rich media we speculated that these genes would not produce a detectable signal using Northern hybridization. Thus, a more sensitive approach was to use RT-PCR to confirm the expression of this putative gene family. Sequence analysis of cDNA from both fragments confirmed the presence of three introns in the 800 bp fragment and no introns in the 500 bp fragment. 5' RACE was used to obtain the cDNA sequence upstream of the initial PCR fragments. The downstream region was amplified using a gene specific primer and an oligo dT primer on the 3' end. We are currently conducting expression studies of these genes under different conditions of growth.

182. Evidence that methylation is involved in the *U. maydis* mating response. Karen Snetselaar, Kevin Dougherty, Joseph Curry, and Michael McCann. St. Joseph's University, Biology, Philadelphia, PA, USA.

Ustilago maydis cells form mating hyphae when they are exposed to appropriate pheromone and nutrient conditions. These hyphae grow toward compatible pheromone-producing cells, and eventually they fuse to form a dikaryon that can infect maize plants. We have found that *Ustilago* methionine auxotrophs are impaired in their ability to form mating hyphae and locate compatible mating partners at a distance, although these auxotrophs can mate efficiently when they are close together. Other amino acid auxotrophs mate normally. Ethionine, a methionine analog known to inhibit activity of methyltransferases, mimics the mating deficient phenotype when added to wild-type cells. When ethionine is added to mating hyphae that have begun elongating in response to pheromone, they cease growth immediately. The same concentration of ethionine has little effect on the growth rate of vegetatively growing cells. This suggests that some type of methylation is involved in the regulation of mating filament formation in *Ustilago*.

183. Characterization of a new "fluffy" mutant isolated by REMI in *Aspergillus nidulans*. Gabriela Soid-Raggi, Olivia Sánchez, and Jesús Aguirre. UNAM, Instituto de Fisiología Celular, Molecular Genetics, México, D.F., México, México.

Aspergillus nidulans mutants showing delayed asexual sporulation (fluffy mutants) have been important to define signal transduction pathways needed for conidiation (Yu et al., 1996). A fluffy mutant named BL001 was isolated by REMI (Sánchez et al., 1998). The "fluffy" phenotype was shown to segregate as a monogenic mutation and to be linked to the *argB* marker used for REMI. Diploid complementation analysis and sexual crosses indicated that the mutation contained in BL001 is not allelic to other known "fluffy genes". The affected gene in BL001 has been provisionally named *fluX*. Plasmid pREMI1, containing the vector used for REMI as well as flanking sequences, was recovered from mutant BL001. pREMI1 insert was used to probe genomic DNA from wild type and fluffy strains derived from BL001. The hybridization patterns demonstrated that pREMI1 contains genomic sequences from *A. nidulans* and that these are altered in the BL001 mutant and its offspring. However, cosmids identified using pREMI1 insert failed to complement *fluX1* phenotype. Sequence analysis of the insert showed that pREMI1 is a chimerical plasmid likely produced during *in vitro* ligation. Nevertheless, sequences adjacent to the REMI insertion point should represent the actual genomic arrangement present in BL001.

Sequence comparision of the identified cosmids and pREMI1 indicate that fluffy mutant BL001 contains a deletion. The *fluX* gene as well as other genes whose mutation causes fluffy phenotypes are required for conidiation induced by nitrogen starvation, but not for coniciation induced by glucose starvation.

184. Clonality and genetic variation in *Amylostereum aerolatum* and *A. chailletii*. Jan Stenlid[1], Rimvydas Vasiliauskas[2], and Iben M. Thomsen[3]. [1]Swedish Univ Agricultural Sciences, Forest Mycology and Path., Uppsala, S-750 07, Sweden. [2]Lithuanian Univ Agricultu, Plant Protection, Kaunas, LT-4324, Lithuania. [3]Danish Forest and Landsc., Research Institute, Horsholm, DK-2970, Denmark.

Genetic variation was investigated within and between vegetative compatibility groups (VCGs) of the basidiomycetes *Amylostereum aerolatum* (Fr.) Boid. and *A. chailletii* (Pers.:Fr.) Boid. DNA fingerprints were made using PCR and M13 core sequence as a primer. A total of 53 of *A. aerolatum* and 57 of *A.chailletii* from Sweden, Denmark, Lithuania, and Great Britain. In all cases, isolates belonging to the same VCG also showed identical fingerprints, suggesting that VCGs correspond to clones. In *A. aerolatum*, wide geographical spread of the VCGs was detected, corresponding well with oidial spread vectored by the wood wasp *Sirex juvencus*. In *A chailletii*, clones with restricted geographical distribution were detected, corresponding to a higher incidence of basidiospore spread, although oidial spread vectored by *Urocerus gigas* is also present.

185. Isolation and characterization of *CMK1*, a gene encoding MAP kinase of *Colletotrichum lagenarium*. Yoshitaka Takano[1], Taisei Kikuchi[1], Yasuyuki Kubo[2], John E. Hamer[3], Kazuyuki Mise[1], and Iwao Furusawa[1]. [1]Kyoto University, Agriculture, Kyoto, Kyoto, Japan. [2]Kubo, Yasuyuki, Kyoto Prefectural Univ., Agriculture, Kyoto, Kyoto, Japan. [3]Purdue University, Biological Sciences, West Lafayette, Indiana, USA.

Colletotrichum lagenarium is a phytopathogenic fungus that causes anthracnose of cucumber. *C. lagenarium* conidia differentiate well developed appressoria pigmented with melanin for penetration into its host plant. In *Magnaporthe grisea*, cAMP and MAP kinase signaling pathways play pivotal roles for pathogenicity including appressorium differentiation. *M. grisea* PMK1, homologous to *Saccharomyces cerevisiae* MAP kinases FUS3/KSS, is involved in appressorium differentiation and pathogenic growth. To investigate roles of MAP kinase signaling pathways in pathogenicity of *C. lagenarium*, we tried to isolate *C. lagenarium* MAP kinase genes by PCR-based screening. We identified a gene showing significant homology to *M.grisea PMK1* and designated *CMK1*. Deduced amino acid sequences of CMK1 shared 96% homology with *M. grisea* PMK1, and 59% homology with *S. cerevisiae* FUS3. To examine functional complementation of *PMK1* with *CMK1*, *M. grisea PMK1* gene disruption mutant nn78 was transformed with *C. lagenarium CMK1*. *CMK1*introduced transformants of nn78 formed appressoria similar to those of *M. grisea* wild type whereas nn78 failed to form appressoria. This result suggests that *C. lagenarium*CMK1 MAP kinase could substitute for *M. grisea* PMK1. Currently, gene disruption analysis is in progress to characterize roles of signal transduction pathways involving the CMK1 MAP kinase in *C. lagenarium*.

186. Cloning and characterization of the colonial, *sbr* mutant of *Neurospora crassa*. John Vierula, Katrina Campsall, Paul Sallmen and Yanhua Yan, Department of Biology, Carleton University, Ottawa, Ontario, Canada..

The colonial, *sbr* strain of *Neurospora crassa* was recovered in a screen of morphological mutants generated by insertional inactivation with a hph^r gene construct. This strain forms compact, slow-growing colonies which darken preceptibly with age but it does not produce conidiophores or conidia. Instead of hyphae, this strain initially forms sausage-shaped cell compartments which give rise to large, randomly positioned, spherical buds. In addition, the *sbr* mutation blocks ascospore germination. The hph^rtag was used to clone the *sbr* gene from a genomic DNA library and subsequently, an apparent full length cDNA clone. Sequence analysis revealed that the *sbr* gene encodes a putative protein with a poly-glutamine domain, a cysteine-rich region and a helix-turn-helix motif suggestive of a function in transcriptional regulation.

187. Ascomycetal mating type genes. Cees Waalwijk[1], Koen Venema[2], Paul Dyer[3], and Gert Kema[1]. [1]DLO Research Institute for Plant ProtectionIPO-DLO, Mycology & Bacteriology, Wageningen, The Netherlands. [2]Wageningen Agricultural U, Phytopathology, Wageningen, The Netherlands. [3]University of Nottingham, School Biological Science, Nottingham, England, U.K.

Recently we have described the mating system of *Mycosphaerella graminicola* (anamorph *Septoria tritici*) as being bipolar and heterothallic. In search of the mating type genes universal primers for the HMG-box, as described by Arie et al., 1997, Fungal Gen. & Biol. 21:118-130, have been employed without success. However, using a heterologous probe from the mating type gene of *Tapesia yallundae*, the causal agent of eye-spot disease of wheat, the corresponding *M. graminicola* allele could be isolated. The most recent data on the genomic organization of this mating type locus will be presented. In *Fusarium* spp. both universal HMG primers as well as primers for the alpha domain produce amplicons in both heterothallic and homothallic species. Moreover, even in *Fusarium oxysporum*, generally recognized as an asexual species these regions could be amplified. Individual strains of this species contain either alpha domain- of HMG- sequences. Sequence analyses of amplicons indicate that only conservative mutations are allowed suggesting that sex may still occur, although rarely in *F. oxysporum*.

188. Characterization of four populations of *Monilinia vaccinii-corymbosi* using AFLPs. Lusike A. Wasilwa, and Peter V. Oudemans. Rutgers University, Blueberry/Cranberry Research Center. Chatsworth, NJ, USA.

The mummy-berry fungus, *Monilinia vaccinii-corymbosi* , is an ascomycete pathogen of blueberry. The sexual phenology (apothecium production) of the fungus is synchronous with the host (*Vaccinium corymbosum*) phenology and fungal populations from early and late blueberry cultivars have distinct phenologies. The purpose of this study was to determine the genetic structure and relationships among mummy-berry populations with similar phenologies. One hundred and twenty isolates representing four populations were evaluated using AFLPs. Thirty isolates from each population were restricted with Pst 1 and ligated to a Pst 1 adapter. The isolates were amplified with five Gibco BRL custom primers with 2-bp extension (GT, GA, GC, AC and CG) at the 3' end. PCR products were separated by electrophoresis using 6.5 % polyacrylamide gels. The primers generated unique fingerprint patterns. Between 10 to 30 bands

were produced by the primers facilitating rapid identification. Fingerprints from three primers (GT, GA, GC) suggest genetic similarity between these four populations congruent with the phenology data. The low level of polymorphisms indicate that these populations are closely related. Several polymorphisms were detected with two primers (AC and CG) that would be used estimate mating systems and gene flow.

189. Relative action of RIP on complementary strands of a duplex and on tandem duplications of various lengths. Michael K. Watters[1], Thomas A. Randall[2], Brian S. Margolin[3], Eric U. Selker[3], and David R. Stadler[4]. [1]Univ of British Columbia, Botany, Vancouver, BC, Canada. [2]Univ. of Ca, Riverside, Plant Pathology, Riverside, Ca, USA. [3]University of Oregon, Biology, Eugene, Or, USA. [4]University of Washington, Genetics, Seattle, Wa, USA.

In *Neurospora crassa*, DNA sequence duplications are detected and altered efficiently during the sexual cycle by a process known as RIP (Repeat Induced Point mutation). To explore the pattern in which base changes are laid down by RIP, we examined two sets of strains. First, we examined the products of a presumptive spontaneous RIP event, that appeared to have been subjected to just a single round of RIP. Results of sequencing suggested that a single RIP event produces two distinct patterns of change, descended from the two strands of an affected DNA duplex. Equivalent results were obtained using an exceptional tetrad from a cross with a known duplication. The mtr sequence data was also used to further examine the basis for the differential severity of Cto T mutations on the coding and noncoding strands in genes. We find that the combination of a bias of RIP toward CA/TG sites in conjunction with the sequence bias of Neurospora can be used reliably to predict the distribution of RIP sites among codon positions, thus fully accounting for the differential effect. Finally, we used a collection of tandem repeats (from 16 to 935 bp in length) withing the mtr gene to examine the length requirement for RIP. No evidence of RIP was found with duplications shorter than 400 bp while all longer tandem duplications were frequently affected. A comparison of these results with vegetative reversion data for the same duplications supports the idea that reversion of long tandem duplications and RIP share a common step.

190. Isolation and characterization of *Aspergillus nidulans* delayed conidiation mutants. Jenny Wieser, and Thomas H. Adams. Texas A&M; University, Biology, College Station, TX, USA.

The formation of conidiophores in *A. nidulans* is a precisely regulated event that occurs within the context of a radially expanding colony. Conidiophores typically differentiate 1-2 mm behind the leading edge of vegetatively growing hyphae. We have previously characterized four *A. nidulans* genes *flbB, flbC, flbD*, and *flbE* that result in fluffy colonies that are delayed at least 24 hours in their ability to form conidiophores. This delay in conidiophore initiation gives rise to colonies in which conidiophore development occurs 12-15 mm behind the leading edge of the radially growing hyphae. *flbB, flbC, flbD*, and *flbE* were four of the six **f**luffy **l**ow ***b****rlA* (FLB) expression mutants isolated in our original screen that identified 122 fluffy mutants with altered levels of *brlA* expression. In this screen, the delayed conidiation mutants were the most abundantly isolated phenotype indicating that proper timing of conidiophore development with in the context of a growing colony is a complex process that requires the combined activities of many genes. Twenty-nine delayed conidiation mutants that express higher levels of *brlA* **f**luffy

medium ***b****rlA* (FMB) and **f**luffy **h**igh ***b****rlA* (FHB) but otherwise are phenotypically identical to *flbB, flbC, flbD*, and *flbE* were isolated. These FMB and FHB delayed conidiation mutants represent at least 4 new alleles and multiple alleles of three of the genes were isolated. Sequence analysis of three of these new delayed conidiation mutants is in progress and their genetic interactions with the previously characterized fluffy mutants *flbA, fluG, flbB, flbC, flbD*, and *flbE* will be presented.

191. Sugar sensing and conidiation in Neurospora. Xin Xie, Jed Parker, and Daniel J. Ebbole. Texas A&M; University, Plant Pathol. & Microbiol, College Station, Texas, USA.

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

192. Evolutionary divergence and recombination in the human fungal pathogen *Cryptococcus neoformans*. Jianping Xu[1], Rytas Vilgalys[2], and Thomas G. Mitchell[1]. [1]Duke University, Microbiology, Durham, North Carolina, USA. [2] Duke University, Botany, Durham, NC, USA.

We applied a gene genealogical approach to investigate the evolutionary relationships among strains of the opportunistic human pathogen, *Cryptococcus neoformans*. Four genes, one from the mitochondrial genome (mtLrDNA) and three from the nucleus (ITS, Laccase and Ura 5), were sequenced for 34 strains obtained from diverse geographic locations. The strains included representatives of both varieties (vars. neoformans and gattii) and all 4 major serotypes (A,B,C,D). Phylogenetic analyses of individual gene sequences demonstrate considerable molecular divergence which largely concordant with traditional classifications of varieties and serotypes. However, significant incongruencies were observed among all gene genealogies. Both permutation compatibility test and partition homogeneity test provided evidence for recombination within and between varieties and among serotypes. Gene phylogenies also show little evidence for geographic patterns of molecular variation, which suggests that this species has the ability for long distance dispersal.

193. Molecular analyses of mating type loci of Gibberella/Fusarium spp. and *Hypocrea spinulosa* (*Chromocrea spinulosa*) .S-H. Yun[1], T. Arie[2], M. Klein [3], S-H Lee[1], O. Yoder[1], and G. Turgeon[1]. [1]Cornell, Plant Pathology, Ithaca, NY, USA. [2] RIKEN, Japan. [3]UC Davis, Davis, CA, USA.

We are examining structural and functional organization of *MAT* in heterothallic, homothallic and asexual members of the *Gibberella/Fusarium* group. The idiomorphs of heterothallic *G. fujikuroi* (*F. moniliforme*) have been cloned and sequenced. *MAT-1* encodes three genes (*MAT-1-1, MAT-1-2, MAT-1-3*), while *MAT-2* encodes one (*MAT-2*); all four are counterparts of the

*MAT*genes encoded by *Neurospora crassa* and *Podospora anserina*. Asexual *F. oxysporum* encodes sequences corresponding to the four *F. moniliforme MAT*genes; the *MAT-2* (*Nc mat a-1, Pa FPR1*) and *MAT-1-1* (*Nc mat A-1, Pa FMR1*) genes have no obvious mutations, *MAT-1-2* (*Nc mat A-2, Pa SMR1*) and *MAT-1-3* (*Nc mat A-3, Pa SMR2*) sequences have several frame shift mutations. Seven diverse *MAT-1* field isolates were sequenced and found to carry the same mutations. Heterologous expression studies of *F. oxysporum* genes in *F. moniliforme* are underway to test if they are functional. The *MAT* locus of homothallic *G. zeae* (*F. graminearum*) has been partially sequenced and a *MAT-2* gene identified. Studies of *MAT* organization in the *Gibberella/Fusarium* group form the foundation for unraveling molecular mechanisms of mating in fungi that exhibit non-standard mating behavior. One example is *Hypocrea spinulosa* (*Chromocrea spinulosa*). Strains of this fungus are either strictly heterothallic or are homothallic. Crosses of heterothallic isolates by homothallic isolates or selfs of homothallic isolates produce 50% heterothallic and 50% homothallic progeny. Using sequence information from closely related *Gibberella/Fusarium* we have partially cloned and sequenced *MAT* genes of a homothallic *H. spinulosa* isolate and identified both *MAT-2* and *MAT-1-1* genes. Comparison of *MAT* from homothallic versus heterothallic strains will be used to analyze the mechanism by which this fungus produces both heterothallic and homothallic progeny from a single homothallic progenitor.

194. Mapping vegetative incompatibility (*vic*) loci in *Gibberella fujikuroi* (*Fusarium moniliforme,* MP-A) with AFLP markers. Kurt A. Zeller[1], James E. Jurgenson[2], and John F. Leslie[1]. [1]Kansas State University, Dept. of Plant Pathology, Manhattan, KS, USA. [2]University of N. Iowa, Dept. of Biology, Cedar Falls, IA, USA.There are 8-10 vegetative incompatibility (*vic*) believed to be segregating in the established mapping population of *Gibberella fujikuroi* (*Fusarium moniliforme*, MP-A). Selection for vegetative compatibility between two strains should simultaneously select for identity in all segregating *vic* loci and should skew segregation ratios for linked neutral markers that flank each *vic* locus. We crossed two *nit1*- strains (A0924 and A4644) derived from the mapping population's parental genetic backgrounds, and selected progeny from that cross that could form heterokaryons with individuals of known AFLP / RFLP genotype. We collected these heterokaryons, resolved each to recover the *nit1*-component, and retested each isolate to confirm its ability to form a heterokaryon. Once confirmed, we retained these isolates for AFLP analyses. We have used AFLP markers to localize genomic regions that display skewed segregation ratios in the vegetatively compatible progeny of this cross. In our preliminary analysis, we have identified 8-9 genomic regions that display segregation ratios significantly (alpha < 0.05) skewed from 1:1. Such regions are found on at least 7 of the 12 linkage groups of *G. fujikuroi*.

195. AFLP markers reveal genetic variation in Egyptian populations of *Cephalosporium maydis*. Kurt A. Zeller[1], James E. Jurgenson[2], and John F. Leslie[1]. [1]Kansas State University, Dept. of Plant Pathology, Manhattan, KS, USA. [2]University of N. Iowa, Dept. of Biology, Cedar Falls, IA, USA.*Cephalosporium maydis* causes late wilt; perhaps the most serious fungal disease of maize in Egypt. Genetic and pathogenic variation among isolates, and population structure in this fungal pathogen are poorly understood. Our objective was to identify molecular markers for use in studies of this pathogen. We characterized genetic diversity in *C. maydis* with AFLP (amplified fragment length polymorphism). We made AFLP fingerprints of 48 strains (10 reference + 38 field isolates) with 68 selective primer pair combinations. Average UPGMA

similarity among all strains was about 89%. Statistical analyses indicate that these 48 strains cluster into four distinct subgroups (lineage groups), each containing 7-19 of the isolates. Pairwise UPGMA similarities within each lineage are generally greater than 95%, while similarities between these lineages are less than 91%. We have identified a subset of these selective AFLP primer-pairs that produce estimates of genetic distances that are highly correlated (r> 0.85) to that of the pooled data. We have begun to use data from these AFLP primer-pairs to characterize variation among additional *C. maydis* samples.

196. A long terminal repeat retrotransposon Cgret from the phytopathogenic fungus *Colletotrichum gloeosporioides* on cranberry. Peiliang Zhu, Peter V. Oudemans. Rutgers University, Plant Pathology, Chatsworth, New Jersey, USA.

A repetitive DNA element cloned from the cranberry fruit rot pathogen *Colletotrichum gloeosporioides* has been characterized. Sequence data indicate that it is a long terminal repeat (LTR) retrotransposon of 7916 base pairs in length. Long terminal repeat sequences of 544 base pairs occur at either end of an internal region 6828 base pairs in length. This element, designated Cgret (*Colletotrichum gloeosporioides* retrotransposon), encodes two putative polypeptides that have high homology to the gag and pol genes of other fungal retrotransposons. The sequence and structure suggest that Cgret is a member of the gypsy group of LTR retrotransposons. The Cgret retrotransposon was present in all of the cranberry isolates of the fungus C. gloeosporioides from New Jersey and Massachusetts, but not in the cranberry isolates from Wisconsin or Chile. None of the genomic DNA samples from isolates of the related fungus *Colletotrichum acutatum* hybridized with probes from this transposable element. Polymorphisms were detected among field isolates of *C. gloeosporioides* from various hosts using hybridization probes derived from the long terminal repeat and the reverse transcriptase domain of Cgret. The structural integrity of Cgret suggests that it is still a functional retrotransposon and may be used as a molecular marker to study the distribution of fungal genetic diversity.

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197. A viral infection upregulates fungal virulence in *Nectria radicicola*. Il Pyung Ahn. Seoul National University, Agricultural Biology, Suwon, Kyunggi, South Korea.

Four distinct sizes of double-stranded RNA (dsRNA) molecules, 6.0, 5.0, 2.5, and 1.5kbp, were detected from 24 out of 81 isolates of *Nectria radicicola* (anamorph: *Cylindrocarpon destructans*), the causal fungus of ginseng (*Panax ginseng*) root rot. They are present singly or in combinations. Curing tests of each dsRNA molecule suggested that the presence of 6.0kbp dsRNA is responsible for increased virulence, sporulation ability, laccase activity, and pigmentation in this fungus. To understand the role of this dsRNA molecule further, 6.0kbp dsRNA was reintroduced to cured isolates (dsRNA free) by hyphal anastomosis. Acquisition of this dsRNA molecule by dsRNA free isolates recovered all virulence-related phenotypes. Ultrastructural observation of mycelia by transmission electron microscope also supported the

physiological changes by curing and reintroduction of this dsRNA molecule. These results are clear evidence that 6.0kbp dsRNA upregulates fungal virulence in *N. radicicola* by demonstrating the cause and effect relationship. To characterize this 6.0kbp dsRNA at molecular level, cDNA library was constructed. Sequencing of several cDNA clones revealed that this molecule harbors RNA-dependent RNA polymerase (RDRP) gene. Phylogenetic analysis of this gene to other RDRP genes indicated that this gene is closely related to those of plant cryptic viruses. Although this dsRNA molecule is believed to be the genome of fungal virus, all efforts to detect typical virus particles were failed. We are in progress to elucidate the biochemical mechanisms how 6.0kbp dsRNA upregulates fungal virulence in *N. radicicola*. Preliminary data suggested that this dsRNA molecule is involved in the regulation of cAMP-dependent protein kinase.

198. Characterization of ABC transporters from *Aspergillus nidulans*. Alan C. Andrade[1], Johannes G.M. Van Nistelrooy[1], Giovanni Del Sorbo[2], Paul L. Skatrud[3], and Maarten A. De Waard[1]. [1]Wageningen Agricultural University, Lab. of Phytopathology, Wageningen, , The Netherlands. [2]University of Naples, Patologia Vegetale, Portici, NA, Italy. [3] Eli Lilly and Company, Infectious Diseases, Indianapolis, IN, USA.

ATP-**B**inding **C**assette (ABC) transporters constitute a large protein superfamily with members involved in **m**ulti-**d**rug **r**esistance (MDR) in a variety of organisms, ranging from bacteria to human. These proteins are transport ATPases which may have a wide range of substrates such as natural products (*e.g.*antibiotics or mating pheromones) or xenobiotics (*e.g.* drugs or fungicides). We use the saprophytic wild-type fungus *Aspergillus nidulans* and azole-resistant mutants to study the role of ABC transporters in fungicide sensitivity and MDR. Four single copy genes, designated *atrA* (**A**BC **tr**ansporter A), *atrB*, *atrC* and *atrD* were cloned and characterised. The deduced proteins contain two groups of six transmembrane regions (TM6) and two nucleotide binding folds (NBF). The topology of AtrAp and AtrBp is (NBF-TM6)2 whereas that of AtrCp and AtrDp (TM6-NBF)2. By Northern analysis experiments, a number of compounds were identified that upregulate the expression of specific *atr* genes. We have also found a differential effect of these compounds on transcription of the *atr* genes among the isolates tested. Single knock-out mutants of all four *atr* genes have been generated and the drug-sensitivity phenotype to several chemicals analysed. For two of these mutants, *delta atrB* and *delta atrD*, a hypersensitive phenotype to some chemicals has been observed suggesting a role of these ABC proteins as drug tranporters. The characterization of the physiological function of the *atr*genes is also of interest and a putative role in the secretion of secondary metabolites and fitness under stress-conditions is being investigated. The results indicate that ABC transporters can play an important role in protection of the fungus against naturally toxic compounds and xenobiotics. Current research focuses on the generation of overexpressing mutants and multiple knock-outs of *atr* genes.

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199. Sequences expressed primarily during filamentous growth in *Ustilago maydis*. David L. Andrews, Scott E. Gold. University of Georgia, Plant Pathology, Athens, Georgia, USA.

The corn smut pathogen (*Ustilago maydis*) exhibits dimorphism in that the haploid stage grows in a budding, yeast-like state while the dikaryon develops a filamentous morphology before/during pathogenic infection. The filamentous form of *U. maydis* requires an interaction

with the maize host before the teliospore-containing galls are formed. This dimorphic transition has been associated with both the cyclic AMP pathway and the MAP kinase pathway. We have employed the technique of suppressive-subtractive hybridization to isolate sequences differentially expressed in the filamentous form of *U. maydis*. RNA for these experiments was isolated from a well-characterized, constitutively filamentous, haploid adenylate cyclase (uac1) mutant and from a wild-type budding haploid. This method has yielded greater than thirty clones which were more highly expressed in the target tissue. Of the clones identified so far, one was found to be the previously reported, filament specific, rep1 sequence. The characterization of other clones obtained by this method will be discussed.

200. Characterization of a low pathgenicity mutant of *Botrytis cinerea*.Anne Vidal-Cros[1], Florence Chapeland[2], Marie-Christine Soulié[2] Martine Boccara[2,3] .[1]Chimie Organique Biologique, [2]Biochimie et Pathologie Végétales Université Pierre et marie Curie - 4, place Jussieu - 75252 PARIS CEDEX 05 - FRANCE. [3]Pathologie Végétale INA P/G 16 rue Claude Bernard 75005 PARIS FRANCE

Botrytis cinerea is a pathogenic fungus which attacks a broad range of plants. It provokes grey mould on grapevine and is responsible of losses on several other crops. Fungicides are used to control this pathogen but become less effective because of fungicide resistance in fungal population. To develop new strategies against *B cinerea* it is important to decipher the pathogenicity mechanisms of this fungus. We first developed tools to approach molecularly the pathogenicity of *B cinerea. B cinerea* can be transformed (1) and Faretra et al. (2) showed that it is possible to cross strains in the laboratory. We have also observed, as others, that homologous recombination can occur at a frequency of 20% which is encouraging for gene disruption experiments. We are testing the role of cell wall biosynthesis, in pathogenicity with two approaches: on one hand, an a priori approach involving chitin synthase gene disruption (see poster of MC Soulie) and on the other hand, random mutagenesis using REMI method. Results and discussion We have isolated a mutant (G32) among a collection of transformants with the pAN7-1 plasmid (hygromycin resistance) which is poorly pathogenic on several hosts, like apple fruit or vine leaf. However, this mutant grows in vitro similarly to the wild type strain. In addition, G32 is resistant to calcofluor, a character that has been associated in the case of yeast with an impairment in chitin biosynthesis. G32 was found to synthesize as much chitin as the wild type strain and to exhibit the same chitin synthase activity. These results are consistent with the observation that the chitin synthase genes are not disrupted. Segregation analysis suggests that calcofluor resistance and low pathogenicity are cosegregating in G32. Analysis of culture filtrates of the mutant shows that it produces 5 times more oligosaccharides than the wild type strain, suggesting that the low pathogenicity phenotype is due to hyperproduction of plant defence reactions elicitors. These oligosaccharides are not chitooligomers, their further charcterization is in progress. Altogether, this piece of work describes a new type of calcofluor-resistant mutant.

201. Fungal recognition of legume flavonoids: potential action through inhibition of cAMP phosphodiesterase activity. Savita Bagga. University of Maryland, Cell Biol. & Molec. Genet, College Park, MD, USA.

Flavonoids are exuded by legume roots and seeds as a host-specific signal for initiating interaction with symbiotic rhizobial bacteria. Recent studies in a number of systems suggest that soilborne fungi which interact with legumes may similarly use the flavonoids as cues for host recognition. We have described germination of the macroconidia of pea pathogenic strains of *Nectria haematococca* MPVI (anamorph *Fusarium solani*) in response to specific flavonoids which induce *nod* genes in the pea-specific rhizobia. Germination is also induced by the pea isoflavonoid phytoalexin pisatin, which is similarly exuded from pea roots. Previous inhibitor studies indicated that flavonoid-responsive germination in *N. haematococca* involves the cAMP pathway. This is supported by an observed rise in cAMP levels upon exposure of spores to flavonoid. Common models of the cAMP pathway would suggest that the flavoinoids are recognized by a receptor. We present an alternate hypothesis, that flavonoids may increase cAMP levels by direct inhibition of cAMP phosphodiesterase activity. The cAMP phosphodiesterase (PDE) activity was isolated from *N. haematococca* spores. Naringenin, a flavanone which induces germination, displays very strong inhibition of PDE activity, with a K_i close to the K_M of PDE for cAMP. A range of flavonoids were compared for germination stimulation and inhibition of PDE activity. The results indicate a general correlation of specificity. This suggests that inhibition of cAMP hosphodiesterase may be the molecular component of *N. haematococca* which provides flavone and flavanone recognition and integration into the cAMP pathway. The entry point for pisatin, an exception in the correlation, may be a different component.

202. Molecular basis of mitochondrial hypovirulence in a *Cryphonectria parasitica* strain obtained from nature. Dipnath Baidyaroy, David H. Huber, Dennis W. Fulbright and Helmut Bertrand[1], Department of Botany and Plant Pathology and Department of [1]Microbiology, Michigan State University, East Lansing, MI 48823.

Although dsRNA viruses often are hypovirulence agents in the chestnut blight fungus *Cryphonectria parasitica*, many hypovirulent strains contain no viruses but instead have dysfunctional mitochondria. KFC9 is one such strain which was isolated from a healing canker on a tree in Michigan. Analysis of the mitochondrial DNA (mtDNA) of KFC9 and its derivatives (virulent strains rendered hypovirulent by hyphal contact with KFC9) revealed that a particular region of the mtDNA of KFC9 is responsible for the infectious hypovirulence phenotype. That region of the mtDNA contains the small subunit rRNA gene with an inserted piece of DNA (InC9) located just 67-bp downstream of the beginning of the mature rRNA transcript. InC9 is 973-bp long, does not resemble introns or transposons, has no sizable open reading frame and does not have homology to any existing sequence. Molecular analyses have revealed that InC9 is either not spliced or spliced with great difficulty from the precursor rRNA. As a result, KFC9 is deficient in the assembly of mitochondrial ribosomes. The molecular basis of the infectious nature of InC9 is not clear at this time. The KFC9 strain was also found to contain an infectious 1366-bp long, circular, plasmid-like element (PLE), parts of which have been derived from the mtDNA. Loss of this PLE gives rise to a slightly faster-growing culture which remains hypovirulent. The observations indicate that InC9, rather than the PLE causes the infectious mitochondrial hypovirulence syndrome found in KFC9. Supported by USDA grant 95-37303-1785 and MAES Project No. MICL01662.

203. Analyses of pathogenicity by *Mycosphaerella* pathogens of bananas.Peter J. Balint-Kurti[1], Mi Jin[2], Alice C. L. Churchill[l], Jon C. Clardy[2], Gregory D. May[1]. [1]Boyce Thompson Institute for Plant Research, Molecular Mycology Center, Ithaca, NY, USA. [2]Cornell University, Chemistry/Chem. Biology, Ithaca, NY, USA.

A genetic transformation system was developed for three related fungal pathogens of banana, *Mycosphaerella fijiensis* and *M. musicola*, causal agents of Black Sigatoka and Yellow Sigatoka disease, respectively, and the newly discovered pathogen *M. eumusae* (J. Carlier, personal communication). Stable transformants expressing green fluorescent protein were isolated from all three species, and growth on susceptible hosts was examined. Growth both on the surface and within leaves was observed. Leaf penetration was exclusively via stomata. Profuse necrotrophic growth was observed in dead leaf tissue. Evidence for the production of both a diffusible toxin and a self-inhibitor of conidiospore germination was noted. Growth of transformants on the non-host plants tomato, tobacco, and *Arabidopsis* was also characterized. Extracts were prepared from mycelium and culture fluids of the *Mycosphaerella* pathogens and screened for plant toxicity. Several fractions from *M. musicola* exhibited non-specific phytotoxicity, and in some cases the toxicity was light-activated, as revealed by a banana cell culture-based assay. Preliminary results suggest that *M. musicola*, which produces an anthraquinone-derived photoactivated toxin, is more resistant to singlet oxygen-generating photosensitizers than is *M. fijiensis*, which does not produce the toxin in detectable amounts.

204. Analysis of pathogenicity and genome organization in *Ophiostoma ulmi* and *O. novo-ulmi*. Louis Bernier[1], Clive M. Brasier[2], Josee Dufour[1], Mathieu Dusabenyagasani[1], and Abdelali Et-Touil[1]. [1]Universite Laval, C.R.B.F., Sainte-Foy, Quebec, Canada. [2]Forest Research, Alice Holt Lodge, Farnham, Surrey, U.K.

Ophiostoma ulmi and *O. novo-ulmi* are the heterothallic ascomycetes responsible for the two highly destructive pandemics of Dutch elm disease that have occurred in this century. We are developing a genetic map of these pathogens by mendelian analysis of RAPD polymorphisms in the F_1 progeny from a *O. novo-ulmi* x *O. ulmi* cross. Close to 150 RAPD loci have so far been mapped on 10 linkage groups. Pulsed-field gel electrophoresis has allowed us to detect chromosome polymorphisms in *O. ulmi* and *O. novo-ulmi* and to physically separate 7-8 chromosomes depending on the strain analyzed. Field inoculations of moderately resistant English elms with F_1 progeny of a cross between two *O. novo-ulmi* strains differing in pathogenicity established that the difference in phenotype between these strains was controlled by a single nuclear locus. We have designated this locus *Pat1*, as it is the first putative pathogenicity gene identified in *O. novo-ulmi*. Southern hybridizations with probes prepared from RAPD markers linked to *Pat1* showed that *Pat1* was located on a 3.5 Mb chromosome. Furthermore, our results suggest that the *Pat1*-m allele conferring lower pathogenicity may have been acquired from *O. ulmi* via introgression.

205. Avirulence genes cloning in the rice blast fungus *Magnaporthe grisea*.Heidi Bohnert[1], Waly Dioh[2], Didier Tharreau [3], Jean-Loup Notteghem[4], and Marc-Henri Lebrun[1]. [1]UMR41 CNRS-RPA, Physioloige Cellulaire, Lyon, 69009, France. [2]Universite Paris-Sud, IGM, Orsay, 91405, France. [3]CIRAD, CA, Montpellier, 34032, France. [4] ENSAM, Pathologie Végétale, Montpellier, 34032, France.

Genetic studies with *M. grisea* isolates pathogenic on rice and fertile in crosses revealed three genetically independent avirulence genes [1] that are likely to interact with so far undescribed rice resistance genes. We initiated the positional cloning of these three genes using RAPD markers screened by bulk segregant analysis and RFLP markers. Two avirulence genes mapped near chromosome ends (avr1-MedNoï and avr1-Ku86). The avirulence gene avr1-Irat7 mapped on chromosome one at 30 cM from avr1Co39. We identified 11 RAPD markers closely linked to these avirulence genes (0-10 cM). Most RAPD markers corresponded to junction fragments between M. grisea genome and known transposons [2]. Positional cloning was started by the screening of a cosmid genomic library from an avirulent progeny by hybridisation with single copy sequence RAPD markers. For RAPD markers containing repeated sequence, we performed a RAPD analysis on cosmid pools and clones [3]. Cosmids contigs were constructed for these three loci. Starting from RAPD marker OPE-Y13 completly linked to avr1-Irat7, we constructed a contig of 100 kb. Two cosmids from this contig conferred avirulence on rice cultivar Irat7 when introduced by transformation in a virulent recipient strain, defining a 30 kb region where avr1-Irat7 is located. Up to now, we have not found a smallest subclone from this region able to complement virulence. The two other contigs are in the process of extension towards avirulence genes. 1. Silue D. *et al.* 1992. Phytopathology 82: 1462-1467. 2. Dioh W. *et al.* 1996. In Rice Genetics 3, IRRI, pp. 916-920. 3. Dioh W. *et al.* 1997. Nucleic Acid Research 25: 5130-5131

206. Transfer of *Neurospora kalilo* plasmids among species and strains by introgressions. Jin-Woo Bok, Cynthia He, and Anthony J.F. Griffiths. University of British Columbia, Department of Botany, Vancouver, BC, Canada.

There are four different variants of the kalilo "family" of linear mitochondrial plasmids. The family is found in several heterothallic species and one pseudohomothallic species of Neurospora, and in one homothallic species of Gelasinospora. The mode of dispersal of these plasmids is not known. Horizontal transmission has proved difficult to demonstrate. Another possibility is transfer by introgression, and this is modelled in this paper. We have used introgression and subsequent heterokaryosis to successfully transfer the prototypic kalilo plasmid from a Haitian strain of *N. crassa* to the standard Oak Ridge N. crassa background, the LA-kalilo plasmid from the pseudohomothallic *N. tetrasperma* to *N. crassa*, and the kalilo plasmid from *N. crassa* to *N. tetrasperma*. Thus introgression is a possible avenue of dispersal between species. The recipient strains were all senescent but the mechanism of this senescence is not known. It could be caused by the plasmids, but if so the mechanism is novel since plasmid/mtDNA junction fragments of the type found in the standard mode of mtDNA insertion could not be detected. However, mtDNA changes are observed in the senescent recipients.

207. Identification of a polyketide synthase gene (*pksP*) of *Aspergillus fumigatus* involved in conidial pigment biosynthesis and virulence. Axel A. Brakhage[1], Kim Langfelder [1], Axel Schmidt[2], Gerhard Wanner[3], Heike Gehringer[1], Sucharit Bhakdi [4], and Bernhard Jahn [4]. [1]Institut für Mikrobiologie und Genetik, Technische Universität Darmstadt, D-64287 Darmstadt, F.R.G.; [2]Institut für Chemotherapie, D-42096 Wuppertal, F.R.G.; [3]Botanisches Institut, Universität München, D-80338 München, F.R.G.; [4]Institut für Medizinische Mikrobiologie, Universität Mainz, D-55101 Mainz, F.R.G. .

Aspergillus fumigatus is an important pathogen of the immunocompromised host, causing pneumonia and invasive disseminated disease. We have tried to identify factors which contribute to conidial survival in the host, which is essential for causing disease. An *A. fumigatus* mutant strain ("white", W), lacking conidial pigmentation, was isolated by UV-mutagenesis. Scanning-EM revealed the surface morphology to be different from that of the wild type (WT). Luminol-dependent chemiluminescence was ten-fold higher when human neutrophils or monocytes were challenged with W conidia, compared to WT conidia. In addition, W conidia were more efficiently damaged by monocytes in *vitro* and they were more sensitive to attack by oxidants in *vitro*. In a murine mouse model the W mutants exhibited reduced virulence. A genomic cosmid library was used to clone a gene complementing the W phenotype. Because it encodes a putative polyketide synthase, it was designated *pksP* (polyketide synthase involved in pigment biosynthesis). The W mutant complemented with *pksP* displayed all the WT characteristics, indicating that the conidial pigment is of major importance for virulence.

208. Propionate metabolism of *Aspergillus nidulans*: Characterization of the methylcitrate synthase. Matthias Brock[1], Reinhard Fischer[2], and Wolfgang Buckel[1]. [1]Philipps-Universität Marburg, Microbiology, Marburg, Hessen, Germany. [2]Max-Planck Institute, Biochemistry, Marburg, Hessen, Germany.

Propionate is used as a preservative against molds in food, grain-silage and cell cultures. *Aspergillus nidulans*, as a model organism for eucaryotic research, is able to grow on propionate as sole carbon and energy source. *A. nidulans* metabolises propionate via the methylcitrate pathway which was discovered in *Candida lipolytica* and more recently in *Escherichia coli*. The methylcitrate synthase as a key enzyme of this pathway was purified and biochemically characterized. The protein was shown to consist of two identical subunits (45 kDa each). The methylcitrate synthase can use both propionyl-CoA or acetyl-CoA for the condensing reaction with oxaloacetate to form methylcitrate or citrate, respectively. A DNA probe was derived from the *N*-terminal sequence. A genomic cosmidbank was screened and a corresponding 3.2 kb restriction fragment was sequenced. By analysing the reverse transcribed cDNA sequence two introns were found. The derived amino acid sequence showed more than 50% identity to most eucaryotic citrate synthases and revealed a mitochondrial import leaderpeptide. The *mcs* gene was fused with the *alc*A promotor of *A. nidulans* and overexpressed in the same organism. The overproduced protein was purified and showed the same activity as the original methylcitrate synthase. A deletion mutant of the *mcs* gene was constructed. The mutant did not show any residual growth on propionate as sole carbon and energy source, proving that the methylcitrate pathway is crucial for propionate metabolism in *A. nidulans*.

209. Withdrawn

210. Complementation of an *Aspergillus nidulans* mutant defective in *aflR* expression. Robert A.E. Butchko[1], Thomas H. Adams[2], and Nancy P. Keller[1]. [1]Texas A&M; University, Plant Pathology and Microbiology, College Station, TX, USA. [2]Texas A&M; University, Biology, College Station, TX, USA.

aflR encodes a conserved pathway specific transcription factor that regulates the expression of gene clusters involved in the biosynthesis of aflatoxin by *Aspergillus flavus* and *A. parasiticus*

and sterigmatocystin (ST) by *A. nidulans*. We have already shown that *aflR* expression is developmentally regulated in *A. nidulans*, mutants that fail to conidiate are also defective in *aflR* expression. In an effort to more fully understand the regulation of the production of ST by *A. nidulans*, we have identified *A. nidulans* mutants that no longer express *aflR* transcript but retain wild type development. These mutants were easily identified by looking for strains that no longer accumulated the orange colored intermediate in the ST pathway, norsolorinic acid (NOR). One of the mutant strains contained a single locus mutation unlinked to the ST cluster. Transformation of this strain with a wildtype cosmid library returned the strain to NOR production and also restored the production of the green conidia. This preliminary data suggests that the gene responsible for regulating *aflR* expression is linked to *wA*. Here we describe the complementation and characterization of one of these mutants.

211. Genetic, morphological and biochemical investigations of a "cryptic species" within *Aspergillus flavus*. Dee A. Carter[1], and John I. Pitt[2]. [1]University of Sydney, Microbiology, Sydney, New South Wales, Australia. [2]Food Science Australia, Food Mycology, Sydney, New South Wales, Australia.

Independent studies undertaken by Geiser et al (PNAS 95:399-393, 1998) using DNA sequencing, and Tran-Dinh et al (in press) using RAPD analysis, found that isolates of *Aspergillus flavus* from Australian peanut-growing soils divided into two very distinct groups. In both studies the two groups were uneven, with the larger group (Group 1) containing approximately 70% and 60% of isolates, respectively. *A. oryzae* isolates also fell into this group, and both groups were distinct from *A. parasiticus*. The current study was undertaken to investigate the difference between these two groups, and to analyse isolates belonging to the smaller group (Group 2) more thoroughly. Morphological, biochemical and genetic tests were able to clearly separate the two groups. Group 1 appears to be the "standard" *A. flavus*, with a ubiquitous distribution, but Group 2 seems restricted in distribution and may represent a new species of *Aspergillus*.

212. The influence of surface characteristics on contact-mediated spore germination in *Colletotrichum graminicola*. Jennifer L. Chaky and Lisa J. Vaillancourt. University of Kentucky, Plant Pathology, Lexington, KY, USA.

Spore germination is an essential step in the disease cycle of most fungal pathogens, yet little is known of the molecular mechanisms regulating this process. Conidia of *Colletotrichum graminicola* can easily be produced in culture, and they germinate efficiently (between 90 and 98%) within five hours when placed in drops of plain, sterile water on a hard, hydrophobic surface. Spore germination was measured on a variety of surfaces using this bioassay. A strong positive correlation was observed between germination rate and surface hydrophobicity (r = 0.938). Spores which remained ungerminated in the assays were arrested at a point after spore hydration but prior to septation. The hypothesis that stronger adhesion of the spores to more hydrophobic surfaces is related to the increase in germination on those surfaces is presently being tested. Various dilute salts in the water drops inhibited germination of spores on otherwise inductive surfaces, perhaps by disrupting the adhesive interactions between the spore and the surface. Several mutants with altered germination phenotypes in the bioassay have been

identified, and characterization of these mutants is underway in an effort to learn more about the mechanism of contact-mediated spore germination in *C. graminicola*.

213. The development of DNA-mediated transformation of *Erysiphe graminis* f.sp. *hordei*. Pushpalata T. Chaure, Sarah J. Gurr and Pietro Spanu. University of Oxford, Plant Sciences, Oxford, Oxford, UK.

The barley powdery mildew fungus, *Erysiphe graminis* f.sp. *hordei* is the obligate biotroph which is responsible for the most consistently damaging foliar disease of barley. The role in pathogenicity of stage-specific genes and the identification of avirulence genes requires functional validation by modulation or ablation of the respective genes. For this reason there is a need to develop technologies for the stable genetic transformation of *Erysiphe*. We have used two selectable markers, a mutant beta-tubulin gene and a BAR gene to attempt stable DNA-mediated transformation of *E. graminis*. Mutations E 198 A and F 200 Y in *E. graminis* beta-tubulin result in benomyl resistance. The introduction of this mutated beta-tubulin confers resistance to benomyl in *Erysiphe*. Rare stable transformants have been identified. The *Streptomyces* BAR gene was cloned downstream of the beta-tubulin promoter and this construct was used to transform *E. graminis in planta*, to confer resistance to the herbicide BASTA. The data from these studies will be presented.

214. Molecular cloning and biochemical analysis of two genes for HC-toxin biosynthesis in *Cochliobolus carbonum*. Yi-Qiang Cheng and Joong-Hoon Ahn. Michigan State University, DOE Plant Research Lab, East Lansing, MI , USA.

HC-toxin, a cyclic tetrapeptide produced non-ribosomally by *Cochliobolus carbonum* race 1, is the disease determinant of Northern corn leaf spot. It is also cytostatic against mammalian cells by inhibiting histone deacetylase. A major goal of research in our lab is to clone and characterize all of the necessary genes for toxin biosynthesis and transport. So far four genes have been identified. *HTS1* encodes a 570-kDa tetrapartite cyclic peptide synthetase; *TOXA* encodes an efflux carrier; *TOXC*, a fatty acid synthase (FAS) beta subunit; and *TOXE*, a regulatory protein. All these genes are present only in toxin-producing isolates (Tox2+) of *C. carbonum*, and map to a single 3.5-Mb chromosome within a ca. 550 Kb region (called the *Tox2* locus). Here we present the cloning of two new clustered genes (*TOXF* and *TOXG*) with possible roles in HC-toxin biosynthesis. They were cloned by screening a cDNA library with a BAC clone that covers part of the *Tox2* locus. Both of them are unique to $Tox2^+$ isolates of *C. carbonum*. *TOXF* is predicted to encode a branched-chain amino acid aminotransferase (BCAT); *TOXG* likely encodes an alanine racemase (AR). Targeted gene-disruption/deletion of *TOXF* or *TOXG* abolished the ability to produce HC-toxin and pathogenicity on susceptible maize lines. Both BCAT and AR are being overexpressed in *E. coli*, and the biochemical reactions catalyzed by the two proteins will be investigated.

215. The chestnut pathogen *Cryphonectria parasitica* exhibits resistance to singlet oxygen-generating photosensitizers. Alice C. L. Churchill. Boyce Thompson Institute for Plant Research, Molecular Mycology Center, Ithaca, NY, USA.

The orange and yellow aromatic polyketide pigments of *Cryphonectria parasitica* comprise 5-10% of the dry weight of the fungus and, as such, represent a significant proportion of its biomass. A review of the literature indicates that these anthraquinone-derived pigments exhibit antimicrobial and antiviral activities, as well as causing induction of apoptosis, DNA damage, and inhibition of signal transduction pathway enzymes in mammalian cells. Quinones represent the largest class of redox cycling compounds and have the potential to be highly toxic through the formation of oxygen radicals. Many species of *Cercospora*, which produce the photoactivated perylenequinone toxin cercosporin, are resistant to photosensitizers that generate singlet oxygen. The gross similarities in chemical structure between cercosporin and the *C. parasitica* pigments prompted us to test the chestnut pathogen for resistance to photoactivated dyes that produce singlet oxygen. We have demonstrated that wild type *C. parasitica* is resistant to several singlet oxygen-generating photosensitizers, whereas an albino mutant is significantly more susceptible to the same light-activated compounds. These results suggest that *C. parasitica* has an active defense mechanism against singlet oxygen-generating compounds, perhaps linked to its own production of similar molecules. We are interested in determining whether the *C. parasitica* pigments are involved in the production of toxic reactive oxygen species. Ultimately, our goal is to understand whether the pigments play roles in pathogenicity and/or chemical defense in the tritrophic interactions among the chestnut tree, its fungal pathogen, and the Hypoviruses that suppress both fungal virulence and pigment production.

216. Cloning of pathogenicity genes by insertional mutagenesis in *Magnaporthe grisea*.Pierre-Henri Clergeot[1], Didier Tharreau [2], Jean-Loup Notteghem [3], Stephanie Sibuet [4], Marie-Pascale Latorse [5], and Marc Henri Lebrun[1]. [1]UMR41 CNRS-RPA, Physiologie Cellulaire, Lyon, 69009, France. [2]CIRAD, CA, Montpellier, 34032, France. [3]ENSAM, Pathologie Végétale, Montpellier, 34032, France. [4]Rhone-Poulenc Agro, Biotechnologies, Lyon, 69009, France. [5]Rhone-Poulenc Agro, Fongicides, Lyon, 69009, France.

Unraveling functions implicated in the infection process of plant pathogenic fungi is an important challenge for crop protection in the future. We are searching for pathogenicity genes of the rice blast fungus Magnaporthe grisea using a REMI-based plasmid insertional mutagenesis strategy (1). We analyzed 3000 REMI transformants for their pathogenicity defects by individual spore inoculation on detached rice and barley leaves as a preliminary screen. Their defect in pathogenicity was confirmed by inoculation on rice plants. We recoverd around 25 mutants either non-pathogenic (8) or significantly reduced in their pathogenicity (17). Among ten mutants crossed with a compatible wild type strain, three (M421, M700 and M763) were tagged by one copy of the plasmid. The mutant M700 corresponded to an insertion of the plasmid in the melanine pathway gene buf1. The number of lesions caused by mutant M763 was dramatically reduced compared to wild type (-95%). The non-pathogenic mutant M421 (no lesions) was able to differentiate appressoria with a reduced osmotic pressure. (2) that did not penetrate through the cuticle. Flanking regions to the plasmid were cloned by inverse-PCR for M421 and by plasmid rescue for M763. Two cosmids hybridizing with the M421 flanking region were identified from which a genomic subclone of 3,5 kb complemented the mutation. Cloning and sequencing of its cDNA highlighted a 0,67 kb ORF interrupted two short introns. The 225 aa protein deduced from this ORF is likely to be a new small membrane protein involved in appressorium function. (1) Sweigard et al. (1998), Mol. Plant Micr. Interact., 5: 404-412. (2) de Jong et al. (1997), Nature, 389: 244-245.

217. Supernumerary chromosomes in filamentous fungi. Sarah F. Covert. University of Georgia, Forest Resources, Athens, GA, USA.

Several fungal genomes are known to contain supernumerary chromosomes. These chromosomes are composed primarily of DNA that is present in only a subset of the individuals within a given species, and thus they contain genetic information that is not essential for life. Both the structural and functional characteristics of supernumerary chromosomes make them interesting components of fungal genomes. The structural features of some supernumerary chromosomes suggest that they evolved in a different genetic background than the essential chromosomes in the same genome. Functional genes on the supernumerary chromosomes in at least two species contribute to the disease causing capacity of the isolates that contain them. If a complete understanding of fungal genome structure, function and evolution is to be attained, it will be essential to include analysis of the supernumerary elements within fungal genomes.

218. Development of an efficient system for insertional mutagenesis of *Penicillium chrysogenum*. Katarzyna Czechowska and Ulrich Kück. Ruhr-Universität Bochum, Allgemeine Botanik, Bochum, NRW, Germany.

The filamentous fungus *Penicillium chrysogenum* is of great industrial importance as major producer of penicillin. To elucidate the penicillin biosynthesis in more detail, the identification of genes controlling antibiotic production is essential. One way to identify these regulatory genes is the isolation of mutants affected in penicillin biosynthesis. In order to obtain this type of mutants for genetic analysis we intend to tag genes by insertion of foreign DNA. Here we present the development of an efficient system, known as restriction enzyme-mediated integration (REMI) [1], for insertional mutagenesis of *P. chrysogenum*. We analysed the transformation efficiency in the presence and absence of restriction enzymes during transformation. An increase of transformation efficiency was found up to 9-fold in the presence of the restriction enzyme during transformation and the efficiency varies with the amount of the enzyme used. The mode of plasmid integration was determined by Southern analysis of randomly chosen transformants. Perfect REMI events occur in more than 65 % of the analysed transformants and in more than 35 % of the transformants we found a single integration event. Finally we succeeded in isolating vector flanking sequences from individual transformants by plasmid rescue. Further experiments will be aimed to identify the function of those genes, which are disrupted by the plasmid molecule. [1] Schiestl RH, Petes TD (1991) Proc Natl Acad Sci USA 88: 7585-7589

219. Evidence for a new mating population of *Fusarium subglutinans* from teosinte and maize from Mexico and Central America. Anne E. Desjardins[1], Ronald D. Plattner[1] and Thomas R. Gordon[2]. [1]USDA/ARS, NCUAR, Peoria, IL, USA. [2]University of California, Davis, CA, USA.

Seed samples from maize (*Zea mays* ssp. mays) from Mexico and of teosintes (Zea spp.), the nearest wild relatives of maize, from Mexico and Central America were assessed for infection with Fusarium species. Both maize and four teosinte species were infected with strains similar in morphology to *F. subglutinans* and more than half of the strains were sexually intercompatible. None, however, were sexually compatible with standard testers of *Gibberella fujikuroi* mating

populations B and E, which are the teleomorphs associated with *F. subglutinans* from Graminae worldwide. These data indicate that these strains from Mexico and Central America may comprise a distinct mating population. A female fertile strain from teosinte was also sexually compatible with a strain of *F. subglutinans* that causes pitch canker disease of pines in California, indicating that strains of *F. subglutinans* from teosinte and maize have a close relationship to *G. fujikuroi* mating population H, to which the pine strain has been assigned.

220. The role of *ras* and G_{alpha} homologs in *PTH11*-mediated signaling in *Magnaporthe grisea*. Todd M. DeZwaan, Anne M. Carroll and James A. Sweigard. Dupont, Experimental Station, Wilmington, DE, USA.

PTH11 encodes a novel membrane protein that is required for pathogenicity by the blast fungus *Magnaporthe grisea*. Pth11p appears to be involved in signal perception that leads to infection structure formation. Since *PTH11* has no obvious homologs, we searched for parallels in which an atypical receptor transduces an intracellular signal. Like Pth11p, the *MEP2* ammonium permease of budding yeast appears to function as an atypical receptor. Whereas Pth11p appears to initiate infection structure differentiation in response to cues on the host leaf surface, Mep2p appears to initiate yeast pseudohyphal differentiation in response to ammonium starvation. The defects of *M. grisea pth11* strains and *S. cerevisiae mep2* strains are both suppressed by exogenous activation of intracellular signaling with cAMP. Based on these similarities we hypothesize that the function of Pth11p during pathogenesis may be analogous to that of Mep2p during yeast nutrient signaling. To test this we are examining genetic interactions between *PTH11* and homologs of yeast signaling components that function during pseudohyphal differentiation. Specifically, *mep2* mutations are complemented by constitutively active alleles of a *ras* protein homolog and a G-protein alpha subunit homolog. We have identified and cloned two *M. grisea ras* homologs (*RAS1* and *RAS2*) and a G_{alpha} subunit homolog that was previously implicated in pathogenesis (*MAGB*). We are currently examining whether *RAS1* and *RAS2* are required for pathogenesis and plan to explore the relationship of the *RAS* genes and *MAGB* to *PTH11* by constructing constitutively active and dominant negative alleles of these small G-proteins, and expressing them in wild type and *pth11* mutant cells.

221. The *PTH11* gene encodes a novel upstream component of pathogenicity signaling in the rice blast fungus *Magnaporthe grisea*. Todd M. DeZwaan, Anne M. Carroll, Barbara Valent, and James A. Sweigard. Dupont, Experimental Station, Wilmington, DE, USA.

The blast fungus *Magnaporthe grisea* forms appressoria in response to specific cues on the host leaf surface. Multiple intracellular signaling components have been identified that mediate appressorium differentiation and function but none of these are thought to act at the cell surface. Thus, it is unclear how extracellular cues are transduced into a pathogenicity-specific signaling cascade. Through insertional mutagenesis we have identified the *PTH11* gene encoding a novel protein with multiple membrane spanning domains. *pth11* mutants fail to produce significant disease and are impaired in their ability to form appressoria. However, *pth11* mutants grow invasively, conidiate *in planta*, and exhibit wild type vegetative growth and sexual differentiation. *pth11* mutants of three distinct *M. grisea* strains show a similar pathogenicity defect and the *PTH11* gene is functionally conserved in distantly related *M. grisea* haplotypes. To determine whether *PTH11* plays a signaling role in pathogenesis we examined the effects of

exogenous cyclic AMP and diacylglycerol. Cyclic AMP suppressed the *pth11* appressorium defect and restored wild type levels of disease whereas diacylglycerol only suppressed the appressorium defect. These findings suggest that *PTH11* plays an upstream role in pathogenicity signaling, possibly by acting at the cell membrane to respond to host surface cues.

222. The role of calcineurin in dimorphism and pathogenicity in the corn smut pathogen *Ustilago maydis*. John W. Duick and Scott E. Gold. University of Georgia, Plant Pathology, Athens, GA, USA.*Ustilago maydis* is a dimorphic basidiomycete and is the causal agent of corn smut disease. It exists as a budding saprophyte in the haploid stage and as an obligate filamentous parasitic dikaryon when two compatible haploid cells fuse. The dikaryon ramifies within the tissue of maize plants eventually causing the formation of galls containing the sooty zygotic teliospores, from which the name smut originates. Dimorphism in *U. maydis* is controlled through signal transduction pathways involving cAMP and the mitogen-activated protein (MAP) kinase cascade. Protein phosphatases have the role of reversing the effects of protein kinase phosphorylation. Post translational modification and specifically protein phosphorylation is a ubiquitous form of regulation in signal transduction pathways. Calcineurin (also referred to as protein phosphatase 2B or PP2B) is a serine/threonine protein phosphatase and, in other systems, has a role in reversing the phosphorylation of the substrates of cAMP dependent protein kinase A. Through a reverse genetic approach, degenerate primers based on the calcineurin catalytic subunit were used to clone a 795 base pair putative calcineurin fragment. Screening of a cosmid library by sib selection led to the recovery of the full-length cosmid clone UMPP2B. Characterization of the calcineurin gene and progress toward an understanding of its role in dimorphism and pathogenicity in *U. maydis* will be discussed.

223. Identification of a gene *pacC* homologue from the dermatophyte *Trichophyton rubrum*. Monica Stropa Ferreira[1], Walter Maccheroni Jr.[2], Sérgio Ricardo Nozawa [3], Antonio Rossi [3], and Nilce Maria Martinez-Rossi [4]. [1]University of Sao Paulo, Genetica, Ribeirao Preto, Sao Paulo, Brazil. [2]University of Sao Paulo, Genetica/ ESALQ, Piracicaba, Sao Paulo, Brazil. [3]Quimica, Ribeirao Preto, Sao Paulo, Brazil. [4]Genetica, Ribeirao Preto, Sao Paulo, Brazil.

T. rubrum is a filamentous fungus whose capability to invade human keratinized tissue (skin, hair and nails) to produce infections is probably related to the secretion of enzymes. Thus, because enzyme secretion may also be influenced by ambient pH, this physiological response could be determinant for the virulence of dermatophytes. Based on regions of homology found between the PacC protein sequences of *A. nidulans, A. niger* and *P. chrysogenum*, degenerate oligonucleotides were designed and used to amplify genomic fragments from *T. rubrum* by PCR. A search with the deduced amino acid sequence of a 219 bp amplification product showed high similarity to the A. nidulans and other PacC proteins, which are responsible for the general control of ambient pH response. Furthermore, the complementation of the pacC14 mutant of *A. nidulans* was achieved by co-transformation of the pAB4-ARp1 plasmid (a fungal autonomously replicating vector) with total genomic *T. rubrum* DNA, with the recovery of most of the extracellular acid phosphatase isoforms of a transformant being observed by isoelectric focusing (IEF). On the other hand, the physiological role of the pacC homologue in *T. rubrum* pathogenicity remains to be clarified.

224. Organization and variation of subtelomeric regions of the rice blast fungus *Magnaporthe grisea.* Weimin Gao and Seogchan Kang. Pennsylvania State University, Plant Pathology, University Park, PA 16802, USA.

Management of rice blast through breeding of blast-resistant cultivars has had only limited success due to the frequent breakdown of resistance under field conditions. Considering the mainly clonal population structure of rice blast fungus *Magnaporthe grisea* in many rice-growing areas, frequent appearance of new pathotypes with the ability to infect previously resistant rice cultivars suggests a high degree of genetic variation. The main objective of this work is to elucidate the nature and mechanisms of genetic changes at the chromosome ends of *M grisea*. Two telomeres of O-137, a rice pathogen isolate, were cloned and characterized. One of them, Tel-2, contains a gene, designated as *TLR-1*, encoding a protein of 744 amino acids that shows significant similarity to RecQ helicases from various organisms and to yeast SGS1</>. The *TLR-1* gene was used as a probe for RFLP mapping. The sequence homologous to this probe was present from nine to eleven copies and segregated with at least six different telomeres, suggesting that many telomeric regions of the mapping parents have sequences homologous to the *TLR-1* gene. Flanking regions of *TLR-1* were also amplified in telomeric regions. Eighteen field isolates from various hosts were screened with *TLR-1* as a probe. Most isolates from grass species other than rice did not contain the gene, but all rice pathogens had multiple copies of the gene. This result supports the idea that rice pathogens are genetically isolated from isolates from other hosts. We will report how *TLR-1* and its flanking sequences are organized in rice pathogens.

225. AOS-scavenging enzymes in the phytopathogen *Claviceps purpurea*. Victoriano Garre, Sabine Moore, Klaus B. Tenberge, and Paul Tudzynski. Westf. Wilhelms-Universitaet, Institut für Botanik, Muenster, Germany.

Claviceps purpurea, a (hemi-) biotrophic pathogen of cereals, infects and colonizes young ovaries, and taps the vascular tissue located at the base of the ovary. There, a stable host-pathogen interface is established. During colonization the fungus is confronted with species of active oxygen (AOS), which are generated in the course of normal ontogenetic processes and during defense reactions (e.g. lignification). We are interested in the ability of fungus to overcome this oxidative stress and in the importance of such mechanisms for pathogenicity. We have started the characterization of AOS-scavenging enzymes in *C. purpurea*, which involves the cloning of the corresponding genes, the study of their expression *in planta* and the analysis of deletion mutants. *In planta*, the fungus secretes at least three catalases (1), and one SOD. We have cloned one SOD and two catalase genes so far. One of the catalase genes (*cpcat1*) encodes two extracellular catalases (CAT C/D); deletion of this gene has no effect on pathogenicity (2). Expression studies and functional analysis of the other genes is in progress. (1) Garre V, Tenberge KB, Eising R: Phytopathol. 88, 744-753 (1998). (2) Garre V, Müller, U, Tudzynski P: MPMI. 11, 772-783 (1998). Present address of the first author: Dpto. Genetica y Microbiologia, Facultad de Biologia, Universidad de Murcia. Apdo.4021, 30071 Murcia, Spain

226. The *ubc3* gene of *Ustilago maydis* encodes a MAP kinase that is required for filamentous growth. Scott E. Gold, Maria E. Mayorga, and David L. Andrews. University of Georgia, Plant Pathology, Athens, Georgia, USA.

Ustilago maydis, the causal agent of corn smut disease, displays dimorphic growth in which it alternates between a budding haploid saprophyte and a filamentous dikaryotic pathogen. Previously we identified a role for the cAMP signal transduction pathway in the dimorphic switch of this fungus. Haploid strains mutant in the *uac1* gene encoding adenylate cyclase display a constitutively filamentous phenotype. Mutagenesis of the uac1 disruption strain allowed the isolation of a large number of budding suppressor mutants. These mutants are named *ubc*, for **U**stilago **b**ypass of **c**yclase as they no longer require the production of cAMP to grow in the budding morphology. Analysis of one of these suppressor mutants led to the identification of *ubc3* (Mayorga and Gold, 1998), which is required for filamentous growth and encodes a MAP kinase most similar to those of the yeast pheromone response pathway. This is the third member of this putative MAP kinase cascade along with *ubc4* (MAPKK kinase) and *ubc2* (see Mayorga and Gold abstract) that we have identified. Mutations in the earlier identified *fuz7* MAPK kinase (Banuett and Herskowitz, 1994) also suppress the filamentous phenotype of the *uac1* disruption mutant providing additional corroborating evidence that *fuz7* is likely a member of this same MAP kinase cascade. We describe the role of the *ubc3* gene in mating and in pathogenicity.

227. A homologue of a gene implicated in the virulence of human fungal diseases is present in a plant fungal pathogen and expressed during infection. Neil Hall, John P.R. Keon, and John A. Hargreaves. IACR Long Ashton, Cell Biology, Long Ashton, Bristol, UK.

The gene encoding SnodProt1 (SP1), an extracellular protein secreted by the wheat fungal pathogen, *Stagonospora (Septoria) nodorum*, has been isolated and characterised. The deduced amino acid sequence exhibited significant similarity to antigenic and allergenic proteins encoded by genes cloned from the human fungal pathogens, *Coccidioides immitis* and *Aspergillus fumigatus*, respectively. SP1 also exhibited similarity to an EST cDNA derived from the rice blast fungus, *Magnaporthe grisea*, grown on isolated rice cell walls. Northern and western blot analyses showed that the gene encoding SP1 is expressed and that the protein is secreted during infection of wheat leaves.

228. An ATP-driven efflux pump is a novel pathogenicity factor in rice blast disease. John E. Hamer, Martin Urban, and Tishina Bhargava. Purdue University, Biological Sciences, West Lafayette, IN, USA.

Cells tolerate exposure to cytotoxic compounds through the action of ATP-driven efflux pumps belonging to the ATP-binding cassette (ABC) superfamily of membrane transporters. Phytopathogenic fungi encounter toxic environments during plant invasion as a result of the plant defense response. Here we demonstrate the requirement for an ABC transporter during host infection by the fungal plant pathogen *Magnaporthe grisea*. The ABC1 gene was identified in an insertional mutagenesis screen for pathogenicity mutants. The ABC1 insertional mutant and a constructed deletion mutant are dramatically reduced in pathogenicity towards various hosts but show no discernible defects in vitro. ABC1 is most similar to yeast ABC transporters, Pdr5 and Cdr1, implicated in multidrug resistance. Consistently, expression of the ABC1 gene is inducible by toxic drugs including a rice phytoalexin. The insertional mutation in ABC1 appears to disrupt the regulatory element required for induction by metabolic poisons. These data strongly suggest that fungal pathogens require ABC transporters to protect themselves against plant defense mechanisms.

229. Genome wide mutagenesis and sequencing for filamentous fungi. Lisbeth Hamer, Amy Page, Jeff Woessner, Matt Tanzer, Kiichi Adachi, Scott Uknes, and John E. Hamer. Paradigm Genetics, Fungal Research, RTP, NC, USA.

Genome wide mutagenesis and gene function analysis is facile in baker's yeast due to the availability of the entire genomic sequence, and the low level of ectopic recombination during DNA-mediated transformation. Typically, PCR fragments with just ~40 bp of target sequence flanking an appropriate marker are sufficient to direct an insertion into a specific target in the yeast genome. None of the above advantages are available in filamentous fungi; complete genome sequences for filamentous fungi are lacking and targeted recombination requires larger flanking DNA fragments (> 1-2 kb), which makes construction of gene knockout vectors a laborious task. Finally, the low level of homologous recombination means that knockouts must be identified against a background of ectopic recombination events. The Fungal Research Group at Paradigm Genetics is committed to develop a high through-put gene function discovery process for filamentous fungi and to inventory and resource this information to discover new antifungal chemicals and fungal derived products. We have developed an assembly line process that combines genome sequencing and knockout vector construction. This technology also permits multiple rounds of mutagenesis to be performed on target sequences facilitating construction of leaky and conditional alleles. We are currently applying this technology to the rice blast fungus, with the goal of elucidating the function of numerous uncharacterized genes.

230. Isolation of the avirulence gene *AVR2-MARA* of *Magnaporthe grisea*. Travis M. Harper, and Marc J. Orbach. University of Arizona, Plant Pathology, Tucson, Arizona, USA.

Genetic analysis of cultivar specificity in the rice blast fungus, *Magnaporthe grisea,* has identified a second gene, *AVR2-MARA*, that confers avirulence toward the rice cultivar Maratelli. A series of crosses demonstrated that *AVR2-MARA,* which originated in strain 4091-5-8, segregates independently from *AVR1-MARA*. Through RFLP analysis, *AVR2-MARA* was mapped to Chromosome 7, between the 2 markers, cos196 and cos209. A chromosome walk from the cos209 marker, which is 7 recombination crossover points (rcps) from *AVR2-MARA*, was initiated. Because *M. grisea* strains demonstrate a high degree of synteny, the walk was started using a BAC library of strain 4224-7-8, which does not contain *AVR2-MARA*. The first two steps of the walk crossed 4 and 2 rcps, respectively. The physical distance of these two steps is ~50 kb and ~80 kb respectively, leaving the walk 1 rcp from the *AVR2-MARA* locus. Progress during the walk has been hampered by the high degree of repetitive DNA, making BAC end clones generally ineffective for walk progression. Repetitive elements have been identified by hybridization to BAC clones. In conjunction with the BAC library, a cosmid library of strain 4091-5-8 is being probed to cross the remaining rcp. In addition to the efforts to clone *AVR2-MARA*, attempts to isolate virulent mutants of the gene will also be reported.

231. Repetitive DNA elements as genetic markers in *Chondrostereum purpureum*. William E. Hintz, Elisa Becker, Paul de la Bastide, and Louise Hahn. University of Victoria, Biology, Victoria, BC, Canada.

Productivity in conifer reforestation sites is reduced by competition by fast-growing hardwood species. The phytopathogenic fungus *Chondrostereum purpureum* is well suited to forest

vegetation management as it has a broad-spectrum pathogenicity towards many hardwoods and prevents resprouting of cut trees. While *C. purpureum* occurs naturally throughout North America, little is known about its population structure. We therefore developed a series of molecular markers to (1) identify specific isolates and (2) assess the impact of release of selected isolates. Amplification of *C. purpureum* genomic DNA with the RAPD primer GGGGTGACGA resulted in the amplification of 3 to 12 fragments certain of which were conserved at the population level. We determined the DNA sequence of a unique RAPD fragment from the lead isolate and designed a SCAR primer pair (GGGGTGACGAGGACGACGGTG and GGGGTGACGACATTATACTGCAGGTAGTAG). It was anticipated that this primer pair would amplify a single DNA fragment from the lead isolate however several fragments, ranging in size from 300 bp to 2000 bp, were simultaneously amplified from genomic DNA of all *C. purpureum* isolates tested. The SCAR amplification patterns have proven to be distinctive for each genetic individual tested making this a particularly useful fingerprinting tool. Sequence comparison of the amplified SCAR DNAs revealed extensive DNA similarity between fragments and we hypothesize that these DNAs represent members of a family of dispersed repetitive DNA elements.

232. Cloning and disruption of protein kinase genes in *Aspergillus nidulans*. Louise M. Hoare[1], A. Renwick [2], A. Dickson[1], and G.D. Robson[1]. Manchester University, School of Biol.Sci., Manchester, UK. [2]Zeneca Agrochemicals, Jealotts Hill, Bracknell, Berkshire,UK.

Novel antifungal agents are constantly required due to the build up of disease resistance in fungal pathogens and the toxic effects of existing agents. Targets must be identified in the pathogens which when disrupted have a debilitating effect on the fungi, inhibiting their ability to infect the host and cause disease. Map kinases have been found to be involved in hypo-osmolarity signal transduction and cell wall integrity in *Saccharomyces cerevisiae* and may therefore be putative antifungal targets. Using *Aspergillus nidulans* as a model, we have designed PCR primers based on consensus to the PKC protein kinase family. As a second strategy, osmotically sensitive mutants are being produced by UV mutagenesis which only grow on osmotically buffered medium. The long term strategy is to clone the full length genes and to study the effect of knockouts on the phenotype of *A. nidulans*.

233. Homologs of aflatoxin biosynthetic genes in *Aspergillus oryzae* and *A. sojae*. David Jeenes, Adrian Watson, Linda Fuller, and David Archer. Institute of Food Research, Norwich Research Park, Norwich UK.

Although aflatoxin biosynthesis has been documented for *Aspergillus flavus* and *A. parasiticus*, the closely related species *A. oryzae* and *A. sojae* which are widely used in food and ingredient manufacture have no history of producing aflatoxins. Despite this lack of aflatoxin production, several groups have reported the presence of genes associated with aflatoxin biosynthesis, including the pathway-specific regulatory gene *aflR*, in strains of *A. oryzae* and *A. sojae*. We confirm by sequencing the presence of 3 structural genes (*nor-1, ver-1* and *omtA*) and the regulatory gene, *aflR*, in strains of *A. oryzae* and *A. sojae*. We also report the sequence of the entire *aflR* gene from *A. oryzae* ATCC 14895. Northern blot and RT-PCR analysis show that none of these genes are actively transcribed in the chosen strains of *A. sojae/A. oryzae*. Sequence analysis reveals that the AFLR proteins are truncated by ca. 60 aa in all the *A. sojae* strains

examined, a mutation that appears to be linked to a small duplication at the N-terminal end of the protein.

234. Loss of a dispensable chromosome from *Alternaria alternata* apple pathotype leads to a toxin minus phenotype and loss of pathogenicity. L.J. Johnson[1], R.D. Johnson[1], H. Akamatsu[2], M. Kodama[1], H. Otani[2] and K. Kohmoto[1]. [1]Faculty of Agriculture, Tottori University, Tottori 680, Japan, [2]United Graduate School of Agricultural Sciences, Tottori University, Tottori 680, Japan.

We have cloned a cyclic peptide synthetase gene, *AMT*, from *Alternaria alternata* apple pathotype that catalyses the production of AM-toxin, a host-specific toxin responsible for pathogenicity. Pulsed-field gel electrophoresis (PFGE) and Southern analysis showed that the gene is located on a small chromosome 1.7Mb or smaller depending on the strain. Expression of *AMT* was investigated by RT-PCR using pathogenic and non-pathogenic isolates, including AM-toxin minus mutants. AM-toxin production was determined by a leaf necrosis bioassay on susceptible and resistant apple cultivars and expression of *AMT* was correlated with AM-toxin production. In addition a laboratory sub-cultured strain previously shown to produce AM-toxin was negative by RT-PCR and a leaf necrosis bioassay confirmed a toxin minus phenotype. However, an original isolate of this strain which had not undergone sub-culture gave a positive result by both RT-PCR and bioassay. CHEF analysis and Southern hybridisation demonstrated the loss of a 1.1 Mb chromosome in the non-toxin producing isolate. Both isolates have identical colony morphologies and RAPD profiles indicate that they share common origins. This indicates that the 1.1 Mb chromosome is non-essential for normal growth but necessary for pathogenicity.Supported by a research grant from the Ministry of Education, Science and Culture of Japan.

235. Cloning of a cyclic peptide synthetase gene whose product catalyses the production of AM-Toxin responsible for pathogenicity of *Alternaria alternata* apple pathotype. R. D. Johnson[1], L. J. Johnson[1], Y. Itoh[3], M. Kodama[1], H. Otani[2], J. D. Walton[4] and K. Kohmoto[1]. [1]Faculty of Agriculture, Tottori University, Tottori 680, Japan, [2]United Graduate School of Agricultural Sciences, Tottori University, Tottori 680, Japan, [3]Faculty of Science, Shinshu University, Matsumoto 390, Japan, [4]Department of Energy Plant Research Laboratory, Michigan State University, MI48824, USA.

Alternaria alternata apple pathotype causes Alternaria blotch of susceptible apple cultivars through the production of a cyclic peptide host specific toxin, AM-toxin. PCR, using primers designed to conserved domains of cyclic peptide synthetase (CPS) genes, amplified several products from *A. alternata* apple pathotype whose deduced amino acid sequence showed high similarity to other fungal CPSs and were specific to the apple pathotype. Screening of a Lambda Zap genomic library with these PCR generated probes identified overlapping clones containing a complete CPS gene, designated AM-toxin synthetase gene (*AMT*), of 14kb in length with no introns. RT-PCR on toxin minus mutants generated by REMI demonstrated that expression of this gene was eliminated. To confirm that AM-toxin synthetase is the primary determinant of virulence and specificity in the *A. alternata* apple pathotype/apple interaction, disruption of *AMT* by transformation of wildtype *A. alternata* apple pathotype with disruption/replacement vectors

based on the plasmid pAN7-1 is being carried out.Supported by a research grant from the Ministry of Education, Science and Culture of Japan.

236. A genetic map using AFLP markers of *Fusarium moniliforme* (*Gibberella fujikuroi*, MP-A). James E. Jurgenson[1], K. A. Zeller[2], and J. F. Leslie[2]. [1]University of Northern Iowa, Biology, Cedar Falls, IA, USA. [2] Kansas State University, Plant Pthology, Manhattan, KS, USA.

Fusarium moniliforme (*Gibberella fujikuroi*, MP-A) is primarily a pathogen of maize, but can also cause disease in other crop species. This pathogenicity, as well as mycotoxin production, causes billions of dollars of economic losses annually. We have continued to develop *F. moniliforme* as a model genetic system for understanding these plant pathogenic fungi. However, the dissection of important biological characteristics in this fungus has been hampered by the lack of a uniformly dense genetic map. The existing RFLP-based map contains significant gaps, making it more difficult to locate biologically important genes, such as those involved in mycotoxin production, with precision. We have utilized AFLPs (Amplified Fragment Length Polymorphisms) to supplement the genetic map. We have added more than 500 AFLP markers to the ~150 markers in the existing map. The resulting map has an average marker interval of 5 map units, and averages only 10-11 kb / map unit. This map has a length of >3500 map units divided into the 12 linkage groups described previously. There are no holes in the map greater than 25 map units. We have identified AFLP markers at only 2 map units from the mating type locus, and from the spore killer (*Sk*) locus. Another AFLP marker maps only 6 units from the *fum1* (fumonisin biosynthetic) locus. The increased saturation of this map will facilitate further development of *F. moniliforme* as a model system for the genetics and population genetics of these plant pathogenic fungi.

237. Isolation of genes related to the appressorium formation from a differential library of *Magnaporthe grisea*. Takashi Kamakura[1], Kenichiro Saitoh[2], Tohru Teraoka[2], and Isamu Yamaguchi[1]. [1]RIKEN Institute, Microbial Toxicology Lab., Wako, Saitama, Japan. [2]Tokyo Univ. Agric. Tech., Agruculture, Fucyu, Tokyo, Japan.

The conidial germ tube of *Magnaporthe grisea* differentiates a specific infection structure, an appressorium, for penetration into the host. Formation of appressorium was observed not only on rice leaves but also on synthetic solid substara such as polycarbonate. We found that a plant lectin, concanavalin A, specifically suppressed the appressorium formation without affecting the germling adhesion if it was applied within 2-3 hours after germination. From the result, we have constructed a cDNA library which represents the appressorium differentiation stage from the 2.5 hours-old germ tubes. For the construction of the library, a double-subtractive cDNA cloning strategy was taken to condense the differential cDNAs expressed during early stage of the development of conidial germ tube and/or appressorium formation. Out of 686 colonies of the library, about 150 distinct clones' nucleotide sequence were determined partially. A melanine biosynthesis gene (BUF1) was found in the library and this strongly suggests that the library contains genes expressed during appressorium forming stage. We have further analyzed some of candidates which showed differential expression by RT-PCR analysis. Gene disruption of one unknown gene, clone A4, caused remarkable change of appressorium differentiation on the artificial solid substrates but not on the plant surface. This gene was expressed specifically in the

young germling and we could not detect any transcripts in the vegetative growth mycelia by RT-PCR. The structure and presumable function of the gene will be discussed.

238. Dissection of nonhost resistance of *Nicotiana* to *Phytophthora infestans* using a Potato Virus X vector. Sophien Kamoun[1], Shujing Dong[1], Edgar Huitema[1], Vivianne G.A.A. Vleeshouwers[2], Francine Govers[2]. [1]The Ohio State University-OARDC, Plant Pathology, Wooster, OH, USA. [2]Wageningen Agric. Univ., Phytopathology, Wageningen, The Netherlands.

Specific recognition events, defined by the perception of pathogen elicitors by plant receptors, trigger defense responses including the hypersensitive response (HR), a form of programmed cell death in plants. *Phytophthora infestans*, the causal agent of late blight of potato and tomato, produces INF elicitins, a diverse family of extracellular proteins that induce the HR in a restricted number of plants, particularly in the genus *Nicotiana* within the Solanaceae. Resistance of *Nicotiana* to *P. infestans* is always associated with a rapid HR of epidermal and mesophyll cells, which restricts the pathogen to the infection site. Our studies aim at determining whether this response is mediated by the recognition of elicitins. *P. infestans* strains deficient in the major elicitin INF1 induced disease lesions on *Nicotiana benthamiana*, suggesting that INF1 functions as an avirulence factor that conditions resistance in this species (S. Kamoun, P. van West, V. Vleeshouwers, K. de Groot, and F. Govers, 1998, Plant Cell, 10:1413). In contrast, INF1 deficient strains remained unable to infect other *Nicotiana* species, such as tobacco. Tobacco may therefore respond to additional elicitors, perhaps other members of the complex INF elicitin family. To dissect the response of *Nicotiana* to elicitins, functional expression of *inf* genes in plants was conducted using a recombinant Potato Virus X (PVX) vector. These PVX-inf constructs triggered a number of responses such as local and systemic HR lesions, allowing a quantitative evaluation of the response of *Nicotiana* to the various INF elicitins. This study shows that species-specific elicitors can be used to dissect nonhost resistance into discrete components.

239. Establishment of differentially expressed gene profile from hypovirulent strain of *Cryphonectria parasitica* . Hyun-Seok Kang, Moon-Sik Yang, and Dae-Hyuk Kim. Chonbuk National University, Biological Sciences, Chonju, Chonbuk, South Korea.

Ordered differential display which has advantages of high sensitivity, reproducibility, proportional representation, and limited number of primer combinations has been conducted to have a profile on the differently expressed genes between a hypovirulent strain of *Cryphonectria parasitica* (UEP1) as well as its isogenic wild type (EP155/2). RNAs were prepared from 1 and 5 days after the liquid culture of both strains and they were further verified with the known marker genes of *C. parasitica* such as laccase, cryparin, and MF2-1. Expressed genes were categorized to five groups according to their temporal expression patterns and those fives groups are CPC, CPE, CPL, CPD, and CPU which indicate constitutive, early-expressed, late-expressed, down-regulated, and up-regulated, respectively. Those genes belong to five groups were amplified, cloned and sequenced. Characterization of 50 clones were conducted and more are under investigation.

240. Expression of an active cytokine from the recombinant *Aspergillus niger*. Myoung-Ju Kim, Tae-Ho Kwon, Yong-Suk Jang, Moon-Sik Yang, and Dae-Hyuk Kim. Chonbuk National University, Biological Sciences, Chonju, Chonbuk, South Korea.

Aspergillus niger has been developed as a host organism for the production of foreign protein and becomes an attractive candidate for large-scale production of heterologous proteins. Granulocyte-macrophage colony stimulating factor(GM-CSF), along with G-CSF, is one of the first hematopoietic regulators to be deployed clinically, and is now used widely to ameliorate chemotherapy-induced neutropenia and to enhance hematopoietic recovery after bone-marrow transplantation. Thus we made an effort to express and secrete an active murine GM-CSF(mGM-CSF) from the recombinant *A. niger*. Five hundred bp fragment encoding mGM-CSF was cloned inbetween promoter and terminator of glyceraldehyde 3-phosphate dehydrogenase (*gpd*) and hygromycin phosphotransferase gene (*hph*) was used as a selection marker. The expression vector was introduced into *A. niger* ATCC 2119 and Northern blot analysis indicated a considerable expression of the introduced mGM-CSF. The biological activity of recombinant mGM-CSF from the culture filtrate was confirmed by measuring the proliferation of the GM-CSF dependent FDC-P1 cell line. The productivity estimated by ELISA was increased up to 640 ng/L of mGM-CSF and its specific activity was further increased more than three-times than that of commercial preparation.

241. Isolation of *Fusarium moniliforme* mutants with reduced fusaric acid production. Gretchen A. Kuldau, James K. Porter, Emma Wray, Rita Bennet, Anthony E. Glenn, Charles W. Bacon. USDA-ARS, TMRU, Athens, GA, USA.

Fusarium moniliforme (*Gibberella fujikuroi*) is an agronomically important ascomycete that synthesizes mycotoxins such as fumonisins and fusaric acid during both asymptomatic and symptomatic associations with its primary host, maize. Fusaric acid is produced in moderate to high levels by all isolates of *F. moniliforme* and by many other Fusarium species. It is considered a phytotoxin, but its role in potentiating asymptomatic and symptomatic infection of maize is largely unexplored. We have found that in vitro production of fusaric acid is stimulated in low iron conditions and repressed in conditions of iron abundance. Modification of a thin layer chromatography (TLC) method has allowed us to detect fusaric acid production directly from agar plugs containing fungus. Using the low iron medium and TLC method for screening we have isolated two *F. moniliforme* UV-induced mutants with low and very low fusaric acid production. Confirmation and quantitation of fusaric acid production by the mutants was accomplished using GC/MS analysis. We are currently performing genetic crosses to determine if the production phenotypes observed are due to single gene mutations. The UV mutants will be used to study the role of fusaric acid in interactions between *F. moniliforme* and maize.

242. Investigation of *hgl1*, a gene involved in cAMP-dependent morphogenesis in the corn smut *Ustilago maydis*. R. David Laidlaw[1], Franz Durrenberger[2], and James W. Kronstad[1]. [1]University of British Columbia, Biotechnology, Vancouver, B.C., Canada. [2]Discovery Technologies , Innovation Center, Allschwil, Switzerland.

During sexual and pathogenic development, *Ustilago maydis* undergoes a morphological switch from a yeast-like budding growth morphology to a branched filamentous morphology. Control of

the phenotypic switch is known to be regulated by both a pheromone response pathway and a cAMP-dependent protein kinase A (PKA) pathway. The morphological switch in *U. maydis* involves a gene named *adr1*, which encodes a catalytic subunit of PKA that is responsible for the majority of PKA activity (1). *adr1*$^-$ cells display a constitutively filamentous phenotype; in contrast, cells with elevated PKA activity are budding. A novel gene (*hgl1*) was identified through complementation of a yeast-like *adr1*$^-$ suppressor mutant back to the filamentous *adr1* morphology. Efforts are now underway to further examine the role of the *hgl1* gene in morphogenesis and pathogenicity. *hgl1*$^-$ cells display a variety of phenotypes, including an interesting defect for teliospore formation during completion of the sexual life cycle in the host plant. PSI-BLAST and Blocks searches using the *hgl1* sequence have not identified any significant homologs to suggest a function for Hgl1. Here, we present results investigating the regulation of *hgl1* transcription in both haploid and diploid *U. maydis* strains, as well as data gathered utilizing *in vitro* expressed proteins to address the possibility of Hgl1 phosphorylation by Adr1. The preliminary characterization of mutants which suppress the yeast-like cell morphology of both *hgl1*$^-$ and *adr1*$^-$*hgl1*$^-$ mutant strains will also be presented. 1) Dürrenberger *et al*. (1998) *Proc. Natl. Acad. Sci.* USA **95**, 5684-5689

243. Characterization of the chromosome that contains *ToxA* of *Pyrenophora tritici-repentis* and its homolog from a non-pathogenic wheat isolate. Amnon Lichter[1], Ganey M. Gaventa[2], and Lynda Ciuffetti[2]. [1]The Volcani Center, Dep. of Postharvest Sci., Bet Dagan, Israel. [2]Oregon State University, Botany & Plant Pathology, Corvallis, OR, USA.

The protein encoded by *ToxA* of *Pyrenophora tritici-repentis* is a unique host-specific toxin. Once introduced into a non-pathogenic wheat isolate of *P. tritici-repentis* the *ToxA* gene was shown to transform it to a pathogenic fungus. Comparative karyotype analysis was conducted to determine the chromosomal localization of *ToxA* and to determine if a cognate chromosome is present in a non-pathogenic Pyrenophora strain. Major karyotype polymorphisms between pathogenic and non-pathogenic isolates, and to a lesser extent among pathogenic strains, made it impossible to assign homologous chromosomes based on size. *ToxA* was localized to a 2.85Mb chromosome and PCR-based subtraction was carried out with the *ToxA* chromosome used as tester DNA and genomic DNA from a non-pathogenic isolate, as driver DNA. Seven of 8 single copy probes that originated from the 2.85Mb chromosome could be assigned to a 2.75Mb chromosome of the non-pathogenic strain while one probe was unique to the pathogen. Six of the probes revealed identical restriction patterns in comparative analysis of the pair of pathogenic and non-pathogenic strains, while 2 probes identified restriction-length polymorphisms. Extension of this analysis to several other isolates with selected probes revealed identical as well as polymorphic patterns. Nine different repetitive DNA probes originated from the 2.85Mb chromosome including sequences that correspond to known fungal transposable elements, but none were confined to it. Interestingly, they were either present at low copy number or absent from the non-pathogens and displayed polymorphic patterns among the isolates. In conclusion, a homolog to the chromosome containing *ToxA* is present in non-pathogenic *P. tritici-repentis*, although karyotype and repetitive DNA analyses indicate it is distinct from pathogenic *P. tritici-repentis*

244. Genetics of Laccase of *Gaeumannomyces graminis*. Anastasia P. Litvintseva, Kathy B. Sheehan, and Joan M. Henson. Montana State University, Microbiology, Bozeman, MT, USA.

Six genes coding for the multi-copper phenol oxidase, laccase, were isolated from the ascomycete *G. graminis*. Three genes were subcloned from a genomic library of *G. graminis* var. *graminis* and the others were from a genomic library of *G. graminis* var. *tritici*. All six laccase genes and their predicted polypeptide sequences were typical of fungal laccases with conserved copper binding sites. Only two of the predicted polypeptides shared high amino acid homology (about 95%) with each other and other predicted gene products were significantly different. Promoters of three of these genes were sequenced. All of them contained putative Metal Responsive Elements (MRE), CreA-like and Nit-2-like protein binding sites and two of them had Xenobiotic Responsive Elements (XRE). One also contained an Antioxidant Responsive Element (ARE). The presence of these putative regulatory elements suggests that different environmental cues are involved in the regulation of expression of different laccase genes. This expression was studied by competitive RT-PCR. So far we have observed that at least two of the genes are constitutively expressed at low levels in the fungus, when it grows in Luria- Bertani (LB) broth, and their expression is induced by the presence of 400 mM Cu2+ and/or 40 mM xyladine.

245. A gene cluster from the corn pathogen *Cochliobolus heterostrophus* required for nonribosomal peptide biosynthesis and general virulence of fungi. S. W. Lu, B. G. Turgeon, and O. C. Yoder. Cornell University, Plant Pathology, Ithaca, NY, USA.

The gene *CPS1* from the corn pathogen *Cochliobolus heterostrophus* encodes a putative multifunctional peptide synthetase. Two overlapping cosmid clones covering 33.7 kb of genomic DNA were isolated. Sequence analyses revealed, in addition to *CPS1*, at least 13 ORFs, nine of which had no apparent homologs in the database. Translation of each of the remaining four ORFs revealed significant similarity to a known protein, i.e., a thioesterase (TES1; 1.1 kb), a DNA-binding protein with a zinc cluster motif, a leucine zipper domain and a phosphorylation site for cAMP-dependent protein kinase (DBZ1; 1.8 kb), a coenzyme A transferase (COT1; 1.9 kb), and a decarboxylase (DEC2; 1.7 kb); partial sequencing of ORF15 suggests a transport protein. Comparison of genes in the *CPS1* cluster with those in known peptide synthetase gene clusters shows substantial similarity, supporting the hypothesis that the *CPS1* cluster is responsible for biosynthesis of a nonribosomal peptide (as yet unidentified). Homologs of *CPS1* were found in other *Cochliobolus* spp. and in other fungal genera. Disruption of either *CPS1* or DBZ1 caused drastically reduced virulence in each of three different pathogens, both races (O and T) of *C. heterostrophus*, *C. victoriae* (a pathogen of oats). Since race T and *C. victoriae* each requires a known host-specific toxin (the polyketide T-toxin and the cyclic peptide victorin, respectively) for virulence to host plants, the results suggest that the hypothetical *CPS1*-controlled peptide is a general virulence factor in pathogenic fungi, perhaps required to facilitate action of other virulence factors such as host-specific toxins.

246. Mapping of the Avr1a avirulence gene in *Phytophthora sojae*. Terry MacGregor [1], Madan Bhattacharyya[2], and Mark Gijzen. [1]University of Western Ontario, Plant Sciences, London, Ontario, Canada. [2]Noble Foundation, Ardmore, Oklahoma, USA. [3]Agriculture Canada, London, Ontario, Canada.

The Avr1a locus was mapped in two F2 populations derived from four different parental isolates. A combination of RAPD analysis and bulked segregant analysis resulted in the identification of

five linked markers. Although these markers were linked to Avr1a in both populations, the arrangement of the markers around Avr1a was different for the two crosses. In one case the markers cover an 11 cM span and map to one side of Avr1a, while the second cross produced a map that spans 53 cM with markers on either side of Avr1a. Southern blots of the four parental strains were probed with the RAPD markers to determine any differences in RFLP patterns. In most cases, the RFLP pattern was consistent for each pair of virulence phenotypes. Taken together, these results suggest that the Avr1a genomic region is similar but not identical in the two avirulent parental isolates. To identify more markers and to produce a high density linkage map around Avr1a, we resorted to AFLP analysis. Many new markers were successfully identified and mapped. Most of the DNA markers, both RAPD and AFLP, appear as single copy sequences when cloned and used as probes in Southern blots. Thus, we are now ready to begin the physical mapping of Avr1a.

247. The *ubc2* gene, a putative member of the MAP kinase cascade, is required for gall formation but not for cell fusion in the pathogenic fungus *Ustilago maydis*. Maria Elena Mayorga, and Scott E. Gold. University of Georgia, Plant Pathology, Athens, GA, USA.

Ustilago maydis, the causal agent of corn smut disease, displays dimorphic growth in which it alternates between a haploid unicellular, non-pathogenic yeast-like form and a dikaryotic, pathogenic filamentous form. We have identified a role for the cAMP signal transduction pathway in the dimorphic switch of this fungus. Earlier, a constitutively filamentous haploid mutant was obtained. Complementation of this mutant led to the isolation of the gene encoding adenylate cyclase, *uac1*. Secondary mutagenesis of a *uac1* disruption strain allowed the isolation of a large number of suppressor mutants, termed *ubc*, for *Ustilago* bypass of cyclase, lacking the filamentous phenotype. Analysis of one of these suppressor mutants led to the identification of *ubc2*, which is required for filamentous growth and appears to encode a protein capable of interacting with other proteins. We describe the possible roles of *ubc2* and its probable physical interaction with *ubc4*, a MAPKK kinase of *U. maydis*. An interaction between *ubc2* and *ubc4* suggests *ubc2* functions in the pheromone responsive MAP kinase cascade. *ubc2* is important not only for dimorphic switching but also in pathogenicity: *ubc2* mutant dikaryons are capable of colonizing maize but are unable to induce gall formation. In cytoduction assays we show that fusion of two compatible *ubc2* mutants occurs at a similar frequency as compatible wild type strains. Therefore, the inability to produce galls *in planta* is probably a post cell fusion defect caused by the *ubc2* mutation.

248. Detoxification of avenacosides by *Stagonospora avenae*. John Morrissey, Jos Wubben, and Anne Osbourn. Sainsbury Laboratory, Norwich, UK.

The steroidal saponins, 26-desglucoavenacosides A and B, are present in oat leaves and may play a role in protecting the plant against fungal attack. The toxicity of 26-desglucoavenacosides towards fungi is dependent on the presence of a branched sugar side chain at the C3 position of the steroidal backbone. Characterisation of a collection of *Stagonospora* isolates revealed that isolates of the oat-attacking species *S. avenae* f. sp. *avenae* secreted an enzyme activity which was capable of detoxifying avenacosides by the sequential hydrolysis of L-rhamnose and D-glucose residues from the sugar chain whereas non-oat-attacking isolates did not. A beta-glucosidase which hydrolyses D-glucose residues from the side-chain was purified and the

cognate gene cloned. This enzyme is related to saponin-detoxifying enzymes from two other phytopathogenic fungi, *Septoria lycopersici* and *Gaeumannomyces graminis*. Mutants generated by targeted disruption of the beta-glucosidase gene in one isolate of *S. avenae* retained the ability to hydrolyse the desglucoavenacoside sugar chain and were unaffected in pathogenicity. These data show that more than one enzyme is involved in the hydrolysis of these saponins and work is progress to characterise the other enzymes involved and to determine their role in the pathogenicity of *S. avenae* to oats.

249. Cloning and functional characterisation of ornithine decarboxylase in *Tapesia yallundae*. E Mueller[1,2], A Bailey[1], A Corran[2], and P Bowyer[1]. [1]Plant Molecular Pathology, IACR Long Ashton Research Station, University of Bristol, Long Ashton, Bristol BS41 9AF, UK; [2]Zeneca Agrochemical, Jeallots Hill, Berks., UK.

Ornithine decarboxylase (ODC), is a key enzyme in the polyamine biosynthetic pathway. Polyamines such as spermidine and spermine are present in high amounts in differentiating cells such as germinating spores and sporulating mycelia and are essential for these processes. Plants use an alternative pathway for polyamine biosynthesis via arginine decarboxylase (ADC) whereas fungi soley use the ODC pathway. Polyamine biosynthesis (and in particular ODCase) is thus a good target for developing new fungicidal chemicals because plants should not be affected by ODC inhibitors. In order to evaluate ODC as a possible fungicide target, we set out to clone ODC from *Tapesia yallundae* (eyespot) and generate mutants by gene disruption. The mutants subsequently would be evaluated for their virulence. The ODC gene of *Tapesia yallundae* was isolated by PCR amplification. Primers were designed against conserved motifs in the central part of the gene. The PCR product was cloned and sequenced to confirm its identity and was subsequently used to screen a genomic library. The gene has been completely sequenced and a line-up of the deduced protein sequence showed high homology to those from other fungi. The coding sequence of the native ODC gene was replaced with the hygromycin resistance cassette and that construct was used to transform *T. yallundae*. Hygromycin resistance transformants were selected for putrescine dependant growth. Of 250 transformants screened, 7 were auxotrophic for putrescine. Southern analysis confirmed that the coding sequence in those mutants was deleted. The functionality of the *T. yallundae* ODC gene was confirmed by complementing mutants in *A. nidulans* and in *T. yallundae*. Both mutants are now able to grow in the absence of polyamines and the ability to produce spores was completely restored. Preliminary pathogenicity studies on the *T. yallundae* ODC mutants showed that the virulence of those mutants is possibly slightly reduced. Further detailed analysis is in progress.

250. LINE retrotransposon caused conidium morphogenetic mutation in *Magnaporthe grisea*. Marie Nishimura[1], Nagao Hayashi[2], Num-Soo Jwa[1], John E. Hamer[3], and Akira Hasebe[1]. [1]Nat Inst of Agrobiological Res, Genetic Diversity , Tsukuba, Ibaraki, Japan. [2]Nat Agri Res Center, Rice Desease, Tsukuba, Ibaraki, Japan. [3]Purdue University, Biological Sciences, West Lafayette, Indiana, USA.

The rice blast fungus, *Magnaporthe grisea*, produces a cluster of conidia in sympodial array. We obtained three morphological mutants of *M. grisea*, designated CAC (CAtenated Conidia), by crossing two wild type rice-pathogenic strains. In CAC mutants, conidiogenesis pattern was altered that conidia were blasted from former produced conidia and catenated in chain, while, the

branching pattern of conidiophore did not altered. Compared to the parental strains, 12 % of CAC mutants could form appressoria, in number, but they required extra 24 hours. CAC mutants lessened pathogenicity towards rice by 90%. Genetic segregation test suggested that the mutation was caused by one gene locus. By southern hybridization and sequencing analysis, it was appeared that MGL, a *M. grisea* LINE transposon, inserted in Acr1 gene in all CAC mutants. MGL duplicated 19 nucleotide-target sequences. These results indicated that active MGL caused morphological mutation and pathogenicity reduction in *M. grisea*.

251. Molecular characterization of a putative aryl-alcohol dehydrogenase from *Botrytis cinerea.* Hannah Noel[1], Neil Hall[2], Alexander Schouten[3], John A. Lucas[2], and Paul M. Wood[1]. [1]University of Bristol, Biochemistry, Bristol, Avon, UK. [2]IACR- Long Ashton, Cell Biology, Bristol, N. Somerset, UK. [3]Wageningen University, Phytopathology, Wageningen, Netherlands

Botrytis cinerea (grey mould) is a ubiquitous pathogenic fungus causing pre-and post-harvest disease in many crops. The pathogen secretes a variety of extracellular enzymes including -galactosidases, pectinases and cellulases. However, there is no evidence for a direct phytotoxic effect of these enzymes in *Botrytis*, and the exact mechanisms of infection and induction of host cell death by the fungus are still obscure. Recent research has linked production of oxidative species (H_2O_2 and OH) during infection with the virulence of the fungus. We are investigating the possible involvement of aryl-alcohol dehydrogenases in the production of oxidative species. A sequence alignment of aryl-alcohol dehydrogenase genes, from other fungi was used as a source of degenerate primers. A 390 bp fragment of *Botrytis cinerea* genomic DNA was amplified by PCR using these primers. This fragment has been sequenced and found to have close similarity with the other aryl-alcohol dehydrogenases. It was then used to probe a genomic library, in order to retrieve the full-length sequence. Northern blot analyses of this transcript are now underway.

252. Transformants of *Phialophora gregata* for easy detection and visualization in soybeans. Teruo Nonomura, and Charlotte R. Bronson. Iowa State University, Plant Pathology, Ames, Iowa, USA.

Pathogenesis by *Phialophora gregata*, the causal agent of brown stem rot of soybeans, has been difficult to study cytologically because of its small, colorless conidia and hyphae. The fungus also grows slowly on media and is difficult to distinguish from other fungi that commonly inhabit soybean roots and stems. To facilitate observations of this fungus *in planta*, we transformed it with genes for green fluorescent protein (GFP), beta-glucuronidase (GUS), and hygromycin resistance (*hyg*B). Transformants showing high levels of expression of the genes were tested for fitness by measuring colony diameter, dry mass and conidiation on media, and virulence in soybeans, compared to the non-transformed strain. Three of the transformants expressing GFP and *hyg*B were indistinguishable from the non-transformed strain by all criteria. All tested transformants expressing GUS, however, were severely compromised in conidiation on media. Transformants expressing GFP and *hyg*B will therefore likely be the most useful for studies of pathogenesis. Preliminary studies indicate that visualization of the fluorescent transformants *in planta* is reliable, although still challenging due to the small size of the fungus. However, spread of the fungus *in planta* is easily monitored by plating pieces of infected

soybean tissue on hygromycin containing media. We are now using these transformants to determine how, where and when the fungus enters and moves within soybeans.

253. Genes induced in *Fusarium graminearum/Zea mays* interactions. Therese Ouellet, Audrey Saparno, Anju Koul, Sharon Allard, and Linda Harris. Agriculture and Agri-Food Canada, ECORC, Ottawa, Ont, Canada.

Fusarium graminearum attacks a wide range of plant species including maize (ear and stalk rot), barley, and wheat (head blight). Favorable environmental conditions (conducive temperatures and high humidity) can result in *Fusarium* epidemics and millions of dollars lost in crop revenues. *F. graminearum* infection in the cereals reduces both grain yield and quality. The grain is also contaminated with mycotoxins such as the trichothecenes. We have initiated a study of the molecular interactions between *F. graminearum* and maize during infection of the silk channel and ear in susceptible and resistant inbreds. Differential RNA display- RT-PCR has been used to identify genes, from *F. graminearum* and maize, that are elicited in the early stages of infection of maize silk by the fungus. Additionally, infection in resistant inbreds has been compared to that from highly susceptible inbreds using this technique. Unique cDNA fragments originating from either *F. graminearum* or *Zea mays* have been cloned and characterized. A summary of the findings will be presented.

254. Genetic diversity of fumonisin-producing isolates of *Fusarium moniliforme* (*Gibberella fujikuroi*) in Korea. Sook Young Park, and Yin Won Lee. Seoul National University, Agricultural Biology, Suwon, Kyunggi, South Korea.

Eighteen hundred and fifty two nitrate non-utilizing (*nit*) mutants were recovered from 130 isolates of *Fusarium moniliforme* (Teleomorph : *Gibberella fujikuroi*). The majority (76%) of *nit* mutants recovered was *nit1,* followed by NitM (17%) and *nit*3 (7%). These isolates were grouped into vegetative compatibility groups (VCGs) by demonstrating heterokaryosis using *nit* mutants. All 130 isolates were paired in all possible pairwise combinations (15,559 combinations) and were divided into 80 nonoverlapping VCGs. VCG analysis of the isolates obtained from a single maize stalk indicated that most maize stalks were infected by more than one isolates of *F. moniliforme* belong to genetically distinct groups. In the tests of mating population and type, 62 isolates were mating type A^+, 29 isolates were A^-, 3 isolates were E^-, 2 isolates were D^-, and 22 isolates were not reproducibly fertile with all mating type testers. Most isolates produced fumonisins and average production levels of fumonisin B_1, B_2, and B_3 were 342, 74, 37 ppm, respectively. Isolates Gf 33, Gf 35, Gf 36, Gf 37, Gf 38 and Gf 40 of *F. moniliforme* produced high levels of fumonisin B_3 compared to B_1 or B_2. All of these 6 isolates were mating type A^+. Moreover, these isolates were grouped in the same VCG 59. Several VC groups were distinguished by amplified DNA band patterns. Furthermore, isolates that produce high levels of fumonisin B_3 exhibited the same band pattern by primer OPF09, and these isolates were clearly differentiated from other isolates by RAPD analysis.

255. Analysis of trichothecene gene expression using a defined DNA microarray. Andrew W. Peplow[1], Andrew G. Tag[1], Tzung-Fu Hsieh[2], Terry L. Thomas[2], and Marian N. Beremand[1]. [1]Texas A&M; University, Plant Pathology, College Station, TX, USA. [2]Texas A&M; University, Biology, College Station, TX, USA.

The trichothecene mycotoxins represent a large group of toxic secondary metabolites produced by a variety of fungi including *Fusarium sporotrichioides*. The trichothecenes are produced via a complex biosynthetic pathway. Most of the genes responsible for their production are coordinately regulated, and many of them are tightly linked in a cluster. Here we report the construction of a DNA microarray and demonstrate its use to monitor expression of genes involved in trichothecene biosynthesis as well as genes thought to be associated with or affected by trichothecene biosynthesis. To construct the microarray, forty distinct DNA sequences were printed on poly-L-lysine coated microscope slides using a gridding robot. Hybridization of arrays with Cy Dye-labeled cDNA synthesized from poly(A) RNA isolated from wild-type and selected mutant strains spanning different timepoints reveals important information about the regulatory network and components that play a role in trichothecene gene expression and toxin production. Since our data from two microarrays are equivalent to 80 northern analyses, this system offers the opportunity of rapidly assessing qualitative differences in expression of many genes involved specifically with or affected by trichothecene production.

256. *Cih1*, a biotrophy-related gene expressed at the intracellular interface formed between *Colletotrichum lindemuthianum* and french bean. Sarah E. Perfect[1], Richard J. O'Connell[2], and Jon R. Green[1]. [1]University of Birmingham, Biological Sciences, Birmingham, Edgbaston, UK. [2]IACR-Long Ashton, University of Bristol, Long Ashton, Bristol, U.K.

Colletotrichum lindemuthianum is a hemibiotrophic ascomycete which causes anthracnose of bean, *Phaseolus vulgaris*. During the initial biotrophic stage of infection, the fungus differentiates infection vesicles and primary hyphae within host epidermal cells. Monoclonal antibodies (MAbs) have been raised to isolated biotrophic infection structures to facilitate the identification of biotrophy-related molecules. One of these MAbs, designated UB25, recognises a glycoprotein present in the infection peg, and the fungal walls and matrix surrounding the intracellular hyphae. However, the antigen does not appear to be present in secondary necrotrophic hyphae, which suggests that it is specific to the biotrophic stage of the interaction. This glycoprotein may therefore be involved in the establishment and maintenance of biotrophy. The MAb UB25 has been used to immunoscreen a cDNA library constructed from infected bean hypocotyls and positive clones have been isolated and sequenced (Perfect *et al*., 1998. *The Plant Journal* 15(2): 273-279). Southern analysis indicates that the CIH1 glycoprotein recognised by UB25 is fungally encoded. Analysis of the deduced amino acid sequence of revealed the presence of two distinct domains, one of which is proline-rich and contains short repetitive motifs with tyrosine-lysine pairs. Tyrosine residues have been implicated in the oxidative cross-linking of plant cell-wall glycoproteins such as extensins. Cross-linking studies of CIH1 indicate that this glycoprotein has the potential to be oxidatively cross-linked by peroxidase in the presence of hydrogen peroxide. *CIH1* homologues have been identified in a range of *Colletotrichum* species by Southern analysis. The expression patterns of these homologues in other fungal-plant interactions has been investigated by northern analysis and indirect immunofluorescence using UB25. Gene replacement studies are underway to assess the functional importance of *CIH1*.

257.A polyketide synthase gene required for the biosynthesis of fumonisin mycotoxins in *Gibberella fujikuroi* Mating PopulationA. Robert H. Proctor, Anne E. Desjardins, Ronald D.

Plattner, and Thomas M. Hohn. USDA ARS NCAUR , Mycotoxin Research Unit, Peoria, Illinois, USA.

Fumonisins are toxins produced by the maize pathogen *Gibberella fujikuroi* mating population A (MP-A) and are associated with mycotoxicoses in both humans and animals. Structural and precursor feeding studies indicate fumonisins are products of either polyketide or fatty acid metabolism. We attempted to isolate a polyketide synthase (PKS) gene involved in fumonisin biosynthesis via PCR with degenerate PKS primers and a cDNA template prepared from a fumonisin-producing culture of *G. fujikuroi*. Nucleotide sequence analysis of the single PCR product and flanking DNA from a cosmid clone revealed a gene (*FUM5*) with a 7.8 kb coding region interrupted by 5 introns. The predicted *FUM5* translation product had conserved amino acid sequences indicative of the ketoacyl synthase, acyl transferase, ketoacyl reductase, dehydratase, enoyl reductase, and acyl carrier protein domains present in Type I bacterial and fungal PKSs. Transformation of a cosmid clone that included FUM5 into G. fujikuroi increased the levels of production in three strains and restored wild-type production in a fumonisin-nonproducing strain. Disruption of FUM5 reduced fumonisin production by over 99% in *G. fujikuroi* MP-A. These results indicate that *FUM5* is a PKS gene required for fumonisin biosynthesis and, further, that fumonisins are primarily products of polyketide rather than fatty acid metabolism.

258. Expressed sequence tag analysis of gene expression in *Phytophthora sojae*. Dinah Qutob[1], and Mark Gijzen[2]. [1]University of Western Ontario, Microbiology and Immunol, London, Ontario, Canada. [2]Agriculture Canada, London, Ontario, Canada.

Phytophthora sojae is an oomycete pathogen that causes stem and root rot on soybean plants. We have constructed three cDNA libraries using mRNA isolated from axenically grown mycelia and zoospores, and tissue isolated from plant hypocotyls 48 h after inoculation with zoospores. More than one thousand expressed sequence tags (ESTs) were generated from each library. The ESTs were compared to existing sequences in the NCBI database by BLASTX searches. The resulting hits were assigned to functional categories and the distribution of the ESTs were compared for each source library. Distinct expression patterns were observed for each library. Although it is not possible to unequivocally distinguish *P.sojae* from soybean ESTs in the infected plant library, we have found that the G+C content of the sequence provides a useful measure for this purpose. When ESTs are classified based upon G+C content and plotted according to frequency, normal distributions are observed for ESTs from axenically grown *P. sojae* (58.1% mean G+C) or soybean (45.9% mean G+C). In contrast, a binomial distribution of two distinct populations of clones are present in the infected plant library. We estimate that 60 to 70% of the ESTs from the infected plant library are derived from *P. sojae* cDNA transcripts. *P. sojae* has always been considered an aggressive and fast growing pathogen, nonetheless, this very high representation of pathogen genes in the infected plant library is surprising.

259. Understanding fungal symbiotic lifestyles: Isolation of pathogenicity genes from Colletotrichum species and deciphering the basis of plant protection afforded by non-pathogenic mutants. Regina S. Redman, and Rusty J. Rodriguez. USGS, BRD, Seattle, WA, USA.

Previously, we isolated and characterized a mutant (path-1) of *C. magna* which does not elicit disease symptoms but retains the ability to infect and grow through host tissues. In addition, plants colonized with path-1 are protected from disease caused by *C. magna, C. orbiculare,* and *Fusarium oxysporum*. Disease protection in this system is localized, tissue specific, and is correlated to a rapid and strong activation of host defenses when path-1 colonized plants are exposed to virulent fungi. To begin deciphering the genetic complexity of pathogenicity, we isolated 176 non-pathogenic mutants of *C. magna* by DNA disruption using restriction enzyme mediated integrative (REMI) transformation. Collectively, these mutants express three phenotypes: A - colonization and full protection of watermelon plants against virulent fungi, B - colonization and partial protection of plants against virulent fungi, and C - colonization and no protection of plants against virulent fungi. Molecular and segregation analysis of two non-pathogenic REMI mutants (R1 and R21) indicated that these non-pathogenic phenotypes resulted from integration of the transformation vector into single sites in the fungal genomes. Recently, we cloned the integrated vector and 4.5 kb of flanking DNA from REMI mutant R1. To verify that the cloned DNA, designated pGMR1, contained a pathogenicity gene, pGMR1 was transformed into a wild type (wt) isolate in an attempt to induce gene disruptions. 47% of the pGMR1 transformants (Tx's) were nonpathogenic on watermelon seedlings and expressed the same phenotype as the R1 mutant.

260. The homoserine utilization gene from a CD chromosome of *Nectria haematococca*: a habitat-defining gene? Marianela C. Rodriguez, Esteban D. Temporini, Catherine C. Wasmann, and Hans D. Van Etten. University of Arizona, Plant Pathology, Tucson, Arizona, USA..The species *Nectria haematococca* mating population VI exists in a wide range of habitats although individual isolates have a limited habitat range. Previous research from our lab has indicated that part of this habitat diversity is due to the presence of supernumerary chromosomes that contain unique habitat defining genes. Since these chromosomes are not needed for axenic growth but are important for habitat specificity, they have been called "Conditionally Dispensable" (CD) chromosomes. One of these CD chromosomes contains a cluster of genes for pathogenicity on pea called the PEP cluster (for pea pathogenicity). In the current study we show that all isolates pathogenic on pea are able to utilize homoserine as a sole carbon and nitrogen source. Homoserine is one of the chemicals present in larger amounts in pea root exudates. We found that the homoserine utilization gene (HUT) is located on the same CD chromosome that contains the PEP cluster but is not part of this cluster. Preliminary results comparing near isogenic strains differing in the region of the CD chromosome containing HUT show that an isolate with this region has a competitive advantage in the pea rhizosphere. Cosmid clones containing HUT have been identified by complementation of homoserine utilization in *Neurospora crassa*. Once HUT has been isolated we will determine if it is an additional habitat-defining gene on the CD chromosome.

261. Involvement of cAMP and pH in signaling sclerotial development and oxalic acid production in *Sclerotinia sclerotiorum*. Jeffrey A. Rollins. University of Nebraska, Plant Pathology, Lincoln, NE, USA.

Sclerotinia sclerotiorum is a broad host range, filamentous, plant pathogen which produces oxalic acid (OA), an essential pathogenicity determinant. Previously, we isolated mutants which are deficient in both OA production and sclerotial development and demonstrated that elevated

levels of cAMP increase OA production and inhibit sclerotial development. We are interested in further characterizing the molecular signaling pathways which may link these secondary metabolic and developmental pathways. We now demonstrate that ambient pH also plays a role in regulating OA production and sclerotial development. Similar to the effects of high cAMP, neutral or alkaline ambient pH inhibits sclerotial development while increasing OA production. To examine the roles of pH-dependent and cAMP-dependent signaling in sclerotial development and OA biosynthesis we are cloning and characterizing genes which encode components of these signaling pathways. We have cloned and characterized the sequence of a gene encoding the catalytic subunit of cAMP-dependent protein kinase A (PKA-S) from *S. sclerotiorum*. Functional analysis of this gene is being carried out by targeted gene disruption and by constitutive overexpression. Characterization of transformants from targeted gene disruption experiments will be reported. The first step towards overexpressing PKA-S, the identification of a constitutive promoter driving reporter gene expression in *S. sclerotiorum*, has been accomplished. This reporter gene system utilizes the toxA promoter (Ciuffetti et al. 1997. Plant Cell 9:135-144) to drive GFP expression. Characterization of GFP expression in *S. sclerotiorum* and efforts to clone genes involved in ambient pH signaling will be presented.

262. Phycomyces genes for the metabolism of 3-hydroxy-3-methylglutaryl-Coenzyme A.Javier Ruiz-Albert, Enrique Cerdá-Olmedo, and Luis M. Corrochano. Universidad de Sevilla, Departamento de Genetica, Sevilla, Sevilla, Spain.

Terpenoids form a chemical family that includes sterols, carotenoids, and gibberellins. All terpenoids share the early biosynthetic steps, including the synthesis of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) and its conversion to mevalonate. These two steps are catalized by the enzymes HMG-CoA synthase (HMGS) and HMG-CoA reductase (HMGR), respectively. The Zygomycete *Phycomyces blakesleeanus* has been a subject for research of carotene biosynthesis. We have cloned and sequenced the *Phycomyces* genes *hmgS* and *hmgR*, encoding HMG-CoA synthase and HMG-CoA reductase, respectively. Gene *hmgR* is 3.9 kb long and encodes a protein of 1171 aminoacids with a transmembrane domain, a linker, and a cytoplasmic catalytic domain. The first has eigth predicted transmembrane segments. The catalytic domain of *Phycomyces* HMGR is very similar to that of other fungi. The *hmgS* gene encodes a cytoplasmic protein of 473 amino acids. *Phycomyces* contains a single *hmgR* gene and a single *hmgS* gene, as judged from Southern hybridizations. The *hmgR* gene is expressed at similar levels throughout vegetative growth. The catalytic domain of HMGR has been overexpressed and the resulting protein has been purified and characterized. Mutants with an increased carotene biosynthesis accumulate more *hmgS* and *hmgR* mRNAs than the wild type. These genes are used to determine the mechanisms of action of external agents and mutations that modify carotene biosynthesis.

263. Monitoring structural changes in heterogeneous populations of the head blight pathogen *Fusarium culmorum* with molecular fingerprint markers .Angela G. Schilling [1], Thomas Miedaner [2], and Hartwig H. Geiger [1, 1]University of Hohenheim, Institute of Plant Breeding, Seed Sciences and Population Genetics, and [2] State Plant Breeding Institute, D-70593 Stuttgart, Germany.

Fusarium culmorum is a phytopathogenic and mycotoxigenic fungal species causing ear and foot rot diseases of cereals world-wide. Genetic variation has been described for aggressiveness, mycotoxin production, and molecular markers among isolates from diverse habitats and individual field populations. In this study, field experiments were conducted to analyse changes in the composition of isolate mixtures as a function of aggressiveness, mycotoxin production of the mixture components and host resistance. Pairs of isolates expressing similar or contrary levels of aggressiveness and different types of mycotoxins were inoculated onto two rye genotypes at anthesis. Host resistance differed between the rye genotypes. All isolates were also inoculated individually. The low aggressive isolates produced nivalenol and the high aggressive ones deoxynivalenol. At the onset of infection, pathogen genotypes were re-isolated from ears and identified with RAPD markers characteristic for the isolates used. On both rye genotypes, disease severities and yield reduction assessed as aggressiveness measures were significantly lower following infection with mixtures than with single isolates. Thus, competition between fungal genotypes may have occurred explaining the weaker colonization of the host. The ratios of re-isolated genotypes from mixed infections deviated significantly from 1:1 when a particularly high aggressive isolate was inoculated together with either a lower or similarly high aggressive one. Similar observations were made with mixtures of a particular low aggressive isolate indicating that pathogen genotypes display additional fitness characteristics other than aggressiveness. Changes in the composition of fungal populations were also inferred from analysing mycotoxin profiles and accumulation in the harvested grain.

264. The role of *BcatrB* , a new ATP-binding cassette transporter, in pathogenesis of *Botrytis cinerea*.Henk-jan Schoonbeek[1], Giovanni Del Sorbo[2] and Maarten A. De Waard[1], [1] Laboratory of Phytopathology, Wageningen Agricultural University. P.O. Box 8025, 6700 EE Wageningen, The Netherlands. [2] Institute of Plant Pathology, Via Università, 100, 80055 Portici (Naples) Italy.

ATP-binding cassette (ABC) transporters are membrane transport ATPases which may play a role in the secretion of exogenous toxic products. We propose that in plant pathogens these transporters can be involved in the secretion of toxic plant defence products which accumulate in fungal cells upon colonisation of plant tissue. In addition, ABC-transporters may function in secretion of fungal pathogenicity factors. We study the role of ABC-transporters in pathogenesis of *Botrytis cinerea*. Two homologues of *PDR5*, a gene involved in pleiotropic drug resistance in yeast, have been isolated from a genomic library of *B. cinerea* strain SAS56. The sequences of the genes *BcatrA* and *BcatrB* predict proteins with a (NBF-TMD)2 organisation and a length of 1562 and 1439 amino acids, respectively. The inducing activity of several fungitoxic compounds of natural and synthetic origin was tested using Northern blot analysis. Only cycloheximide induced *BcatrA* Its effect on the expression of *BcatrB* was weak. Interestingly, this gene was strongly induced by the grapevine phytoalexin resveratrol. Neither of the genes was induced by other plant defence compounds tested (pisatin, tomatin and quercetin) at physiological concentrations. Knock-out mutants of both genes were made by replacement of the major part of the coding region by a hygromycin resistance cassette. No phenotype could be determined for D*BcatrA* mutants. However, D*BcatrB* mutants proved to be more sensitive to resveratrol. These results indicate that BcatrB plays a role in protection of the fungus against resveratrol and possibly other plant defence compounds which have not been tested yet.

265. Genetic transformation of the basidiomycete *Trametes versicolor*.Frank Schuren, Hannie Hessing, Marian van Muijlwijk, Cora van Zeij[1], Rupert Pfaller[1] and Cees van den Hondel. TNO Nutrition & Food Research Institute, P.O. Box 360, 3700 AJ Zeist, The Netherlands [1]Consortium für Elektrochemische Industrie, München, Germany.

The basidiomycete *Trametes versicolor* has the ability to secrete significant amounts of laccase into its growth medium. Increased expression of a *T. versicolor* laccase gene in *Aspergillus niger* turned out to be problematical. Therefore, a transformation system was developed for *T. versicolor* to enable homologous expression. By using FOA-selection a *pyrG* mutant was isolated which could be transformed with the *Schizophyllum commune ura1* gene. Transformation frequencies were rather low but the addition of restriction enzymes during transformation (REMI) and the fusion of the *ura1* coding sequence to the *T. versicolor gpd* promoter resulted in an enormously increased transformation frequency, up to 30 transformants µg-1 DNA. Overexpression of laccase was tested both by analyzing transformants containing multiple copies of the laccase genomic clone and by analyzing transformants containing gpd promoter driven laccase constructs. A simple plate assay was developed for quick analysis of transformants. Best expression was found in transformants containing gpd promoter driven laccase constructs. Laccase expression in liquid cultures of well-expressing transformants was 5-10 higher compared to non-transformed strains.

266. Analysis of xylanase and pectinase genes in *Cochliobolus carbonum*.John S. Scott-Craig, Nyerhovwo J. Tonukari, Sigrun Wegener, and Jonathan D. Walton. Michigan State University, DOE Plant Research Lab, East Lansing, Michigan, USA.

The maize pathogen *Cochliobolus carbonum* secretes a large number of plant-cell-wall-degrading enzymes when grown with maize cells walls as the sole carbon source. Xylan-containing polymers constitute almost 50% of the dry weight of the maize cell wall while pectin constitutes about six percent. At least five xylan-degrading and three pectin-degrading enzymes are produced by the fungus. Genes encoding four endo-xylanases (*XYL1,2,3,4*), one ß-xylosidase (*XYP1*), an endo-polygalacturonase (*PGN1*), an exo-polygalacturonase (*PGX1*) and a pectin methylesterase (*PME1*) have been cloned and used to create strains containing deletions of each gene. These deletions, singly and in combination, are being examined for their effects on the growth of the fungus in vitro on xylan and pectin and on the ability of the fungus to infect maize.

267. Factors affecting pathogenicity of *Metarhizium anisopliae*.Steven E. Screen, and Ray J. St. Leger. University of Maryland, Entomology, College Park, MD, USA.

Infection by *Metarhizium anisopliae* requires a precise sequence of events, encompassing spore adhesion, germ-tube development, appressorium formation, penetration, and directed growth down through the insect cuticle. We have been studying the host-related signals that direct this development/differentiation using the co-ordinated formation of infection structures and secretion of the major cuticle degrading protease (PR1) as our models. A complex interaction between the fungus and host, involving the topography and hydrophobicity of the cuticular surface, and the concentration and nature of available nutrients, ultimately determines whether a pathogenic interaction is established. These primary host related signals are transmitted into the cell via second messengers (cAMP and Ca^{2+}) causing changes in the expression/activity of both

global (eg. CRR1 and NRR1) and pathogenicity specific transcription regulator proteins. Response to host signals is also influenced by pH which can override the inductive effects of cuticle components.

268. Characterisation of a gene encoding cyanide hydratase in the phytopathogenic fungus *Leptosphaeria maculans* .Adrienne C. Sexton, and Barbara J. Howlett. The University of Melbourne, Botany, Parkville, Vic, Australia.

A gene encoding cyanide hydratase from *Leptosphaeria maculans*, an ascomycete which causes blackleg disease of oilseed Brassica spp. has been cloned. The predicted amino acid sequence of this single copy gene is 77% and 82% identical to cyanide hydratase from phytopathogenic fungi *Gloeocercospora sorghi* and *Fusarium lateritium*, respectively. This enzyme catalyses the breakdown of hydrogen cyanide to a less toxic compound, formamide. The cyanide hydratase gene is induced strongly in vitro by 0.1 mM KCN, and slightly in the presence of 1 mM 2-propenyl glucosinolate, the major glucosinolate of *Brassica juncea*, plus a plant enzyme, myrosinase (which catalyses glucosinolate hydrolysis). This finding suggests that HCN may be released during glucosinolate breakdown in *B. juncea*. Levels of HCN released from macerated leaves of *B. juncea* and *B. napus*, and from 2-propenyl glucosinolate hydrolysis were measured. No HCN was detected from 2-propenyl glucosinolate or from *B. juncea* leaves, however, a small amount (25 nmol/g fresh weight) was released from *B. napus* leaves. *B. napus* has a high content of hydroxy aliphatic glucosinolates, which yield hydroxynitriles upon hydrolysis, and in other plants hydroxynitriles breakdown to HCN. Therefore, *L. maculans* may need to detoxify HCN during infection of *B. napus*. Transcription of cyanide hydratase is detectable in infected cotyledons of *B. juncea* and *B. napus*, but the role of this gene in the infection process is unclear.

269. Cloning of a cAMP-dependent protein kinase catalytic subunit in *Aspergillus nidulans*. Kiminori Shimizu and Nancy P. Keller. Texas A&M; University, Plant Path & Microbiology, College Station, TX, USA.

The filamentous fungus *Aspergillus nidulans* is known to produce both a carcinogenic mycotoxin, sterigmatocystin, and an antibiotic, penicillin, as secondary metabolites. Previous work in our lab has indicated that production of both metabolites is dependent on the activity of G(i)a subunit of a heterotrimeric G protein. To investigate the involvement of the catalytic subunit of cAMP-dependent protein kinase (PkaC), a likely downstream target of G(i)a activity, in the production of these natural compounds, we have initiated the genetic characterization of this protein. We screened an *A. nidulans* cosmid library with the EST clone n8d03a1.r1, predicted to encode the PkaC, and obtained two overlapping cosmids, pWE08C4 and pWE06A11. Southern analysis showed that the putative *pkaC* gene is located on a 5.4 kb HindIII fragment. Sequence analysis of this region revealed that the *pkaC* gene is highly conserved among fungal species on both the nucleotide and the deduced amino acid sequences.

270. The hydrophobin gene family of *Cladosporium fulvum*. Pietro D. Spanu[1], Martina Huber[1], Gert Segers[2], Peter-Stein Nielsen[3], and Richard P. Oliver[2]. [1]Oxford University, Plant Sciences, Oxford, UK. [2]Carlsberg Laboratory, Physiology, Copenhagen, Denmark. [3]Carlsberg Laboratory, Yeast Genetics, Copenhagen, Denmark, Denmark.

Cladosporium fulvum, the causal agent of tomato leaf mould, has at least six hydrophobin genes; we have named these *HCf-1* to *HCf-6*. Based on sequence similarity the first four proteins appear to be class I hydrophobins and HCf-5 and HCf-6 class II hydrophobins. HCf-4 and HCf-6 are "bimodular" hydrophobins: they have a ca. 100 aa C-terminus with a typical hydrophobin sequence and an N-terminus of about the same length with a completely different sequence. The N-terminus of HCf-4 shows no evident similarity to sequences in the databases, while the N-terminus of HCf-6 has an extremely glycine- and asparagine-rich composition. All six hydrophobins are expressed, but the pattern of expression of each gene differs depending on development and nutritional status of the growth medium. *HCf-1* mRNA is the most abundant and together with the mRNAs of *HCf-2*, *HCf-3* and *HCf-6* is present in vegetative mycelium. The levels of mRNA of all hydrophobins increase during sporulation. Depletion of nitrogen and /or carbon from the medium induces differential changes in the levels of mRNA of the various hydrophobins. The presence of so many different hydrophobins in *C. fulvum* raises intriguing questions about the possible functions of these genes. Results on the effect of ablation of some of the hydrophobins will be presented and discussed. The support of the Royal Society (to PS) and Foetek II (to PSN) is gratefully acknowledged.

271. Changes in ambient pH accompanying growth of *Metarhizium anisopliae, Neurospora crassa* and *Aspergillus fumigatus* allow extracellular protease activity. Raymond J. St. Leger and Steven E. Screen. University of Maryland, Entomology, College park, MD, USA.

Mutants of *M. anisopliae* altered in production of oxalic acid were evaluated for interrelationship of ambient pH, buffering capacity added to media, growth, and generation of extracellular proteases and ammonia. Wild-type and acid-overproducing mutants (+) grow almost as well at pH 8 as at pH 6, but acid non-producing (-) mutants showed limited growth at pH 8, indicating that acid production is linked to the ability to grow at higher pH. Production of ammonia by *M. anisopliae* was inducible by amino acids when cells were derepressed for nitrogen and carbon. Likewise, although some ammonia was produced by *Aspergillus fumigatus* and *Neurospora crassa* in minimal media, production was enhanced by low levels of amino acids. Ammonia production by *A. fumigatus, N. crassa,* and *M. anisopliae* increased medium pH and allowed production of subtilisin proteases whose activities are observed only in basic pH. In contrast, protease production by the Acid (+) mutants of *M. anisopliae* was greatly reduced because of acidification of media. This suggests that alkalinization by amino-acid-induced ammonia production is adaptive by facilitating the utilization of proteinaceous nutrients. Consistant with this, Acid(+) mutants of *M. anisopliae* were non-pathogenic to host (*Manduca sexta*) larvae suggesting that virulence is dependent on pH in infected tissues being maintained or increased. The results of this study suggest that ammonia may have functions related to regulation of the microenvironment and it represents a previously unconsidered virulence factor in diverse fungi with the potential to harm tissues and disturb the hosts immune system.

272. Analysis of the distribution of pathogenicity genes in the filamentous fungus *Nectria haematococca*. Esteban Daniel Temporini and Hans D. VanEtten. University of Arizona, Plant Pathology, Tucson, Arizona, USA.

The filamentous fungus *Nectria haematococca* contains a cluster of genes required for pathogenicity towards pea plants. This cluster, called the Pea Pathogenicity or PEP gene cluster

contains at least six genes and is located on what has been termed a "conditionally dispensable" (CD) chromosome. CD chromosomes are dispensable for normal growth in culture because they do not contain genes essential for axenic growth but they carry genes required to colonize specific environments. A comparison of the G+C content and codon usage of the genes in the PEP cluster indicates that they differ from that of genes located on normal chromosomes in this fungus. Our working hypothesis is that the PEP cluster is analogous to bacterial pathogenicity islands and has been acquired by *N. haematococca* by horizontal transfer. In this work we show the distribution of PEP genes homologs in laboratory and field isolates of this fungus and its correlative relationship to host-specific pathogenicity. Our results show that all pea-pathogenic isolates have at least one copy of each PEP gene homolog. There is a high degree of polymorphism among PEP homologs and they were found in at least three different CD chromosomes. We also show that they can be present on normal chromosomes even in non-pathogenic isolates implying that some of the homologs may not function as pea pathogenicity determinants. A single lateral transfer followed by divergence or multiple transfers during the evolution of this pathogen are the proposed mechanisms to explain the origin and evolution of the PEP cluster/CD chromosomes in *N. haematococca*.

273. Expression and cellular distribution of the *Magnaporthe grisea* MAPkinase PMK1.
Fernando A. Tenjo, Jin-Rong Xu, and John E. Hamer. Purdue University, Biological Sciences, West Lafayette, IN, 47907, USA.

Fungal plant pathogens develop infectious structures in response to the interaction with specific surfaces. The involvement of signal transduction pathways in this and other fungal developmental processes has been well documented. Our lab has isolated a MAP kinase *PMK1* from *Magnaporthe grisea* which is essential for appressorium formation and invasive growth in plants. We want to localize Pmk1p during appressorium formation and during the infection for a better understanding of its role during these processes. The activation of Pmk1p may affect its localization within a cell, or can be restricted to a particular cell type as has been demonstrated for other MAP kinases A *GFP-PMK1* translational fusion was used to transform a *pmk1*$^{-}$ mutant. The formation of appressorium was restored in the resulting transformants. The localization of the GFP-PMK1 fusion protein during appressorium formation in inductive and non-inductive surfaces will be presented. Also the pattern of expression will be assessed in germlings growing in conditions that suppress appressorium formation, and during the infection process in vitro using onion skin strips. The results of the localization of Pmk1p will be discussed in relation to the possible mechanism of activation of this MAP kinase.

274. Restriction enzyme mediated insertional mutagenesis of *Colletotrichum graminicola*.
Michael R. Thon and Lisa Vaillancourt. University of Kentucky, Plant Pathology, Lexington, KY, USA.

As part of a project to identify and clone pathogenicity-related genes from *Colletotrichum graminicola*, causal agent of maize anthracnose, we have developed a method for restriction enzyme mediated insertional (REMI) mutagenesis. *C. graminicola* strain M1.001 is transformed to hygromycin resistance with efficiencies of over 30 transformants/microgram DNA. Transformation efficiency varies greatly depending on the type and amount of restriction enzyme used during transformation. Transformation efficiency increases 5-20 fold when restriction

enzymes are added during transformation. Approximately half of the transformants contain single copy plasmid integrations, and about half of these have maintained the recognition sites of the restriction enzyme used during transformation. During assays of the transformants we have identified several putative mutants, including one with altered conidial morphology and two with reduced ability to colonize maize pith tissue.

275. 1,3,6,8-tetrahydroxynaphthalene reductase is involved in melanin biosynthetic pathway of *Colletotrichum lagenarium*. Gento Tsuji[1], Tomomi Sugawara[1], Isao Fujii[2], Yuuichirou Mori[2], Yutaka Ebizuka[2], Yoshitaka Takano[3], Iwao Furusawa[3], Osamu Horino[1] and Yasuyuki Kubo[1]. [1]Kyoto Prefectural University, Lab of Plant Pathology , Kyoto, Japan. [2]Tokyo University, Fac of Pharma. Sci., Tokyo, 113-0033, Japan. [3]Kyoto University, Lab of Plant Pathology, Kyoto, 606-8502, Japan.Melanin biosynthesis of *Colletotrichum lagenarium* is essential for appressorial penetration of its host plants. The involvement of 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN) and 1,3,6,8-THN reductase (T_4HR) in the melanin biosynthetic pathway has been hypothesized, however no evidence has been presented experimentally in *C. lagenarium*. Here we present the evidence by heterologous expression of *C. lagenarium* polyketide synthase gene (*PKS1*) in *Aspergillus oryzae* and isolation of a novel melanin deficient mutant of *C. lagenarium*. *A. oryzae* was transformed with *PKS1* expression vector constructed with pTAex3 carrying TAKA amylase promoter. 1,3,6,8-THN was identified from culture filtrate of transformants by Mass and NMR spectra analysis. Subsequently, to isolate *T_4HR* deficient mutant, 1,3,8-trihydroxynaphthalene reductase gene (*THR1*) deficient mutant was mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine, in view of the possibility that THR1 catalyze the conversion of 1,3,6,8-THN to scytalone. Deduced double mutant, with *THR1* and *T_4HR* mutation, secreted red pigment into the culture. Mass and NMR spectra analysis of secreted metabolites from the mutant identified the melanin shunt products, flaviolin and 3,3'-biflaviolin, derived from 1,3,6,8-THN. These results suggest that T_4HR is involved in melanin biosynthetic pathway of *C. lagenarium*.

276. Molecular genetics of gibberellin biosynthesis in *Gibberella fujikuroi*. Bettina Tudzynski[1], Peter Hedden [2], Veronika Homann [1], Thorsten Voss [1], and Pia Linnemannstoens [1]. [1]University of Muenster, Institut f. Botanik, Muenster, NRW, Germany. [2]IACR-Long Ashton Station, Dept. Agricult. Sciences, Long-Ashton, England, UK.

In order to isolate genes of the gibberellin pathway from *Gibberella fujikuroi*, a differential screening of a cDNA library was performed. The deduced amino-acid sequence of two clones contained the conserved heme-binding motif of cytochrome P450 monooxygenases. By sequencing the corresponding hybridizing 6.7 kb genomic *SalI* fragment, a second cytochrome P450 monooxygenase gene was found to be closely linked to the first one. Gene replacement experiments clearly demonstrated that both genes are involved in gibberellin biosynthesis. Chromosome walking was perfomed to find further genes of this family or other genes involved in the gibberellin pathway. Next to the two P450 monooxygenase genes, a putative geranylgeranyl diphosphate synthase gene, the copalyl diphosphate gene, which is the first specific gene of the gibberellin pathway, and two further P450 monooxygenase genes were identified. Transcription of the 6 genes is co-regulated. These results suggest that at least some of the genes involved in the biosynthesis of gibberellins are closely linked in a gene cluster in *G. fujikuroi*. The function of several genes of this cluster was identified by gene disruption and GC-

MS and HPLC analysis combined with feeding experiments. Furthermore, the ammonium regulation of the gibberellin production was studied on molecular level.

277. Cloning of G protein alpha subunits from *Botrytis cinerea*. Bettina Tudzynski and Daniela Kasulke Westfälische Wilhelms-Universität Münster, Institut für Botanik, 48149 Münster, Germany.

Botrytis cinerea is the causal agent of "grey mould" diseases of many economically important fruits, vegetables and flowers. Our main interest is the study of the genes involved in the interaction process between the fungus and its host plant. Heterotrimeric G proteins play an important role in transducing several extracellular signals from the cell surface to a variety of intracellular targets via a cascade of interacting proteins, such as cAMP cyclase and protein kinases. For some phyto-pathogenic fungi more than one heterotrimeric G protein-encoding genes were obtained. At least one of them was shown to be involved in pathogenesis. On the basis of sequence alignments between G alpha subunits of many eukaryotic organisms we constructed degenerated PCR primers and cloned three different genes (*bcg1, bcg2,* and *bcg3*). One of them showed a high degree of identity with CPG-1 from *Cryphonectria parasitica* and MAGB from *Magnaporthe grisea* and belongs to the G alpha i class. Since especially members of this class seem to play an important role in pathogenesis, differentiation and mating, a gene disruption approach was started. Genetic and pathogenicity analysis of the transformants are under investigation.

278. Active derivatives of Ptr ToxA: Towards the biochemical identification of a ToxA interacting wheat protein. Robert P. Tuori, Linda Hardison, Thomas J. Wolpert, and Lynda M. Ciuffetti. Oregon State University, Botany and Plant Pathology, Corvallis, Oregon, USA

Functional Ptr ToxA was expressed and purified from *Escherichia coli* as an N-terminal poly-histidine fusion protein (NC-FP). NC-FP, consisting of both the N- and C- domains of the *ToxA* ORF, is expressed as an insoluble protein in *E. coli* at approximately 10 mg/l culture. Following in vitro refolding, NC-FP elicits cultivar-specific necrosis in wheat, with a specific activity similar to native Ptr ToxA. A construct containing a single PCR-induced substitution mutation was isolated during the screening of bacterial colonies for expression. The mutant toxin, NCmut-FP, is significantly reduced in its necrosis-inducing ability. The mutation, T_{137} to A, does not disrupt a region of the protein with obvious function as this threonine residue is not phosphorylated. NC-FP and NCmut-FP were affinity-purified with NiNTA-agarose resin and derivatized with a trifunctional reagent, sulfo-SBED, which contains a photoactivatable crosslinking moiety. The majority of the protein was derivatized following the labeling reaction, as determined by electrophoresis. Mass spectral analysis will also be conducted. Labeled NC-FP retains full activity. Derivatized NC-FP and NCmut-FP will be used for both *in vitro* and *in vivo* binding assays to identify a potential interacting wheat protein(s). NCmut-FP will provide a useful control to distinguish between specific and nonspecific interactions. This mutation will also provide a useful control in yeast two-hybrid screens for interacting wheat proteins.

279. Involvement of pectinases from *Botrytis cinerea* in plant pathogenesis. Jan A.L. van Kan[1], Arjen ten Have[1], Wendy J.M. Oude Breuil[1], Jos P. Wubben[2], Wietse Mulder[2] and Jaap Visser[2]. [1]Wageningen University, Laboratory of Phytopathol, Wageningen, Gelderland, the

Netherlands. [2]Wageningen University, section MGIM, Wageningen, Gelderland, The Netherlands

Botrytis cinerea is a fungus that can infect over 200 plants resulting in devastating pre and post harvest diseases of many agronomic crops. At all stages of the infection process the fungus secretes pectinolytic enzymes which have been suggested to be involved in the penetration and the invasion of plant tissue. We have isolated 6 members of the endo-polygalacturonase (endoPG) gene family as well as an endo-pectin lyase. Expression studies, performed in several hosts at 20 degrees C and/or 4 degrees C, have shown a differential expression of the isolated genes. One of the endoPG encoding genes, *Bcpg1*, shows a basal expression both *in planta* as well as in liquid cultures. Elimination of this gene, using gene replacement, resulted in a strain that: (1) Shows a significantly reduced virulence. (2) Shows a reduced induction of the remaining endoPG activities. Therefore we postulate that the activity of the gene product BcPG1 releases oligo-galacturonides that induce expression of (some) other endoPGs. We are therefore studying the expression of the endoPG gene family in both wild type and the *Bcpg1* null mutant by northern blot, quantitative RT-PCR and IEF zymogram analysis. The latest results will be presented and discussed with respect to both regulatory consequences and plant pathological aspects. This research was supported by the Dutch Technology Foundation (STW), grant number WBI 33.3046.

280. Withdrawn

281. Ergot alkaloid biosynthesis genes cloned from *Claviceps* and *Balansia*. Jinghong Wang[1], Caroline Machado[1], Daniel Panaccione[2], and Christopher Schardl[1]. [1]University of Kentucky, Plant Pathology, Lexington, Kentucky, USA. [2]West Virginia University, Plant and Soil Sciences, Morgantown, West Virginia, USA.

Toxic ergot alkaloids include clavines, lysergic acid and ergopeptines. Dimethylallyltryptophan synthase (DMAT synthase) catalyzes the first pathway-specific step in ergot alkaloid biosynthesis, and a peptide synthetase is required for the terminal step to the highly neurotoxic ergopeptines. A gene encoding DMAT synthase was cloned from *Claviceps purpurea* ATCC 20102 by screening a cosmid library with the previously cloned homologue from *Claviceps fusiformis*. The two homologues were compared to identify putatively conserved amino acid sequences. Using degenerate primers designed to these sequences a DMAT synthase gene from *Balansia obtecta* was cloned by PCR. In *C. purpurea* ATCC 20102, the DMAT synthase gene is tightly linked to one of several peptide synthetase genes identified previously. Homologues of this particular peptide synthetase gene were detected in several related fungi that produce different ergopeptines: *C. purpurea*, which produces ergotamine; *B. obtecta*, which produces ergobalansine; and *Neotyphodium* spp. known to produce ergovaline. No such homologue was detected in *C. fusiformis*, a clavine producer but ergopeptine non-producer. These results strongly suggest that this peptide synthetase is involved in ergopeptine biosynthesis. In summary, we have apparently identified genes for two critical steps in ergot alkaloid production in fungi producing structurally distinct ergot alkaloids.

282. Characterisation of an avirulence gene from *Venturia inaequalis*. Joe Win[1], Kim M. Plummer[1], Joanna K. Bowen[2] and Matt D. Templeton[2]. [1]University of Auckland, School of

Biological Sciences, Auckland, New Zealand. [2]HortResearch, Molecular Genetics, Auckland, New Zealand.

Venturia inaequalis causes scab disease in apple (*Malus*). The gene-for-gene nature of the *Malus* and *V. inaequalis* interaction has been demonstrated by genetic analysis of both the host and the pathogen. This system offers an excellent opportunity to investigate avirulence and resistance. We are investigating the interaction between the resistance gene *Vm* originally from *Malus micromalus* and the corresponding avirulence gene from *V. inaequalis*, arbitrarily referred to as *AvrVm*. Host differential reactions are readily distinguished by a hypersensitive response (HR) in host differential h5 and susceptibility (characterised by large sporulating lesions) in host differential h1. We are using reverse genetics to isolate the *AvrVm* gene product. HR is induced when the cell free culture supernatant (CFCS) of an incompatible isolate of *V. inaequalis* is infiltrated into leaves of h5. The HR inducing activity is greatly reduced by proteinase K digestion but is resistant to boiling. No HR is produced by this CFCS in the compatible host (h1). Acetone precipitation and ultrafiltration of the CFCS shows most HR inducing activity is present in a fraction between 3kDa and 30kDa molecular weights. The activity has also been localised to a fraction eluted from Mono-Q ion-exchange column. Protein purification steps were visualised by SDS-PAGE. HR evoking peptide(s) will be sequenced and the sequence information will be used to isolate the cDNA encoding the peptide. The putative avirulence gene will be confirmed by complementation and gene disruption. This project is supported by the Marsden Fund.

283. Heterologous expression of *pel* from *Colletotrichum gloeosporioides* in *C. magna* increased its pathogenicity on avocado fruit..Nir Yakoby[1], Amos Dinoor[2] and Dov B. Prusky[1]. Volcani Center, Postharvest Science , Bet Dagan, Israel. [2]Hebrew University, Department of Plant Pathology and Microbiology, Rehovot, Israel.

C. gloeosporioides is the main postharvest pathogen attacking avocado and other subtropical fruits. Previous work showed that *C. gloeosporioides* secretes pectolytic enzymes, i.e. pectate lyase and polygalacturonase during symptom development. To demonstrate the importance of pectate lyase as a pathogenicity factor during symptom development on avocado fruits, we choose *C. magna*, a pathogen of cucurbits, that cause minor symptoms on avocado fruits, as a expression system for *pel* A 4.4 kb genomic *pel* clone from *C. gloeosporioides* was subcloned into pGEM-7Z at EcoRI creating pPEL1.1. Twenty isolates of transformed *C. magna*, protoplasts, with pPEL1.1, were selected on HM-media supplemented with hygromicin. Four random isolates were tested for *pel* expression and pathogenicity on avocado fruit peel. Southern blot hybridization using pPEL1.1 as a probe detected the integration of the plasmid in *C. magna*, transformants. Western blot analysis of *C. gloeosporioides* and *C. magna*, grown in pectolytic inducing media showed the presence of one single band at 39 kDa and 41.5 kDa respectively. While the transformed *C. magna* isolates showed both bands. Pathogenicity tests of *C. magna* transformants on avocado fruit showed a significant increase in pathogenicity compared to the wild type *C. magna*. Present results clearly suggest the contribution of pectate lyase as a pathogenicity factor of *C. gloeosporioides* in avocado fruits. We acknowledge the support of BARD,GIARA and CDR in the present work

284. Molecular cloning of an indole-diterpenoid gene cluster from *Penicillium paxilli*.
Carolyn Young, Lisa McMillan and Barry Scott. Massey University, Molecular BioSciences, PalmerstonNorth, Manawatu, New Zealand.

Indole diterpenoids such as paxilline and lolitrem B are mycotoxins produced by a range of filamentous fungi including Penicillium species and Epichloë grass endophytes. Little is known about the biosynthesis of these secondary metabolites but geranylgeranyl pyrophosphate (GGPP) and indole are assumed to be the precursors. We report here on the cloning of a cluster of genes involved in the biosynthesis of paxilline in *P. paxilli* using the combined approaches of pAN7-1 plasmid mutagenesis and chromosome walking. Previously we isolated three paxilline negative mutants of *P. paxilli* and all were shown to contain large deletions (Young *et al*. 1998). Lambda clones spanning ~100 kb across this deleted region have been isolated by chromosome walking. A fourth Pax^-mutant, isolated by REMI, was found to contain an untagged deletion of 22 kb that maps to the same locus as the original mutants. Sequence analysis of the region defined by the REMI-induced deletion, identified a cluster of ORFs with similarities to known prenyltransferases and monoxygenases. Targeted disruption of a GGPP synthase confirmed that this gene is essential for paxilline biosynthesis but not essential for growth. A second copy of GGPPS was identified by Southern analysis and this is assumed to provide GGPP for primary metabolism. Extended sequencing over an 80 kb region has identified other genes likely to be involved in this pathway, including a possible transcription factor that contains a $Zn(II)_2Cys_6$ binuclear cluster DNA-binding motif. The boundaries of the cluster have been defined to a maximum size of 60 kb by deletion analysis.

285. Expression of sugar utilization pathway genes links to aflatoxin production. Jiujiang Yu, Perng-Kuang Chang, Deepak Bhatnagar, Thomas E. Cleveland. USDA/ARS, Southern Reg Res. Ctr., New Orleans, LA, USA.

Aflatoxins are secondary metabolites produced by the fungi *Aspergillus parasiticus* and *A. flavus*. These compounds are extremely potent carcinogens. Contamination of agricultural commodities with aflatoxins results in food safety and economic problems worldwide. To understand the molecular regulation of aflatoxin biosynthesis, we have cloned the 70 kb aflatoxin pathway gene cluster. At one end of the cluster we have cloned another cluster of 4 genes (15 kb) that are apparently involved in sugar utilization. (1) A gene, named *nadA*, with homology to NADH oxidase. (2) A gene, named *hxtA*, encodes for a hexose transporter protein. Hydrophobicity plot of the amino acid sequence indicates that this protein contains 12 membrane spanning regions responsible for uptake of hexose such as glucose and galactose. (3) A gene, named *glcA*, potentially encodes an alpha 1-6 glucosidase for the release of glucose from the side chain of amylopectin. (4) The fourth gene, tentatively named *sugR*, has homology to the *amyR* gene in *A. sojae* and *Bacillus subtilis* for the regulation of expression of the sugar utilization pathway genes. Preliminary results of Northern blot analysis demonstrated that these sugar utilization pathway genes are expressed in aflatoxin conducive medium. Data support the hypothesis that the sugar utilization pathway genes are linked to the aflatoxin production not only genetically but biologically.

286. Molecular mapping of loci conferring virulence on barley and wheat in *Cochliobolus sativus*. Shaobin Zhong, and Brian J. Steffenson. North Dakota State University, Dept of Plant Pathology, Fargo, ND, USA.

Two isolates (ND90Pr and ND93-1) of *Cochliobolus sativus* differing in their virulence on barley and wheat were crossed and their progeny analyzed for segregation. Segregation for high and low virulence on each host species approximated a 1:1 ratio indicating that virulence was conferred by a single gene in both cases. A molecular marker map is being constructed on this progeny population with the goal of ultimately cloning the virulence loci. To date, 87 AFLP markers and 3 DNA markers generated with PCR primers for the mating type locus have been placed on the map, which consists of 9 major and 8 minor linkage groups. The locus conferring virulence for barley co-segregated with three AFLP markers on one of the major linkage groups, whereas the one conferring virulence for wheat was localized on another linkage group. The molecular map and the linked AFLP markers will be useful starting points for the map-based cloning of the virulence genes in *C. sativus*.

287. ABC transporters in the wheat pathogen *Mycosphaerella graminicola*. Lute-Harm Zwiers, Marco M.C. Gielkens, Stephen D. Goodall, Ioannis Stergiopoulos, Koen Venema and Maarten A. De Waard. Wageningen Agricultural University, Laboratory of Phytopathology, Wageningen, The Netherlands.

ABC transporters belong to the ATP-binding superfamily of transporters and are involved in the secretion of both toxic and non-toxic compounds. They provide protection against the activity of endogenous and exogenous toxic compounds by reducing their accumulation in cells. In plant pathogenic fungi ABC transporters can also act as pathogenicity factors by (i) protecting the fungus against plant defense products during pathogenesis, (ii) secretion of pathogenicity factors (e.g. toxins), and (iii) secretion of mating factors. These hypotheses are tested for the causal agent of Septoria tritici leaf-blotch of wheat, *Mycosphaerella graminicola* (anamorph *Septoria tritici*). Typical symptoms of diseased leaves are necrotic lesions. Formation of these lesions may be associated with secretion of phytotoxins of the pathogen. Protection may also be required against phytoalexins of wheat. Heterologous hybridisation of a *M. graminicola* genomic library with a probe derived from a *Saccharomyces cerevisiae* ABC-transporter gene and PCR, using degenerate primers directed against ABC domains, led to the cloning of five ABC transporter encoding genes, named *Mgatr1 - Mgatr5*. Northern blot analysis shows that all five genes have a distinct expression profile when treated with different compounds known to be either substrates or inducers of ABC transporters. Interestingly, some compounds able to induce such a differential expression are plant secondary metabolites and fungicides. Expression patterns can be different for the two morphological states (yeast-like and mycelium) of this dimorphic fungus. The results suggest that ABC transporters play a role in pathogenesis and possibly also in activity of certain fungicides.

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288. The swollen cell phenotype of the *swo*A mutant of *Aspergillus nidulans.* Gretel M. Abramowsky, Patrick J. Westfall and Michelle Momany. University of Georgia, Botany, Athens, Georgia, USA.

Temperature sensitive *swo* (swollen) mutants of *Aspergillus nidulans* have been isolated. They represent eight genes that may be involved in polarity establishment, polarity maintenance and hyphal morphogenesis. One of these mutants, *swo*A, grows isotropically at restrictive temperature, and is unable to produce a germ tube. When shifted from restrictive to permissive temperature, multiple normal sized germ tubes emerge. Conversely, a shift from permissive to restrictive temperature halts elongation and isotropic swelling begins. Currently, further characterization and cloning experiments of *swo*A are being conducted.

289. Isolation of the *rad12* gene of *Coprinus cinereus.* Sonia N. Acharya[1], Marilee Ramesh[2], and Miriam E. Zolan[1]. [1]Indiana University, Biology, Bloomington, Indiana, USA. [2]Department of Biology, Indiana University at Purdue, Indianapolis.

The Zolan lab has identified four *Coprinus cinereus* genes necessary for DNA repair and meiosis, *rad3, rad9, rad11,* and *rad12*. *rad11* has been shown to be the Coprinus homolog of Saccharomyces *MRE11*, a gene involved in DNA double-strand break repair and meiosis. In both yeast and humans, Mre11 forms a complex with the Rad50 protein. I cloned the Coprinus homolog of *RAD50* using degenerate PCR. *rad50* maps to the *rad12* locus; RFLP mapping revealed 0/115 recombinants therefore I am testing the hypothesis that *rad50* is *rad12*. Cosmids containing *rad50* will be used in transformation experiments to test for rescue of the *rad12*mutant phenotypes of radiation sensitivity and defective meiosis. Electron and light microscopy of surface-spread nuclei have shown that *rad12*mutants show prophase I defects in chromosome condensation and, depending on the mutant, varying degrees of synaptonemal complex formation. I will examine homolog pairing in the mutants using fluoresence in situ hybridization.

290. Intergeneric Fragmented DNA transfer from stress tolerant Bacillus sp. to improve some economic traits in *S. cerevisiae*. Kamel A. Ahmed[1], Mohamed H. Hamoda[1], Tahany M. El-Kawokgy[2], and Nivien A. Abosereih[2]. [1]University of Cairo, Dept. of Genetics, Cairo, Egypt. [2]National Res. Centre, Microbial Genetics Lab, Egypt.

Fragmented naked DNA was isolated from two local Bacillus sp. strains. one is tolerant to 99% methylated alcohol and the second tolerated 30% Nacl. Each DNA was transfered to four genetically different haploid *S. cerevisiae* strains. The frequency of yeast transformants was found to vary between different recipient strains. Some alcohol tolerance transformants were able to tolerate 22% alochol compared to the level of 13% in the original recipients. In the case of Nacl transformants,their tolerance reached 18% while the tolerance level of recipients did not exceed 10%. 22% alcohol tolerant transformants or 18% saline tolerant ones were found to have higher growth rate, higher wheat meal fermentation ability than their original parents. When crossed to their original parents resulted duploids were also higher in their growth rate and

fermentation ability than their original parents constructed duploids. Attempts were carried out to allocate tolerance genes.

291. Motor proteins involved in nuclear migration in the filamentous ascomycete *Ashbya gossypii*. C. Alberti-Segui, R. Altman, F. Dietrich, and P. Philippsen. University of Basel, Microbiology, Basel, Switzerland.

In order to follow nuclear movement within the hyphae during fungal growth, an in-frame GFP fusion to the histone H4 has been carried out which resulted in a strong fluorescent labeling of nuclei. Video fluorescence microscopy and time-lapse studies revealed an active traffic of nuclei within the hyphae, including continuous oscillations, frequent mitotic events, by-passing of nuclei and movement through septa. This dynamic behaviour results in a uniform distribution of nuclei along the hyphae. To better characterize nuclear movement, we also measured some dynamic parameters. Under time-lapse condition (minimal medium, 24 °C), we estimated the nuclear velocity to be 0.3 µm per minute which was similar to the hyphal tip extension, whereas oscilation velocity could reach 4 µm per minute. By random sequencing of three thousand *Ashbya* DNA clones, we identified five homologs to *S. cerevisiae* genes coding for molecular motors that have been shown to be involved in nuclear migration (4 kinesin-like proteins and 1 dynein heavy chain). PCR targeting based on homologous recombination has been used to delete the corresponding genes. Deletion of two of the kinesin-like proteins did not affect to an observable degree nuclear distribution or migration but caused morphogenic and sporulation defects. The mutant that showed the most severe defect in nuclear migration was the *dhc1* mutant for which the whole dynein heavy chain gene has been removed. In contrast to the dynein mutant of *Aspergilus nidulans* where nuclei fail to move into the germ tube, we observed clumping of nuclei at the tip of the hyphae.

292. *sod*^{VI}*C* is an alpha-COP-related gene which is essential for establishing and maintaining polarised growth in *Aspergillus nidulans*. Susan J. Assinder[1], Susan L. Whittaker[1], Kelly J. Milward[1], and John H. Doonan[2]. [1]University of Wales, Bangor, Biological Sciences, Bangor, Gwynedd, UK. [2]John Innes Centre, Cell Biology, Norwich, UK.Strains of *Aspergillus nidulans* carrying the conditional-lethal mutation *sod*^{VI}*C1* (= Stabilisation Of Disomy) are defective in nuclear division and hyphal extension. The mutation affects both the establishment and the maintenance of polar growth, since mutant spores do not germinate at restrictive temperature and pre-existing hyphae stop growing upon upshift. The defect is reversible within the first 3-4 hours at restrictive temperature but longer periods of incubation are lethal due to cell lysis and morphological abnormalities. There is no evidence for a specific cell cycle lesion, suggesting the existence of a feedback mechanism whereby hyphal extension is co-ordinated with nuclear partitioning. The wild-type *sod*^{VI}*C* gene has been cloned from a chromosome VI-specific cosmid library and its product exhibits strong homology to the alpha-COP subunit of the coatomer complex involved in the secretory pathway in yeast and higher organisms. Molecular disruption of the gene is lethal, indicating that Sod^{VI}C is essential for growth in *A. nidulans*. A codon-modified form of the gene for green fluorescent protein (GFP), adapted for expression in plants, has been used as a vital reporter for protein location in *A. nidulans*. Translational fusions which target GFP to the Golgi or endoplasmic reticulum have been used to study the effect of the *sod*^{VI}*C1*mutation on protein trafficking.

293. Role of homologs of yeast *bud1*, *bud2* and *cla4* in hyphal morphogenesis of the filamentous fungus *Ashbya gossypii*. Y. Ayad-Durieux, F Dietrich, J. Wendland, and P. Philippsen. University of Basel, Biozentrum, Inst of Applied Microbiology, Basel, Switzerland.

The cotton pathogen *Ashbya gossypii* is a promising model system for studying hyphal morphogenesis in filamentous fungi. Its haploid genome of 8.85 Mb encodes no more than 4500 proteins which is one third less than encoded by the *Saccharomyces cerevisiae* genome. Gene manipulations by PCR-based gene targeting work as efficiently in this fungus as in *S. cerevisiae.* The hyphae of *Ashbya gossypii* show apical growth, lateral branching and dichotomous tip branching. Sequence analysis of random genomic clones (with the help of Novartis Ltd) revealed several homologs to *S. cerevisiae* morphogenesis genes. Interestingly, only few of the identified *Ashbya* genes lack homology to *S. cerevisia*e. In order to characterize the role of homologs of yeast morphogenesis genes in *Ashbya*, we analysed as a first step phenotypes of deletion mutants. *Agbud1* mutants grow slower than wildtype. *Agbud2* mutants show increased lateral branching and decreased dichotomous tip branching. *Agcla4* deletant strains develop much thicker hyphae and do not form septa. A detailed description of these phenotypes will be provided. In addition, 17 *Ashbya* genes lacking homology to *S. cerevisiae* were deleted; no morphogenetic defects were observed.

294. Analysis of homologous gene targeting in hyperrecombinant mutants of *Aspergillus nidulans*. Patricia J. Ayoubi, and Rolf A. Prade. Oklahoma State University, Microbiology and Mol Gen, Stillwater, OK, USA.

A number of molecular genetic tools and methods have recently been developed for filamentous fungi which rely on genetic recombination and integration of donor DNA into a chromosomal locus. Genetic recombination is a fundamental cellular process ubiquitous among living organisms and underlying most classical genetics techniques. However, the efficiency of more modern homology-based gene targeting techniques in filamentous fungi such as *Aspergillus nidulans* can be affected by many factors and often results in complex ectopic integration events. In sexually reproducing eukaryotic organisms, homologous recombination is required for pairing of homologs and ensures proper chromosome segregation. Sexual development in *A. nidulans* involves the development of specific sexual structures and probable gene expression patterns different from vegetative mycelia. Further, gene products required for sexual development likely include products involved in meiotic homologous recombination. For example, meiosis-specific RecA homologs have been identified in yeast (Dmc1) and other fungi, plants and animals. Interestingly, examination of sequences upstream of the *uvsC* transcription start site (a gene of *A. nidulans* homologous to *dmc1* from yeast) reveals a putative MluI cell cycle box. In addition, *uvsC* cDNA is detectable in sexual structure-specific ESTs but not in asexual-specific ESTs. Combined, these observations indicate a strong correlation between homologous recombination and sexual development in *A. nidulans*. In an effort to better understand homologous recombination and possibly improve homology-based gene targeting rates in *A. nidulans*, we have compared the frequencies of gene targeting by circular and linear plasmid DNA constructs in various *A. nidulans* hyperrecombinant and DNA repair mutants.

295. A Polo-like kinase in *Aspergillus nidulans*: implications for regulation of mitosis and development. Catherine L. Bachewich and Stephen Osmani. Penn State College of Medicine, Weis Centre for Research, Danville, PA, USA.

Aspergillus nidulans has proven to be an excellent model organism for investigating aspects of general cell cycle regulation and development. In particular, our understanding of the regulation of mitosis was advanced by determining the presence and function of major mitotic regulatory factors, some of which for the first time, in this organism. The Polo-like kinases are a relatively new family of cell cycle regulators implicated in various stages of mitosis, as well as cytokinesis and septation. Despite their widespread occurrence, ranging from yeast to human cells, their precise functions and regulation are not clear. To date, Polo-like kinases have not been described in any filamentous fungus. In order to progress our understanding of mitosis and development in *Aspergillus* and higher organisms, and to elucidate potential functions and interactions of Polo-like kinases with other mitotic factors, such as NIMA kinase, we investigated the presence of a Polo-like kinase in *Aspergillus*. A full length clone was isolated, using RACE PCR with primers designed against sequences in *Aspergillus* EST's with similarity to conserved regions of the Polo sequence. The genomic clone was recovered from chromosome 7 of a cosmid library. The genomic and cDNA clones were similar in size, at approximately 3.8 kb. Sequencing the genomic clone revealed high similarity to the Polo-like kinases across the amino-terminal, catalytic domain, and within the carboxy-terminal "Polo box", a conserved region defining Polo-like kinases. Manipulation of the gene, including overexpression at various stages of the cell cycle and molecular deletion, are underway to help determine the function of the gene and its contribution to cell cycle progression in *Aspergillus*.

296. Mechanisms of replication of circular fungal mitochondrial plasmids and mitochondrial DNA. Dipnath Baidyaroy[1], and Helmut Bertrand[2]. [1]Michigan State University, Botany & Plant Pathology, East Lansing, Michigan, USA. [2]Michigan State University, Microbiology, East Lansing, Michigan, USA.

Fungal mitochondrial plasmids can be of three major groups: (1) linear and usually encoding a DNA polymerase and an RNA polymerase, (2) circular with a gene coding for a reverse transcriptase, and (3) circular with a novel DNA polymerase gene. We are trying to characterize the replication mechanisms of the two types of circular plasmids by analyzing replication intermediates using two-dimensional gel electrophoresis. Contrary to previous studies (Maleszka, 1992, Biochem. Biophy. Res. Comm. 186:1669-1673), we found that the plasmids are present *in vivo* as multimeric circles instead of linear molecules of heterogenous sizes. In addition, we have obtained data indicating that the Mauriceville plasmid of *Neurospora crassa*, despite containing only a reverse transcriptase gene, replicates by three different mechanisms: (1) reverse transcription, (2) replication from an internal origin, and (3) a rolling circle mechanism which effectively generates numerous multimeric forms, some as big as octamers (~28.8 kb), of the unit length plasmid. On the other hand, the pCRY1 plasmid of *Cryphonectria parasitica* and the Fiji and LaBelle plasmids of *Neurospora*, all of which are assumed to replicate by the action of their own DNA polymerases, seem to replicate by a rolling circle mechanism. Mitochondrial DNA-derived circular plasmid-like elements were also observed to replicate via rolling circle mechanisms in *C. parasitica*. Collectively these observations suggest

that replication in fungal mitochondria is predominantly mediated through the rolling circle mechanism. Supported by USDA grant 95-37303-1785 and MAES Project No. MICL01662.

297. Transmission of a mitochondrial plasmid across vegetative incompatibility barriers in *Cryphonectria parasitica*. Dipnath Baidyaroy[1], Jonathan M. Glynn[2], and Helmut Bertrand[2]. [1]Michigan State University, Botany & Plant Pathology, East Lansing, Michigan, USA. [2]Michigan State University, Microbiology, East Lansing, Michigan, USA.

Previous studies have shown that exchange of mitochondrial DNA (mtDNA) occurs between the hyphae of vegetatively compatible strains in *Cryphonectria parasitica*, but little is known about the asexual transmission of mitochondrial elements among incompatible strains. In this study, we have assessed the effect of five vegetative incompatibility (*vic*) genes on the horizontal transmission of the mitochondrial plasmid pCRY1. Out of the five genes tested, an allelic difference between the donor and the recipient strains at only one locus strongly inhibited the transmission of pCRY1. Two genes allowed transmission in one direction (from one specific allele to the other; only two alleles are known for each *vic* locus) while the remaining two genes allowed transmission in both directions. However, the extent of transmission varied from one gene to another. The significance of this study is that none of the *vic* genes completely inhibited the horizontal transmission of pCRY1. In most combinations, at least one recipient isolate acquired the plasmid. The plasmid was also successfully transferred between two highly incompatible strains by protoplast fusion. In every case, the plasmid invaded and stably colonized the recipient after being transmitted. This data suggests that, in nature, mitochondrial genetic elements can be transmitted irrespective of karyotic compatibility or incompatibility and can behave like infectious elements. This infectious behavior suggests that mitochondrial plasmids might be effective as biological control agents of *C. parasitica* in nature, provided they elicit mtDNA mutations like some of the *Neurospora* plasmids. Supported by USDA grant 95-37303-1785 and MAES Project No. MICL01662.

298. Identification and Characterization of the *Neurospora crassa* opsin, NOP-1. Jennifer A. Bieszke[1], Edward L. Braun[2], Laura E. Bean [3], Seogchan Kang[4], Donald O. Natvig [3], Elena N. Spudich[1], John L. Spudich[1], and Katherine A. Borkovich[1]. [1]University of Texas Medical School-Houston, Microbiology , Houston, TX, USA. [2]The Ohio State University, Plant Biology, Columbus, OH, USA. [3]University of New Mexico, Biology, Albuquerque, NM, USA. [4]Pennsylvania State , Plant Pathology, University Park, PA, USA

Opsins are seven-transmembrane helical apoproteins that form light absorbing pigments upon binding retinal. Previously, genes encoding opsins had only been identified in animals and the archaea. Here, we report the characterization of an opsin gene, *nop-1*, from the eukaryotic filamentous fungus *Neurospora crassa*. The NOP-1 protein sequence is 81.1% identical to archaeal opsins in the retinal-binding pocket. Evolutionary analysis revealed clear homology between NOP-1 and several fungal opsin-related proteins; thus, NOP-1 is a plausible link between archaeal and visual opsins. Expression of the *nop-1* gene is highest under conditions that favor conidiation and is also positively influenced by the presence of light. A role for NOP-1 in conidiation is supported by the light-dependent conidiation phenotype of *nop-1* strains in the presence of the mictochondrial H^+-ATPase inhibitor oligomycin. The NOP-1 protein was overexpressed in the methylotrophic yeast, *Pichia pastoris*, in order to study its photochemistry.

Static absorbance spectroscopy showed NOP-1 could bind all-*trans* retinal (l_{max}> = 534nm). In addition, flash photolysis was used to show NOP-1 undergoes a photochemical reaction cycle. The results demonstrate that NOP-1 is an opsin capable of binding and photocycling retinal in a manner most similar to archaeal rhodopsins, and whose function is important for the conidiation process in *N. crassa*.

299. New insights into meiotic recombination from study of the *am* locus in Neurospora.
Frederick J. Bowring, and David E.A. Catcheside. Flinders University, Biological Sciences, Adelaide, South Australia, Australia.

Analysis of the segregation of flanking markers amongst recombinants in heteroallelic repulsion-phase crosses has been used to generate maps of the *am* locus. While three studies are in close agreement concerning the order of alleles within the locus, there is conflict over the orientation of the locus with respect to flanking markers. Alignment of the allele maps with respect to flanking markers was based on the relative frequency of the two classes of prototrophic progeny where flanking markers are recombined. Fincham's (1967) data are in general consistent with the 5' end of the gene being centromere distal, Smyth's (1973) data are consistent with the opposite orientation and Rambosek and Kinsey's (1984) data suggest no specific orientation. Molecular data confirm Fincham's orientation and because different combinations of flanking markers were used in each study, we speculated that the differing conclusions concerning the orientation of this locus may result from incidental crossing over in the flanking intervals (Bowring and Catcheside 1995). The segregation of molecular flankers located approximately 4 kb distal and 6 kb proximal of the *am* locus confirm this. Among prototrophic recombinants from a cross heteroallelic am^1am^6 , 93% of crossovers were in the intervals flanking *am* and thus not directly associated with the recombination event at this locus (Bowring and Catcheside, 1996). We have now shown that there is a clustering of crossovers immediately distal of *am* in prototrophic recombinants suggesting that recombination events in *am* promote crossovers nearby. This appears to be the first demonstration of negative interference reaching statistical significance.

300. SepH: A ser/thr protein kinase required for septation in *Aspergillus nidulans*.
Kenneth Seward Bruno[1], Jennifer L. Morrell[2], John F. Marhoul[3], and John E. Hamer[1]. [1]Purdue University, Biological Sciences, West Lafayette, IN, USA. [2]Vanderbilt University, Dept. of Cell Biology, Nashville , TN, USA. [3]Novartis Crop Protection, Fungal Targets Group, RTP, NC, USA.

The filamentous fungus *Aspergillus nidulans* forms septa in order to partition portions of an extending germ tube thus cellularizing the resultant mycelia. Septum formation is temporally and spatially regulated by nuclear positioning and cell cycle progression. *sepH1* was identified in a conditional mutant screen for cytokinesis mutants. At restrictive temperature *sepH1* mutants polarize a germ tube and undergo many rounds of nuclear division but fail to form septa. Septum formation is reversible in *sepH1* mutants when they are returned to permissive temperature, but remains blocked if nuclei are prevented from traversing mitosis. *SEPH* is a 165kD ser/thr protein kinase. *SepH* is 42% identical and 62% similar to *cdc7*, a gene required for septum formation in *S. pombe.* Targeted disruption of the sepH gene results in a strain (seph::argB) which is unable to form septa at any temperature. Interestingly, the disruption strain displays a thermosensitive-lethal phenotype with cells accumulating defects observed as aberrant nuclear morphology at 42 C. A screen for suppressors of this thermosensitve phenotype has yielded a pool of

seph::argB/mutant strains that are viable at 42 C and capable of forming septa. Termed BSH for **b**y-pass **s**ep **H**, the phenotype of these suppressors may offer some insight into *sepH* function. *Bsh167* is temperature sensitive and produces uninucleate sub-apical cells. This phenotype is strikingly similar to that of a strain highly expressing the *S.pombe cdc7* gene under the control of the inducible AlcA promoter. *SepH* and *cdc7* represent an emerging class of protein kinases involved in cytokinesis.

301. Monitoring of cytosilic Ca^{2+} levels in vivo in *Emericella (Aspergillus) nidulans* expressing Apoaequorin.Hong Cao and Diana C. Bartelt. St. John's University, Biological Sciences, Jamaica, NY, USA.

The focus of our research has been the *A. nidulans* calmodulin-dependent protein kinase (ACMPK) which can serve as both a target and a transducer of calcium-dependent signalling. We have shown that ACMPK is encoded by a single copy gene and is essential for viability. The molecular mechanisms by which Ca^{2+} and ACMPK control growth are unknown. The ability to measure changes in Ca^{2+} concentration in vivo during growth, cell division, and in response to external stimuli would aid in the identification of Ca^{2+}-mediated cellular processes. Several laboratories have developed the approach of creating transgenic organisms that express apoaequorin which can be targeted to various intracellular compartments. Treatment such organisms with coelenterazine reconstitutes functional aequorin within the cytosol and subcellular organelles of their cells, thereby producing luminescence whose light emission directly reports the internal Ca^{2+} concentration. We have constructed strains of A. nidulans that express apoaequorin with a view toward studying Ca^{2+} fluxes *in vivo* as a function of cell division cycle, development and responses to external stimuli. Luminescence requires incubation of the cells with coelenterazine and is calcium-dependent. In both defined and rich media to which no calcim has been added, cytosolic Ca^{2+} levels are maintained below 50 nM. Characterization of the strains will be presented (Supported by N.I.G.M.S.).

302. Isolation and characterization of the gene encoding G-alpha protein homolog from *Aspergillus nidulans*. Mi-Hee Chang, and Kwang-Yeop Jahng. Chonbuk National University, Biological Sciences, Chonju, Chonbuk, South Korea.

Heterotrimeric G proteins, consisting of alpha, beta and gamma subunits, are implicated in major signal transduction pathways governing the various cellular functions in eukaryotic organism. To study the role of G protein in differentiation of the filamentous fungus *A. nidulans*, we isolated *ganA* gene encoding Galpha protein homolog from *A. nidulans* by PCR amplification using degenerate oligonucleotide primer and analyzed its nucleotide sequence. Open reading frame of *ganA* containing 361 amino acids is highly related to alpha-subunits of heterotrimeric G proteins in other organisms. DNA fragment containing *ganA* cDNA could partially complement *gpa1* point mutation of *S. cerevisiae*. By targeted integration we made deletion mutations of *ganA* in which the internal fragment was replaced by the *argB* gene. We also constructed dominant-activating mutation and overexpression clone of *ganA*. However these mutations showed no significant phenotypes in asexual or sexual development. From these mutants, we are determining the cellular function of *ganA* in development and growth. This work was supported by grants for Research for Genetic Engineering from KRF.

303. Double strand break repair in *Coprinus cinereus*. W. Jason Cummings, Chris Johnson, and Mimi Zolan. Indiana University, Biology Department, Bloomington, IN 47405, USA.

Using the basidiomycete fungus *Coprinus cinereus* as an experimental system, we have isolated mutants, representing four distinct genetic loci, which exhibit elevated sensitivity to ionizing radiation and are also defective in meiosis. Epistasis analysis has demonstrated that these genes, called rad 3, 9, 11 and 12, are part of a single gamma-radiation survival pathway. In *Saccharomyces cerevisiae*, members of the RAD52 epistasis group illustrate that mutants exhibiting the dual phenotypes of hypersensitivity to ionizing radiation and defects in meiosis are compromised in their ability to repair DNA double-strand breaks. Since the *C. cinereus* rad mutants demonstrate these dual phenotypes, we are developing an assay for monitoring the repair of a single DSB formed by the rare-cutting endonuclease I-SceI. Strains containing integrated versions of a substrate construct and a transcriptionally regulatable endonuclease construct are used in this assay. The substrate construct contains repeated copies of a hygromycin resistance gene which, following cleavage, can be repaired either by homologous recombination or by an end-joining mechanism. This assay will allow us to asses the efficiency of DSB repair in *C. cinereus*, and will be used to determine the extent to which the mutants in our collection are deficient in DSB repair. In addition, the assay will be used to obtain insight into the specific mechanisms of repair that may be affected in rad mutants by testing the efficiencies of both homology-dependent repair and end-joining repair processes. We will present results obtained using the described assay in *C. cinereus*.

304. Gene map of chromosome I of *Ashbya gossypii*. Fred S. Dietrich[1], Sylvia Voegeli[1], Tom Gaffney[2], Christine Mohr[1], Corinne Rebischung[1], Rod Wing[3], Steve Goff[4], and Peter Philippsen[1]. [1]University of Basel, Applied Microbiology, Basel, BS, Switzerland. [2]Novartis, Research Triangle Park, NC, USA. [3]Clemson University, Genomics Institute, Clemson, SC, USA. [4]Novartis, La Jolla, CA, USA.

We have identified the complete set of genes on Chromosome I of the filamentous ascomycete *Ashbya gossypii*. The genome size of *A. gossypii* is 8.8Mb divided into seven chromosomes. The smallest chromosome, chromosome I, is 680kb in length and encodes 28 tRNAs, 1 structural RNA, and 378 proteins. Also identified are the centromere and telomeres. More than 95% of the chromosome I genes share homology with genes in the public databases, particularly with *S. cerevisiae*. The gene map, which is based on 2-3 fold sequence coverage spanning the chromosome, reveals that nearly all genes are found in blocks of synteny between *S. cerevisiae* and *A. gossypii*. These blocks of synteny contain up to 63 genes when the genome duplication in the *S. cerevisiae* lineage is taken into account. Examination of the gene order indicates that a combination of more than 20 interchromosomal and intrachromosomal exchange events have occurred since the divergence from a common ancestor of *S. cerevisiae* and *A. gossypii*. Many of the exchange events, which can be recognized as break points between blocks of synteny, appear to have occurred at tRNA genes.

305. Cleavage membrane development in *Allomyces macrogynus*. Karen Elizabeth. Fisher, and Robert W. Roberson. Arizona State University, Plant Biology, Tempe, AZ, USA.The zoosporangia of *Allomyces macrogynus* are coenocytic cells containing approximately 24 to 36 nuclei. During zoosporogenesis, membranes develop which sequester nuclei and cytoplasm into

uninucleate, uniflagellate zoospores of approximately equal volume. We have documented the development of cleavage membranes using standard epifluorescence and laser scanning confocal microscopy in living zoosporangia stained with the lipophilic styryl dye, FM 4-64. During initial stages of zoospore formation, FM 4-64 staining was observed only at the plasma membrane. Within 5 to 10 minutes, discrete sites of intense fluorescence were detected along the plasma membrane. From the regions, membranous elements developed and ramified through the sporangial cytoplasm. These membranes eventually converged after approximately 30 to 40 minutes, forming an elaborate network of interconnected membrane sheets, which divided the cytoplasm into hexagonally shaped zoospore initials. Zoosporangia treated with cytoskeletal inhibitors (i.e. nocodazole, cytochalasin D [CD]) displayed irregular membrane development, generating multinucleate, multiflagellate zoospores of disproportionate volumes. Results were most extreme in zoosporangia treated with CD, an inhibitor of actin polymerization. These sporangia exhibited little cleavage membrane development, forming only 2 to 4 large, multinucleate, multiflagellate zoospores. Observations indicate that cleavage membranes originate at the plasma membrane and their proper development and function requires an intact actin cytoskeleton.

306. Characterisation of *Magnaporthe grisea* NTH1/PTH9: a gene encoding a neutral trehalase enzyme with a role in pathogenicity. Andrew J. Foster[1], James A. Sweigard[2], and Nicholas J. Talbot[1]. [1]Exeter, Biological Sciences, Exeter, Devon, UK. [2]E.I. du Pont de Nemours , Central Research & Dev., Wilmington, DE , USA.

The neutral trehalase encoding gene, *NTH1/PTH9*, was identified previously in the rice blast fungus *Magnaporthe grisea* using insertional mutagenesis. Strains carrying an insertion within the gene show reduced pathogenicity toward susceptible grasses as well as a consistently reduced trehalase activity in conidial extracts. Targeted deletion of *NTH1/PTH9* in a rice pathogen of *M. grisea* also gives rise to strains with reduced pathogenicity and reduced conidial trehalase activity. Accumulation of trehalose coupled to increased trehalose turnover has been shown to play a role in response to varied stresses in yeast. Consistent with this view the *NTH1/PTH9* gene is transcriptionally activated in response to hyper-osmotic stress and contains two closely spaced STRE elements within its promoter. This trancriptional induction is independent of the OSM1 MAP kinase, a homologue of *Saccharomyces cerevisiae* HOG1p. We have recently obtained a clone of a putative *M. grisea* trehalose-6-phosphate synthase encoding gene which is also expressed in response to hyper-osmotic stress. Molecular genetic and biochemical analysis together with cytological studies of infection structure formation and function are being employed with a view to ascertaining possible roles for trehalose metabolism in pathogenesis.

307. Expression of mitogen-activated protein kinase, Mkp1, of *Pneumocystis carinii* restores resistance to inhibitors of cell wall synthesis in slt2delta-deficient *S. cerevisiae* and may regulate expression of FKS2, a component of the glucan synthesis complex. Deborah S. Fox[1], and George Smulian[2]. [1]University of Cincinnati, Pathology and Mol. Med., Cincinnati, Ohio, USA. [2]University of Cincinnati, Internal Medicine, Cincinnati, Ohio, USA.

Signal transduction pathways are important in the adaptive response of microbes to their environment. The MAP kinase Mkp1 of *P. carinii* has been shown to complement the slt2delta defect in the cell integrity pathway of *S. cerevisiae*. Mutants of the cell integrity pathway display

increased sensitivity to a variety of compounds including caffeine, which influence some facet of cell wall synthesis since a variety of cell integrity MAP kinase cascade mutants with cell wall construction defects are sensitive. Overexpression of mkp1 in slt2delta-deficient *S. cerevisiae* reduced the toxic affect of caffeine to near wild type (SLT2+) levels. The expression of FKS2, a subunit of beta 1,3-glucan synthase, is regulated by the activation of the transcription factor, RLM1, a target of Slt2p. RLM1 is able to reverse the caffeine sensitivity phenotype when overexpressed in a cell integrity pathway defective strain and rlm1delta-deficient mutants are sensitive to caffeine. The observations that mkp1 expression results in the restoration of resistance to inhibitors such as caffeine demonstrates that Mkp1 is activated upon exposure to conditions which stimulate the cell integrity pathway of *S. cerevisiae* and suggests that Mkp1 may interact with one or more downstream targets of Slt2p, including Rlm1. The examination of the ability of Mkp1 to control the expression of FKS2 via the activation of Rlm1 will determine the role of Mkp1 in the regulation of *S. cerevisiae* cell wall synthesis and will provide a pivotal tool for the examination of cell wall synthesis within *P. carinii*.

308. Isolation and characterization of the *mre11* gene of *Coprinus cinereus*. Erin E. Gerecke, and Miriam E. Zolan. Indiana University, Biology, Bloomington, Indiana, USA.

We are interested in understanding the functions of genes involved in DNA repair and meiosis. The Zolan lab has identified mutants defining at least four genetic loci, *rad3*, *rad9*, *rad11*, and *rad12*, which are necessary both for survival following gamma irradiation and for meiosis. The *rad11-1*mutant is highly sensitive to gamma radiation and fails to produce spores; most meiotic cells fail to undergo the first meiotic division. We have cloned the *rad11*gene; it is a homolog of *MRE11*, a gene involved in DNA repair and meiosis in numerous organisms, including yeast and humans. The mutation present in *rad11-1* (*mre11-1*) generates a stop codon approximately midway through the predicted amino acid sequence. Surface-spread nuclei of the *mre11-1* mutant have been examined using light and electron microscopy. During prophase I of meiosis, chromosomes show defects in condensation, and the synaptonemal complex appears abnormal, although short regions of apparent synapsis have been identified in the majority of nuclei. Using fluorescence *in situ* hybridization (FISH) on spread nuclei, we have demonstrated that pairing of homologous chromosomes is reduced, but not abolished, in *mre11-1* meiotic nuclei. For a single locus on chromosomes 8 and 13, approximately 75% of nuclei demonstrated pairing for at least one of the two chromosomes, but only 30% showed pairing for both chromosomes. At least one of the two chromosomal loci examined is paired in approximately 50% of the nuclei.

309. Phospholipid biosynthetic mutants of *Neurospora crassa*. Marta Goodrich-Tanrikulu. Calgene LLC, Biochemistry, Davis, CA, USA.

Previously, four choline-requiring mutants of *Neurospora crassa* have been identified genetically. Two of these, *chol-1* and *chol-2*, have been characterized biochemically, but *chol-3* and *chol-4* have not. The *chol-1* and *chol-2* mutations affect the biosynthesis of the phospholipid phosphatidylcholine. In addition, two other mutations, *inl* and *un-17*, are known to affect phospholipid biosynthesis. The *inl* mutant has also been characterized biochemically, and cannot synthesize inositol phospholipids. Phospholipid biosynthesis is impaired in the *un-17* mutant, which fails to grow above 34 C, but the affected steps of phospholipid biosynthesis have not

been identified. In this study, phospholipid biosynthesis in the biochemically uncharacterized mutants *chol-3, chol-4* and *un-17* is examined.

310. A Glucoamylase::GFP fusion to study protein secretion in *Aspergillus niger*.Caroline L. Gordon[1], Anthony PJ. Trinci[1], David B. Archer[2], David J. Jeenes[2], John D. Doonan[3], Brian Wells[3], and Geoff D. Robson[1]. [1]Manchester University, Microbiology, Manchester, UK. [2]Institute for Food Research, Genetics and Microbiology, Norwich, East Anglia, UK. [3]John Innes Centre, Cell Biology, Norwich, East Anglia, UK.

Aspergillus niger is used as a host for heterologous protein production, however, yields are generally lower than those obtained for homologous proteins. Mechanisms of protein secretion and the secretory pathway in filamentous fungi are poorly characterised, although there is evidence to suggest that secretion occurs by a similar mechanism to other eukaryotes with proteins destined for secretion being directed to the hyphal tip. A synthetic green fluorescent protein (sGFP(S65T)) was fused to truncated *A.niger* glucoamylase (GLA:499) in order to monitor protein trafficking through the secretory pathway. Southern blot analysis of transformants confirmed that the gene fusion had successfully integrated into the *A.niger*genome. Confocal and fluorescence microscopy has shown that GLA::sGFP fusion protein is fluorescent in *A.niger* and appeared to be directed to the hyphal tip. In young mycelia, hyphal cell wall fluorescence was apparent and immunogold labelling of sGFP confirmed that sGFP was partially localised within the hyphal cell wall. Using Western blotting, extracellular GLA::sGFP was only detected in culture filtrates of young mycelia grown in a soya milk medium. Current work is focusing on the disruption the secretory pathway by secretion inhibitors.

311. Dissection of the calcineurin signal transduction pathway in *Cryptococcus neoformans*.Jenifer M. Gorlach, and Joseph Heitman. Duke University, Genetics, Durham, NC, USA.

We demonstrated that calcineurin (Cn), a calcium/calmodulin-dependent protein phosphatase, is required for growth at 37 degrees C, tolerance to lithium, and pathogenesis of *Cryptococcus neoformans* (Odom et al. 1997, EMBO J 16:2576-2589). In this study, we took a yeast two-hybrid approach to identify genes encoding proteins which interact directly with Cn. In the two-hybrid screen, a protein was found that could interact with CNA1, the catalytic subunit of Cn, from *C. neoformans, S. cerevisiae*, and mouse. This 30 kD calcineurin A-binding protein (CBP1) shares only a small amount of sequence similarity with DSCR1, a protein transcribed from a gene located in the Down Syndrome Critical Region. The first 21 kD of the protein is required for binding to CNA1. CBP1 binding to CNA1 is dependent upon the presence of the CNB1 regulatory subunit of Cn, but does not interact with the calcineurin regulatory subunit alone in the two-hybrid assay. The Cn inhibitor FK506 disrupts the CNA1-CBP1 interaction and this inhibition is dependent upon the presence of an FKBP12-FK506 complex. A CBP1-GFP-tagged protein was constructed for in vivo binding assays and localization in *C. neoformans*. Calcineurin A co-immunoprecipitates with the CBP1-GFP fusion protein in extracts from *CNA1* wild-type cells but not from *cna1* mutant cells that lack calcineurin A. The subcellular localization of CBP1 under standard and stress growth conditions is being determined. In parallel, gene replacement experiments were conducted to identify a role of CBP1 in *C. neoformans*. To date, no observable phenotype has been found for a *cbp1* mutant strain. Phenotypes for strains overexpressing CBP1 are currently being investigated.

312. A mutation within the catalytic domain of COT1 kinase confers changes in the presence of two COT1 isoforms in *Neurospora crassa*.Rena Gorovits[1], Oshrat Prohpeta[1], Mikhail Kolot[2], and Oded Yarden1. [1]The Hebrew University of Jerusalem, Plant Pathol. & Microbiol, Rehovot, Israel. [2]Tel Aviv University, Biochemistry, Ramat Aviv, Israel.

Neurospora crassa grows by forming spreading colonies. *cot-1* belongs to a class of *N. crassa* colonial temperature-sensitive (*cot*) mutants, and encodes a Ser/Thr protein kinase. We have mapped the *cot* mutation to a single base change resulting in a His to Arg substitution at amino acid 351, which resides within the catalytic domain. Antibodies raised against COT1 detected and immunoprecipitated a predominant 73-kDa polypeptide in *N. crassa* extracts, whose abundance was constant under all growth conditions tested. An additional, lower MW, COT1 isoform (67-kDa) present in the wild-type, was not detected in *cot* grown at the restricitve temperature. Similarly, this isoform was not detected in *cot-3* or *cot-5* strains, when grown at restrictive temperatures. Apparent changes in the phosphorylation state of P150Glued (encoded by *ro-3*, a suppressor of *cot*) and evidence of in vitro physical interactions between COT1 and calcineurin indicate a functional linkage between the kinase, phosphatase and cytoskeletal motor protein.

313. The role of SEPB in the *Aspergillus nidulans* DNA damage response.Scott E. Gygax, and Steven D. Harris. University of Connecticut Health Center, Microbiology, Farmington, CT , USA.

Temperature sensitive mutations in the *Aspergillus nidulans sepB* gene dramatically perturb chromosomal DNA metabolism. At restrictive temperature, these mutations cause; i) elevated levels of mitotic recombination and chromosome non-disjunction, ii) progressive delays in nuclear division, and iii) inhibition of septum formation. Molecular characterization of the *sepB* gene demonstrated that it encodes a predicted protein possessing five consensus WD-40 repeats and a motif shared with DNA polymerase alpha. We are currently employing a variety of approached to characterize the role(s) of SEPB in chromosomal DNA metabolism. Here, we report observations that suggest that SEPB is involved in DNA repair.
At the semi-permissive temperature, the *sepB3* mutation caused enhanced sensitivity to DNA alkylating agents such as MMS. Further analysis showed that the DNA damage checkpoint is retained in *sepB3* mutants, but MMS-induced mutagenesis is virtually abolished. Furthermore, levels of *sepB* transcript were slightly higher in MMS-treated cells, and this increase depended upon UVSB function. Phenotypic characterization of double mutants revealed the existence of genetic interactions between *sepB3* and a number of other mutations that affect the response to DNA damage (i.e. *uvsB110*, *uvsD153*, *musN227*, and *musP234*). Notably, the increased levels of mutagenesis caused by the *uvsC114* and *musN227* mutations were diminished by *sepB3*. Collectively, our observations suggest that SEPB may be part of a DNA damage regulon that is required for induced mutagenesis.

314. Transposable elements in *Podospora anserina*.Andrea Hamann, Frank Feller, and Heinz D. Osiewacz. J.W. Goethe University, Botanical Institute, Frankfurt, 60439 , Germany.

During the course of investigations aimed to elucidate the distribution and function of repetitive sequences in the genome of *Podospora anserina*, we identified mini- and microsatellite

sequences as well as sequences related to class I and class II transposons. *Repa*, a repetitive sequence that previously was reported to result from the recombination between long terminal repeats (LTR) of an unidentified complete retrotransposons, was found to be present in about 15 copies in the genome. This sequence turned out to be a highly informative probe for the discrimination of different geographical races. One clone was selected containing two intact LTRs and an open reading frame (ORF) coding for a putative protein with significant homology to reverse transcriptases. However, this ORF contains a number of stop codons indicating that the selected element is inactive. A second element, *Pat 1*, was identified encoding a putative transposase with homology to Pot3, a class II transposable element of *Magnaporthe grisea*. The corresponding sequence is present in about 25 copies in the genome of *P. anserina* and appears to be rather conserved in the 18 investigated races of this ascomycete. Acknowledgment: The experimental work was supported by a grant of the Deutsche Forschungsgemeinschaft (Bonn, Germany).

315. Cloning and characterization of a subtilisin/Kex2p-like endoprotease gene of *Aspergillus nidulans*. Kap-Hoon Han, Sung-Min Ju, Dong-Min Han, and Won-Sin Kim. Wonkwang University, Division of Life Science, Iksan, Chonbuk, South Korea. Limited endoproteolysis of inactive precursor proteins at sites marked by paired or multiple basic amino acids is a widespread process by which biologically active peptides and proteins are produced within the secretory pathway in eukaryotic cells. The identification of a novel family of endoproteases homlologous with bacterial subtilisins and yeasts Kex2p has accelerated progress in understanding the complex mechanisms underlying the production of the bioactive materials. Many distinct proprotein convertases of this family have been identified in mammalian species and non-mammalian eukaryotes. We have cloned and characterized the *Aspergillus nidulans* subtilisin/Kex2p-like endoprotease gene using the amplified 540bp DNA fragment which was identified from a Blast search of the dbEST database with the mouse frurin. Sequence analysis revealed greatest homology to the catalytic domain of subtilisins, Kex2p and furin that extends over ~330 amino acids is highly conserved among the eukaryotic proprotein convertases.

316. The Roles of UVSB and MUSN in the DNA Damage Response in *Aspergillus nidulans.* Amy F. Hofmann, and Steven D. Harris. University of Connecticut Health Center, Microbiology, Farmington, CT, USA.

The DNA damage response (DDR) is a protective mechanism that prevents the transmission of a damaged genome to successive generations. In the filamentous fungus *Aspergillus nidulans*, the DDR encompasses several events, including, i) arrest of nuclear division, ii) inhibition of septation, iii) induction of mutagenesis, and iv) transcription induction of DNA damage-inducible genes. Phenotypic analysis of *uvsB110* mutants has revealed that UVSB is required for each of the aspects of the DDR Molecular characterization of UVSB demonstrates that it is a member of a PI-3 kinase subfamily that includes proteins implicated in cell cycle checkpoint function in species ranging from yeast to humans. Notably, mutations in a human homologue, ATM, are responsible for a hereditary cancer-prone syndrome known as ataxia telangiectasia. Mutations in another *A. nidulans* gene, *musN*, can partially suppress the damage sensitivity of *uvsB110* mutants. Furthermore, the *musN227* mutation suppresses some of the specific defects observed in *uvsB110* mutants, including the failure to inhibit septation and induce mutagenesis. In addition, *musN227* causes a slight increase in *uvsC* transcription in

uvsB110 mutants. Surprisingly, *musN227* does not restore DNA damage-dependent nuclear division arrest to *uvsB110* mutants. Together, these results suggest that MUSN may function to promote recovery from the DDR.

317. Regulation of cell separation in *Ustilago maydis*.Helge Hudel and Michael Boelker. University of Marburg, Biology, Marburg, Hassia, Germany.

The phytopathogenic fungus *Ustilago maydis* exhibits a dimorphic life style. Haploid sporidia grow yeast-like by budding and are non-pathogenic. Only the filamentous dikaryon is able to induce tumors in maize plants. To identify genes that are involved in the regulation of morphogenesis and dimorphism mutants that exhibit aberrant morphologies were generated by UV and REMI mutagenesis. Mutants in two genes, *don1* and *don3*, have been isolated that fail to separate cells after nuclear division and septum formation. The *don1*gene encodes a guanine exchange factor (GEF) specific for members of the Rho/Rac/Cdc42 family, the *don3* gene codes for a member of the STE20 like kinases that are known to be activated by Rho/Rac proteins. The Ras-like GTPase Cdc42 interacts with both Don1 and Don3 and is thus most likely involved in this regulatory cascade. The Don1 protein contains, in addition to the GEF domain, a pleckstrin homology region and a potential Zn finger. Using fusion proteins of the Don1 Zn finger domain with GFP we could demonstrate that the fusion protein is localized to the vacuole membrane. The protein is also found to be accumulated at the septum before cell separation occurs. The implications of this localization for the function of Don1 will be discussed.

318. Analyses of structures and*ras*-superfamily of *Neurospora crassa*. Sayoko Ito-Harashima[1], Mayumi Sugisaki[2], Takashi Ichihara[2], Yasushi Matsui[3], Akio Toh-e[3], and Hirokazu Inoue[2]. [1] Institute of research and Innovation, Biotechnology, Kashiwa, Chiba, Japan. [2]Saitama University, Regulation Biology, Urawa, Saitama, Japan. [3]University of Tokyo, Tokyo, Japan.

ras and *ras*-related genes encode small G proteins that are highly conserved in a wide variety of organisms. The *NC-ras* gene is one of the *ras* homologs of *N. crassa*. We cloned genomic DNA fragment of the *NC-ras* gene and analyzed its structure and function. *NC-ras* ORF was interruped by three introns and the positions of introns were completely conserved between *NC-ras* and *Aspergillus nidulans Aras*. The strains overexpressed the *NC-ras* gene showed abnormality in life cycle. They were female sterile in sexual cycle. In vegetative growth phase they showed morphological abnormalities, and the defects were partially suppressed by addition of theophylline, an inhibitor of phosphodiesterase (a degradation enzyme of cAMP). These data suggest that the NC-RAS protein may be involved in the signal transduction pathway mediated by cAMP. Recently we also isolated another member of *ras*-superfamily, *sar-1*. The *sar-1* is a homolog of *Saccharomyces cerevisiae SAR1*, which is involved in the intracellular vesicle transport. It has been elusidated that Sar1 protein is essential for the formation of transport vesicles from the ER membrane in yeast. *N. crassa* SAR1 protein showed 60-90% identity with *SAR1* homologs of other organisms at the predicted amino acid level. Northern blot analyses indicated that the *sar-1* was constitutively expressed during vegetative growth phase. We are now examining the functional relationship of *sar-1* homologs between *N. crassa* and *S. cerevisiae*.

319. Molecular and genetic analyses of the *nimO* initiator of DNA synthesis in *Aspergillus nidulans*. Steven W. James, Karen E. Messner, and Maureen C. Miller. Gettysburg College, Biology, Gettysburg, PA, USA.

The *nimO* gene of *Aspergillus nidulans* shares structural and functional homology with the budding yeast G1/S regulator, *Dbf4*, being required both for initiation of DNA synthesis and efficient progression through S phase; and involved in a checkpoint linking DNA synthesis with mitosis (James et al., accepted; 19th FGC). The gene is essential, because strains containing *alcA::nimO*$^{+}$ as their only copy of *nimO* become completely ethanol-dependent. Since the greatest similarity between *nimO* and *Dbf4* lies in their C-termini, *nimO* function was investigated using a series of *alcA*-driven C-terminal truncations. *nimO* alleles lacking as much as 244 C terminal amino acids partially rescued *nimO*18 ts-lethality when expression was strongly induced, by permitting vegetative growth but not asexual development. However, these truncated alleles could not complement a deletion of *nimO*, indicating that the C-terminus is essential and suggesting that the *nimO*18 protein and truncated polypeptides somehow interact. Dbf4p triggers DNA synthesis by activating the Cdc7p kinase and escorting it to targets at origins of replication. If *nimO* is a *Dbf4* homolog, it should associate with its cognate Aspergillus partner. We isolated and sequenced an apparent Aspergillus homolog of *Cdc7* (*Cdc7*Asp), and we have identified two new loci, *snoA* and *snoB*, as partial suppressors of *nimO*18. Preliminary studies using *Cdc7*Asp suggest that it may complement *snoA* suppressors, and molecular and genetic studies are underway to ascertain if (1) *snoA* encodes *Cdc7*Asp, and if (2) nimOp and Cdc7pAsp physically associate to control DNA synthesis. (Supported by NSF-RUI# MCB 95-07485).

320. uvsF function, effects on recombination, and interaction with other Aspergillus cell-cycle/repair mutants cell-cycle/repair mutants. Etta Kafer, Tricia John, Jennifer Chow and Linda Sui. Simon Fraser University, Institute for Molecular Biology and Biochemistry, Burnaby, B.C., Canada.

We are interested in the mechanisms of mitotic recombination in *Aspergillus nidulans*. DNA repair-defective mutants from several epistatic groups affect this process in various ways. Such genes may have different primary function and produce very different patterns of recombination, as identified for two hyperrec types, *uvsF* and *uvsB*. *uvsF201* is defective in nucleotide excision-repair (NER) and shows increased mitotic crossing over and UV mutagenesis. Its gene sequence is homologous to human/yeast *RFC1* genes which code for the largest of five subunits of replication factor C. RFCp is essential for DNA replication and for NER, and *cdc44-1* of yeast is cold-sensitive lethal for RFC1. Surprisingly, *uvsF201* grows well, but is highly mutagen sensitive and produces synthetic lethals with many different repair mutations. When *uvsF* cDNA in a yeast vector was expressed in *cdc44-1* cells, Western blots showed that the expected Aspergillus protein was synthesized, but no interspecies "complementation" was found. However, as the *uvsF201* point mutation can be complemented by *uvsF* gene fragments, the *CDC44* gene in Aspergillus may yet rescue *uvsF201*. -- The closely adjacent gene to *uvsF*, which is a homologue of organelle ribosomal protein L16 genes, is transcribed from a common promoter region in opposite direction. The two genes may therefore be co-regulated, as claimed for such cases in yeast. The second hyperrec mutant, *uvsB*, grows poorly and the pattern of mitotic recombination in *uvsB* diploids is uniquely abnormal, differing significantly from *uvsF*

and from controls. The *uvsB* gene was recently found to be homologous to *rad3* of fission yeast and shown to be required for DNA repair as well as cell-cycle checkpoint control (S. Osmani, personal communication).

321. Hyphal morphogenesis and cell survival in *Aspergillus nidulans*. Susan G. W. Kaminskyj. Univ. Saskatchewan, Biology, Saskatoon, SK S7N 5E2, Canada.

Aspergillus nidulans hyphae grow by tip extension, and are later divided by crosswalls into actively growing tip and quiescent basal cells. Basal cells can branch, resuming growth after forming a new tip. Apical growth in tip cells, and growth arrest in basal cells, depend on the function of "*hyp*ercellular" genes, identified using temperature sensitive mutants. All *hyp* ts strains can complete their asexual life cycle at 42°C and produce viable conidia, suggesting that these genes are not essential. Intriguingly, if *hypA* or *hypB* function is perturbed by shifting 28°C-grown germlings to 42°C, basal cells are remodeled to assume the restrictive phenotype, but tip cells die. For *hypA*, only, tip cell survival appears to be related to cell cycle stage. About 20% of control tips survive upshift, and this is increased three-fold by treatment with hydroxyurea. These results suggest that *hypA* is a nuclear-cycle related growth switch for hyphal development. *hypA* has sequence similarity to genes in budding and fission yeasts, and a cosmid containing the fission yeast ortholog was able to complement the *hypA* defect. Current efforts focus on defining the upshift phenotypes and proving homology between the *A. nidulans hypA*, and the yeast genes.

322. Cloning and functional analysis of the gene coding a PLC homologue in *Neurospora crassa*. Yuko Kawahashi[1], Mayumi Sugisaki[1], Akio Toh-e[2], and Hirokazu Inoue[1]. [1]Saitama University, Faculty of Science , Urawa, Saitama, Japan.[2]Tokyo University, Faculty of Science, Bunkyo-ku, Tokyo, Japan.

Phosphoinositide-specific-phospholipase C (PLC) is responsible for the production of two second messengers in a signal transduction pathway. PLCs are usually divided into three types (beta,gamma,delta) based on size, immunological reactivity and amino acid sequence. Each isoform has X and Y regions which are highly conserved and regarded as catalytic regions in PLCs. We obtained a short DNA fragment by PCR using primers based on the two conserved amino acid sequences in the X region. Using the PCR product as a probe, we screened cosmid genome libraries by colony hybridization and isolated a genome DNA fragment of 2.6 kb. We sequenced this fragment and found it to be homologous to the X region. But, it was not the total gene coding a PLC protein; the Y region and the carboxy terminal sequences were deleted. PLC mutants made by RIP were highly sensitive to MBC (carbendazim),TBZ (thiabendazole), and thiophanate-methyl,which are chemical agents blocking polymerization of beta-tublin. This indicated that the PLC homologue of *N.crassa* is involved in organization cytoskelton. In addition , the mutants showed a sensitivity to H_2O_2 , suggesting that the homologue is related to oxidative stress-stimulated signal transduction pathway.

323. Comparative study intra and extracellular pectinases produced by *P. frequentans*. Cristina Yoshiko Kawano, Maria Angélica dos S.Cunha Chellegatti, Suraia Said, and Maria José Vieira Fonseca. Universidade de São Paulo, Ciências Farmacêuticas, Ribeirão Preto, São Paulo, Brasil.

Microbial pectinesterase and depolymerases (hydrolases and lyases), catalysing the degradation of pectic polysaccharides, play important role in the invasion of plant tissues by phytopathogens, and in the spoilage of fruit and vegetables. Furthermore theses enzymes are widely used in the bioconversion as adjuncts to cellulases and/or hemicellulases in treatment of cellulosic biomass and also in biopulping processes for clarifying fruit juices and liquefying fruits and vegetables. The pectic enzymes are synthesized and secreted from intact cells into the surrounding medium by fungi, bacteria and some yeasts. The fungus *Penicillium frequentans* was selected among 100 fungi isolated from soil in Brazil because of its ability to produce high levels of extracellular pectinases in solid medium using citrus pulp pellets.This fungus synthesized eleven PGs (polygalacturonases) and two PEs (pectinesterases) when grown in liquid culture supplement with pectin.These enzymes were separated and detected in polyacrylamide gels containing pectin and sodium polypectate (NaPP). Seven PGs and the two PEs were secreted in the medium while four PGs were not secreted. Among the secreted PGs, the endo-PG (10 band) and exo-PGs (5 band) were the enzymes secreted in highest levels. All secreted PGs bound to Con A and their secretion and/or enzymatic activities were inhibited by TM (tunicamycin), except the constitutive and inducible endo-PG (band 10). The affinity on ConA and TM effect studies suggested that the secreted endo- and exo-PG differed by level and process of glycosylation. The exo-PG was characterized as a N-glycoprotein while the endo-PG, probably, is an O-glycoprotein. The PGs (3 and 4) were neither bound to ConA or secreted and their enzymatic activities were inhibited by TM suggesting that they are, probably, N-glycoprotein with complex oligosaccharides of type three and tetraantennary. The order not secreted and unbound to Con A PGs (6 and 8) were not inhibited by TM. These enzymes presented, chromatographic characteristics and effects to TM, similar to endo-PG (band 10), since these PGs could be unglycosylated or/and aggregate forms of the endo-PG (band 10).

324. Genomics of *Mycosphaerella graminicola*. Gert H.J. Kema, Trudy B.M. van den Bosch, Cees Waalwijk. DLO-Research Institute for Plant Protection, Mycology & Bacteriology, Wageningen, Gelderland, The Netherlands.

Mycosphaerella graminicola, a heterothallic bipolar ascomycete, is the causal agent of *Septoria tritici* leaf blotch disease of wheat. This disease is currently considered to be Europe's most important wheat disease and therefore gains a lot of interest from the agro-industry. We have recently developed the first linkage map of the *M. graminicola* genome with over 300 AFLP and over 100 RAPD markers and including several biological markers such as the MAT gene and an avirulence locus. A BAC library was constructed of the avirulent parental strain of this *M. graminicola* mapping population as a starting point for a sequencing project on this fungus using the latest high troughput sequence technology. The current status of the project will be presented.

325. Mapping the *Mycosphaerella graminicola* genome. Gert HJ Kema[1], Stephen B Goodwin[2], Sonia Hamza[3], Els CP Verstappen[1], Jessica R. Cavaletto[2], Theo AJ van der Lee[4], Marjanne Hagenaar-de Weerdt[1], Peter JM Bonants[1] and Cees Waalwijk[1] [1]DLO-Research Institute for Plant Protection [IPO-DLO], P.O. Box 9060, 6700 GW Wageningen, The Netherlands; [2]Purdue University, Dept. of Botany and Plant Pathology, 1155 Lilly Hall, West Lafayette, IN 47907-1155, USA; [3]Institut National Agronomique de Tunisie (INAT), 43 Av. Charles Nicolle, 1082 Tunis El Mahrajène, Tunisia; [4]Wageningen Agricultural University, Dept. of Phytopathology, Binnenhaven 9, 6709 PD Wageningen, The Netherlands.

We have developed a crossing protocol to obtain a mapping population of *M. graminicola*, the causal agent of septoria tritici leaf blotch disease of wheat. The parental strains are highly contrasting for their avirulences towards a number of wheat cultivars, characteristics that appear to be under monogenic control. Progenies could therefore be used to map avirulence- and MAT-loci and to identify linked markers in order to start map-based cloning strategies to isolate these genes. A F1 progeny of IPO323 [avirulent, MAT1-1] and IPO94269 [virulent, MAT1-2] was isolated and 68 isolates were used to construct a linkage map using AFLP and RAPD technology. Using a LOD threshold value of 4, 410 of the 439 markers showed linkage to at least three other markers, eventually resulting in 26 linkage groups. In addition, we conducted bulked segregant analyses, which resulted in two additional markers that perfectly linked with the avirulence locus. AFLP markers that mapped on the same linkage group as the avirulence locus were used as probes to identify the chromosome on pulsed-field gel electrophoresis [PFGE] blots. Similarly, a heterologous MAT probe was used to identify the chromosome carrying the MAT locus. Although the map indicated that both genes were located on small linkage group, these probes hybridized to much larger chromosomes on PFGE filters, which was also confirmed by marker-derived SCARS on chromosome bands excised from PFGE gels. This strategy is used to relate linkage groups to specific chromosomes in order to establish a physical map of the genome. Acknowledgment Part of this project is funded by EU-BIOTECH PL096-352

326. Disruption of the gene encoding the catalytic subunit of the vacuolar H+-ATPase causes severe morphological changes. Ryan Kendle, Emma Jean Bowman, and Barry Bowman. University of California, Santa Cruz, Biology, Santa Cruz, CA, USA.

Fungal vacuoles are important as storage organelles for basic amino acids and polyphosphate and for maintaining cytosolic homeostasis for protons and calcium ions. To function properly the vacuoles depend upon the activity of the vacuolar H^+-ATPase located in the vacuolar membrane. The enzyme pumps protons into the organelle, acidifying the interior and generating a proton gradient used to drive transport of solutes across the membrane. We have inactivated the V-ATPase of *Neurospora crassa* by RIPing *vma-1*, the gene encoding the catalytic subunit. The resultant *vma-1* null strains are deficient in multiple stages of development. They grow slowly, exhibiting dichotomous branching rather than an apical dominant pattern typical of hyphal fungi. They produce no aerial hyphae or conidia and when crossed with each other from helper strain backgrounds produce no asci or ascospores. The null strains are unusually sensitive to medium pH above 6.0, to heavy metals such as zinc, and to high salt concentrations. Two strains with no detectable V-ATPase activity were characterized. Sequence analysis revealed that one is heavily RIPed and unable to make a protein product, while the other is lightly RIPed and is expected to produce a protein product. Interestingly, ascospores from the first strain rarely germinate, but those from the second strain germinate like wild type.

327. On the ultrastructure of cell wall in null pigmentation mutant of *Aspergillus nidulans.* Jeong-Mi Kim[1], Kwang-Yeop Jahng[1], Dong-Min Han[2], and Yun-Shin Chung[3]. [1]Chonbuk National University, Biological Sciences, Chonju, Chonbuk, South Korea. [2]Wonkwang University, Molecular Biology, Iksan, Chonbuk, South Korea.[3]Washington University, Genetics, Washington, St. Louis, U.S.A.

To investigate the effects of cell wall on the pigmentation and branching in *Aspergillus nidulans*, the ultrastructure and cytochemical composition of cell wall in wild type (FGSC4) and *npgA1* mutant (no pigmentation through the whole life cycle, WX17) were examined. Scanning electron microscope (SEM) showed that the hyphal surface and branching in WX17 were rough and poorly formed, in contrast to those in FGSC4 showing smooth and well developing, respectively. Transmission electron microscope (TEM) and carbohydrate staining showed that the hyphal wall of FGSC4 layered as H1, H2, H3, and H4 were mainly composed of protein, chitin, B-glucan, and the complex of chitin and B-glucan respectively. However the hyphal wall of WX17 was lacking H1 and H3 layers suggesting that the defect in pigmentation and hyphal branching of *npgA1* mutant might be due to the lack of the B-glucan layer in the cell wall. To study the function of the gene npgA in constructing cell wall structure, we isolated DNA fragment that was able to complement npgA1 mutation from the genomic library of FGSC4. From its nucleotide sequence, we found that there was an open reading frame (ORF) in which the amino acid sequence was homologous to that of *SLC1* gene which encoded fatty acyltransferase in *Saccharomyces cerevisiae*. The fragment was also capable of complementing the defect in a 1-acyl-sn-glycerol-3-phosphate-acyltransferase, encoded by the gene *plsC,* of *Escherichia coli*. From these results, it could be concluded that the *npgA1*-complementing element might play a role in constructing cell wall structure or depositing pigment.

328. Cloning and expression of the cDNA encoding an alternative oxidase gene from *Aspergillus niger* WU-2223L.Kohtaro Kirimura, Masashi Yoda, and Shoji Usami. Waseda Institute of Science and Technology, Applied Chemistry, Shinjuku-ku, Tokyo, Japan.

A cDNA fragment encoding the mitochondrial alternative oxidase, the enzyme responsible for cyanide-insensitive and salicylhydroxamic acid (SHAM)-sensitive respiration, from the citric acid producing-fungus *Aspergillus niger* WU-2223L was cloned and expressed in *Escherichia coli* as host strain. Synthetic primers were designed from the conserved nucleotide sequences of the alternative oxidase genes from plants and a yeast. The 210 bp DNA fragment was amplified by PCR with the primers using chromosomal DNA of *A. niger* WU-2223L as template, and used to screen a cDNA library of *A. niger*. One full-length cDNA clone, 1.2 kb, was obtained and sequenced to reveal that the clone contained an open reading frame (ORF-AOX1) encoding a polypeptide deduced of 351 amino acids. The predicted amino acid sequence exhibited 46%, 52%, and 48% homologies to the alternative oxidases of *Hansenula anomala*, *Neurospora crassa* and *Sauromatum guttatum*, respectively. In the 5'-terminus region of the ORF-AOX1, a mitochondrial targeting motif was found. The whole open reading frame ORF-AOX1 was ligated to plasmid pKK223-3 to construct an expression vector pKAOX1. *E. coli* transformant harboring pKAOX1 showed cyanide-insensitive and SHAM-sensitive respiration, and the expression was induced to two fold by addition of IPTG. These results indicated that the ORF-AOX1 encodes an alternative oxidase of *A. niger*.

329. Human TNF-Alpha could be successfully produced in the filamentous fungus *Aspergillus niger* heterologous protein expression-secretion system. N. Krasevec[1], S. Blaas[2], C.A.M.J.J. van den Hondel[2], and R. Komel[1]. [1]National Institute of Chemistry, Ljubljana, Slovenia. [2]TNO, Molecular Genetics, Zeist, The Netherlands.

The expression-secretion system of the GRAS filamentous fungus *A. niger* has proven to be capable of producing heterologous proteins at commercially interesting levels, however, it has also to be taken into account that it has not yet been applied for a wide range of heterologous protein products. Human tumor necrosis factor alpha (hTNF-alpha) monomer is a 17 kDa non-glycosylated protein, containing a single intra-molecular disulfide bond in its structure. In solution it exists as a compact, bell-shaped homotrimer, which is considered to be a biologically active form of this important cytokine with a wide range of biological activities. For heterologous expression of hTNF-alpha in *A. niger* the same strategies were used, which were proved to be the most successful for several other non-fungal proteins: a) gene-fusion with the *A. niger* glucoamylase GII form as a carrier-gene, with KEX2-like in vivo cleavage-site in between the genes; b) strong fungal transcription control regions and efficient secretion signals of the *A. niger* glucoamylase gene; and c) selection of high copy transformants due to the *amdS* selection marker in the protease-deficient host-strain *A. niger* AB1.13. No processed hTNF-alpha detected in the media or even in the cell, although specific mRNA of the expected size was observed. We could detect hTNF-alpha in the form of glucoamylase-fusion protein in the cell, regardless of the presence or absence KEX2-like in vivo cleavage-site. This glucoamylase-hTNF-alpha fusion-protein was N-glycosylated due to the glucoamylase GII part (O-glycosylation was not checked), mostly attached to the membranes, but not to the outside of the cell wall. Only 12% of hTNF-alpha added into maltodextrine production medium was proteoliticaly degraded after 24 hours, so degradation in the protease-deficient host-strain AB1.13 compared to N402 strain was not a problem. hTNF-alpha analogue LK 802 (Cys95/148, His107/108) was also expressed with carrier-gene strategy. Cys95/148 mutation is introducing an intermolecular disulfide bond so that a more compact and stable trimeric molecule is produced, and His107/108 mutation is introducing six histidine residues at the apical site of the molecule, providing an efficient separation method on metal affinity chromatography. However, the glucoamylase-hTNF-alpha analogue fusion-protein with both above mentioned mutations could not be successfully separated from the cell extract. In all these experiments hTNF-alpha synthetic gene optimised for *E. coli* bias was used, but when the gene was exchanged for natural human TNF-alpha cDNA, processed hTNF-alpha was successfully secreted into the medium. After the first screening of small amounts of transformants the yield was estimated to 13 mg/l culture.

330. Regulation of septum formation in *Aspergillus nidulans* by cyclin-dependent kinase activity. Peter R. Kraus, and Steven D. Harris. University of Connecticut Health Center, Microbiology, Farmington, CT, USA.

Strict regulation of cdk activity has been shown to be important for cytokinesis in a variety of eukaryotic systems. In the filamentous fungus *Aspergillus nidulans*, the cdk encoded by the *nimX* gene has been shown to be involved in regulating cytokinesis. Genetic evidence suggests that the ability to separate mitosis and septum formation depends upon a threshold level of NIMX activity. A transient inhibition of NIMX activity by the activation of the DNA damage checkpoint pathway results in a significant delay in septum formation. This supports the idea that germlings must reach a threshhold level of NIMX activity at a certain point during interphase in order to initiate septum formation. In order to identify additional components of the pathway regulating septum formation, we have initiated a genetic screen for extragenic suppressors of the Ts *nimT23* mutation, which results in inactivation of the NIMX-activating tyrosine phosphatase NIMT. We have counter-screened 500 suppressors of the *nimT23* mutation

for HU sensitivity in order to distinguish those mutants which may be defective in the checkpoint pathways which respond to DNA damage or unreplicated DNA. We have isolated 7 HU sensitive mutants which fall into three linkage groups (designated *sntA*, *sntB* and *sntC*) and are distinct from alleles of *nimX* and *ankA* (which encodes the *A. nidulans* homolog of *S. pombe* Wee1p). *snt* mutations allow germlings to undergo septum formation before the critical cell size is reached and in the presence of DNA damage generated by mutation (*sepB3*) and by the bi-functional alkylating agent diepoxyoctane (DEO). The *snt* mutations have different effects on cell cycle checkpoints and result in decreased phosphorylation on Tyrosine-15 of NIMX.

331. The MAPKKK Ste11 regulates vegetative growth through a novel kinase cascade of shared signaling components. Bee-Na Lee. Harvard Medical School, Dept of Biological Chem, Boston, MA, USA.The *Saccharomyces cerevisiae* MAPKKK Ste11 functions in three specialized MAPK cascades that induce cells to either mate, grow invasively or handle stress in response to distinct extracellular stimuli. To determine whether Ste11 functions under uninduced conditions, we screened for mutants that require STE11 for vegetative growth. We found that sls1 (och1) mutants defective in the synthesis of cell wall mannan and other mannoproteins require Ste11 for survival. Ste11 regulates vegetative growth in a pathway that includes Ste20 (MAPKKK), Ste7 (MAPKK) and Ste12 (transcription factor), components also shared by the mating and invasive growth pathways. By contrast, the pathway-specific components Ras2, Tec1, Gb, Ste5, and MAPKs Fus3 and Kss1 are not required. The Ste11 pathway regulates vegetative growth in parallel with the Bck1 and high osmolarity glycerol (HOG) MAPK cascades, functioning in part to maintain cell wall integrity and increase expression of FKS2, encoding (1,3) b-D- glucan synthetase. Proteins known to sequester or inhibit Ste11 pathway components in other cascades (i.e. Ste5, Fus3, Hog1) inhibit the Ste11 vegetative pathway. We propose that vegetative growth is controlled by basal levels of activity through parallel kinase cascades which can reassemble with scaffolding proteins and other regulators for specialized outputs in response to specific stimuli.

332. Analysis of multiple alleles of *Neurospora crassa ro* mutants. In Hyung Lee[1], Santosh Kumar[1], Peter Minke[2], John Tinsley[2], and Michael Plamann[1]. U. of Missouri, Biological Sciences, Kansas City, MO, USA. [2]Texas A&M; University, Biology, College station, TX, USA.

Cytoplasmic dynein is a microtubule-associated, minus-end directed, mechanochemical enzyme, and the dynactin complex has been proposed to regulate the interaction of cytoplasmic dynein with membranous cargo. We have been using *Neurospora crassa* to genetically analyze cytoplasmic dynein and dynactin. Previously, we showed that *ro-1*, *ro-3*, and *ro-4* encode cytoplasmic dynein heavy chain, $p150^{Glued}$ (the largest subunit of dynactin), and ARP1 (the most abundant subunit of dynactin), respectively. The availability of multiple independent alleles of ro genes encoding subunits of cytoplasmic dynein and dynactin provides us with the opportunity to do a detailed analysis of protein interactions and specific functions within the motor complex. The largest subunit of dynactin, $p150^{Glued}$, is not present in a strain deleted for *ro-4*; however, $p150^{Glued}$ is present in a *ro-4*(E8) strain. The *ro-4*(E8) mutant is altered for localization of both cytoplasmic dynein and dynactin, with both complexes colocalizing with microtubule tracks at hyphal tips. The *ro-4*(E8) allele contains a point mutation predicted to affect a surface residue in a conserved region of ARP1. In the *ro-4*(E8) mutant the interaction of cytoplasmic dynein/dynactin with membranous cargo is partially impaired. Western analysis of different *ro-1*

mutants revealed that each *ro-1* allele affects the stability of RO1 and RO3 to a different degree. In a similar manner, each mutation in *ro-3* affects the stability of RO1 and RO3 to a different degree. Our results suggests that both cytoplasmic dynein and dynactin are required for stable interaction with membranous cargo.

333. Identification of cell wall proteins of *Pneumocystis carinii* by 2D gel electrophoresis and MALDI-TOF mass spectrometry.Michael J. Linke, and A.George Smulian. University of Cincinnati, Infectious Diseases, Cincinnati, Ohio, USA.

Regulation of cell wall synthesis is critical in the adaptation to environmental changes and cell wall components are important antifungal therapuetic targets, however, few cell wall proteins of the pulmonary pathogen, *Pneumocystis carinii*, have been characterized. Most proteins identified thus far have been identified based on their immune recognition by the infected host. To identify additional structural proteins and other components of the *Pneumocystis carinii* cell wall, a soluble cell wall extract was obtained by the treatment of *P. carinii* organisms with zymolyase followed by centrifugation at 100,000 x g. Individual protein "spots" within this soluble cell wall extract were resolved by 2D gel electrophoresis and identified by Coomassie Brilliant blue staining. Spots were excised, destained and subjected to in gel trypsin digestion. Peptide fragments were extracted and analysed by MALDI-TOF mass spectrometry. Peptide fragment fingerprints were compared to SwissProt and NCBI protein databases using Protein Prospector software and potential homologues identified. Peptide sequence information will be obtained on signature peptides by MS analysis following Post Source Decay fragmentation and Collision Induced Dissociation to verify the identity of the protein matches. 2D gel electrophoresis followed by MALDI-TOF mass spectrometry should allow high throughput, rapid identification of cell wall components.

334. Modification of fungal N-linked carbohydrates. Marleen Maras, and Roland Contreras. Gent University and VIB , Molecular Biology, Gent, Flanders, Belgium.

The carbohydrate moiety of glycoproteins has been shown to have a role in antigenicity, solubility, targeting, cell-cell contact, homing and pharmocological behaviour. Often, the primary structures of the carbohydrates found on proteins, secreted by mammalian cells, is complex, heterogeneous and difficult to fractionate on the basis of this heterogeneity. In order to obtain proteins with well defined, more homogenous carbohydrate structures, procedures have to be worked out for their synthesis. High-mannose oligosaccharides were characterized on glycoproteins from certain filamentous fungi. These N-glycans were found to have simple structures, that can serve as precursors for the synthsesis of mammalian-like oligosaccharides. Mammalian-like hybrid oligosaccharides were synthesized on glycoproteins from the filamentous fungus *Trichoderma reesei* by in vitro enzymatic modification. Succes of this conversion was found to be dependent on the strain of Trichoderma used. In addition, preincubation of the fungal proteins with *Aspergillus saitoi* alfa 1,2 mannosidase substantially improved formation of the hybrid structure with one strain, while it did not with glycoproteins from another strain. With all strains used, it was found that alfa 1,2 mannosidase did not convert all oligosaccharides to the acceptor substrate for N-acetylglucosaminyl transferase I. This demonstrates that Trichoderma synthesizes a fraction of high mannose-type but not mammalian-like N-glycans. Therefore, further studies were directed to a detailed analysis of the N-lined

glycans of the CBHI protein. NMR data were obtained for two major components and detailed studies were carried out for several minor components. In addition, we cloned an alfa 1,2 mannosidase from *T. reesei* Ity may be responsible for the processing of high-mannose fungal oligosaccharides to Man5GlcNAc2, which is substrate for 'in vitro' N-acetyl glucosaminyl transferase I. The gene was engineered in a *Pichia pastoris* expression plasmid. Upon transformation to the yeast, alfa 1,2 mannosidase activity was easily detected. Initial experiments to characterize oligosaccharides of the transformed Pichia strain showed formation of N-glycans, smaller than Man8GlcNAc2 that are never synthesized in the wild type yeast.

335. Programmed cell death in fungi: Heterokaryon incompatibility involves nuclear DNA degradation. Stephen M. Marek[1], Jennifer Wu[2], N. Louise Glass[2], David G. Gilchrist[1], and Richard M. Bostock[1]. [1]Univ. California, Plant Pathology, Davis, CA, USA. [2]Univ. British Columbia, Biotechnology Laboratory, Vancouver, BC, Canada.

Within species, fungi can fuse to form heterokaryotic mycelia if they possess identical heterokaryon incompatibility (*het*) loci. If fungi attempting heterokaryosis differ at one or more *het* loci, the fused cells self-destruct by a killing reaction process reminiscent of apoptosis in animal cells (Jacobson et al. FG&B; 23:45). In *Neurospora crassa*, ten such *het* loci have been identified in addition to the mating locus. The *het* locus encodes a single nonessential protein and controls specificity as one of three alleles, *OR*, *PA*, or *GR*. In this study, the cytology of transformants carrying incompatible and compatible combinations of *het-c* alleles and pairings between vector control transformants were examined using fluorescent DNA stains and **t**erminal deoxynucleotidyl transferase-mediated d**U**TP-X **n**ick **e**nd **l**abeling (TUNEL). Transformants carrying incompatible *het-c* transgenes were inhibited in growth and contained heavily degraded nuclear DNA in cells also displaying vacuolated or shrunken cytoplasms. Transformants carrying compatible *het-c* transgenes contained little or no degraded DNA. In pairings between incompatible control transformants, fusion cells frequently contained heavily degraded nuclei in disorganized cytoplasms. In pairings between compatible control transformants, fused cells rarely contained degraded nuclei. Cells killed by the incompatibility reaction often contained cytoplasmic and nuclear remnants that often persisted after adjacent cells grew new hyphae into the killed cells. The process by which heterokaryon incompatibility kills and disassembles cells and the extent to which it is genetically controlled can best be described as programmed cell death.

336. Characterization of a vacuolar Ca+2/H+ exchanger (CAX) of *Neurospora crassa*. Emilio C. Margolles-Clark, Stephen Abreu, and Barry J. Bowman. University of California Santa Cruz, Department of Biology, Santa Cruz, California, USA.

A gene encoding a vacuolar Ca+2/H+ exchanger (CAX) was obtained from the Neurospora Genomic Project. This transporter is an integral membrane protein, possibly containing 11 membrane spanning regions, as indicated by hydrophilicity analysis of its amino acid sequence. A strain containing a mutated CAX gene was generated by RIPing. CAX protein proved to be non essential for the survival of the organism. The effect of the mutation on growth in different Ca+2 concentrations at different pH conditions will be discused. Transport experiments showed that Ca+2 transport into vacuolar vesicles of the mutant strain is dramatically reduced. This indicated that the Ca+2/H+ exchanger plays a major role in the vacuolar internalization of the

cation. The low Ca+2 transport background found in the mutant allowed the study of the properties of the transporter in the wild type strain. The kinetic data of the transporter as well as its inhibition by other divalent cations will be presented.

337. Lytic enzymes in *Ustilago maydis*: amplification and cloning of a 1,3-b-glucanase and an acidic protease. Alfredo D. Martinez-Espinoza, and Jose Ruiz-Herrera. CINVESTAV-Unidad Irapuato, Genetic Engeering of Plants, Irapuato, Guanajuato, Mexico.

Ustilago maydis causes the disease named common smut in maize. It is believed that lytic enzymes play a role in mating and pathogenicity. Preliminary evidence showed the presence and activity of glucanases and proteases. We have initiated the search for genes coding for these enzymes. Oligonucleotides were designed based on conserved sequences of 1,3-b-glucanases and proteases from several fungi and were used as primers for PCR. Regarding the glucanases, a 190 bp PCR fragment was amplified, cloned and sequenced. The sequence showed homologies ranging from 75 to 85 % with exo-1,3-b-glucanases from *S. cerevisiae, Y. lipolytica, A. bisporus* and *C. albicans*. The PCR fragment was used to screen a genomic library of U. maydis. Two positive clones were isolated, and found to contain inserts of 9 and 8 kb. Restriction digests and Southern blots showed differences between the clones. Two fragments were identified and subcloned. They were characterized by restriction and sequence analysis. Regarding the proteases, an 800 bp PCR product was amplified, cloned and sequenced. The sequence showed conserved regions of aspartyl-proteases. Library screening is currently under way. Gene disruption will determine the role of these genes in mating and pathogenicity in *U. maydis*. **338. Identification and analysis of clonable extragenic suppressors of the nimXcdc2F223L mutation of *Aspergillus nidulans*.** Sarah Lea McGuire, Melanie D. Schrader, Brett W. Carter, Dana L. Roe, Chad D. Young, Sean P. Grace, Gene A. Lang, and Suzanne E. Wahrle. Millsaps College, Biology Department, Jackson, MS, USA.

The nimX^{cdc2} protein kinase of *Aspergillus nidulans* regulates progression of nuclear division during G_1>, S, and G_2> phases of the cell cycle. To identify genes which encode proteins that interact with nimX^{cdc2}, we have generated a collection of strains with mutations that suppress the temperature-sensitive nimX^{cdc2}F223L mutation. The suppressor strains were screened for the presence of an additional phenotype (cold- or drug-sensitivity) that could be used in cloning. Of 1500 suppressors isolated, 37 contained additional phenotypes. 14 suppressor strains with additional phenotypes have been crossed with a wild-type strain to determine if the suppressor mutations were intragenic or extragenic. This yielded two strains with extragenic suppressor mutations that cosegregate with the additional phenotype. These strains were designated MDS250 and CY17. Both suppressor mutations have been shown to be recessive by diploid analysis, and phenotypic analysis indicates that both mutations stop the cell cycle during interphase. The growth phenotype of CY17 contains aberrant nuclear and cytoplasmic morphologies that indicate deregulation of tyrosine phosphorylation of nimX^{cdc2}. We are currently performing analyses to determine at which point during interphase the nuclear division cycle is halted in these strains. In addition, both suppressor mutations are being mapped to chromosome to facilitate cloning and the remaining 23 suppressor strains are being analyzed genetically. Supported by NIH grant GM55885.

339. Morphological and genetic characterisation of Hbr-2, a hyperbranching mutant of *Aspergillus nidulans*. Stephen Memmott, Sarah Pollerman, and Geoffrey Turner. University of Sheffield, Molec Biol Biotech, Sheffield, South Yorkshire, UK.

Fungal morphology plays a major role in the fermentation industry as it affects the physiological state of the culture. This leads to consequences for both process conditions and final product yield. Fungal growth proceeds via the two basic mechanisms of hyphal elongation and the production of branches either at the hyphal tip or as lateral branches generated from intercalary compartments. The factors which control the production and frequency of branches are known to be both environmental and genetic. In order to discover underlying genetic components involved in the branching of *Aspergillus nidulans*, temperature sensitive, hyperbranching mutants were isolated and phenotypically characterised using branching frequency, septation and nuclear distribution measures. In addition genetic analysis was used to identify 10 loci affecting branching frequency. One such mutation, designated *hbrA2*, was chosen and the affected gene was isolated through functional complementation. A chromosome specific library and a co-transformation system was used to isolate a fragment complementing *hbrA2*. A sequence database search has given promising leads as to the function of *hbrA2*. Characterisation of this fragment will be described. Attempts are now being made to disrupt *hbrA*, and to place the gene under the control of a regulated promoter to study further the role of this gene in fungal growth.

340. Apical growth in *smco7* mutant of *N. crassa*. Yuko Mochizuki, and Tadako Murayama. Kanto-Gakuin Univ., College of Engineering, Kanazawa-ku, Yokohama, Japan.

Transformation of a morphological mutant, *smco7*, with the plasmid carrying NC-ras2, a ras gene homologue of Neurospora, resulted in the recovery of the wild type morphology and a deletion of one nucleotide was detected in the region corresponding to the N-terminal region of the putative ras protein homologue encoded by NC-ras2 of the *smco7* mutant. The apical cells of *smco7* were shorter, thinner, more fragile than those of the wild type. The *smco7* mutation seemed to cause defects in cell wall synthesis at the apex. Te extracellular invertase and trehalase activities were much lower in *smco7* than in the wild type. The extracellular enzymes may be transported to the apex and secreted there through the common mechanism in the transport and exocytosis of the cell membrane and cell wall precursors An actin inhibitor, Cytochalasin A (CA), considerably inhibited the hyphal growth, made hyphae thinner, and lowered the level of extracellular invertase after the shift of the mycelia to the medium containing CA in the wild type. These results suggest that the actin plays important roles in the apical growth and the production of extracellular enzymes and the ras protein plays some roles in the regulation of actin in Neurospora.

341. *sepG*, a gene required for septation in *Aspergillus nidulans*. Maria Victoria Montenegro-Ch, and John E. Hamer. Purdue University, Biology, West Lafayette, Indiana, USA.

Aspergillus nidulans is a multicellular, filamentous fungus that divides by the formation of a crosswall called a septum. Septum formation is temporally and spatially regulated by nuclear positioning and cell cycle progression. *sep G* was identified as a conditional mutant that causes an aseptate phenotype in cells that have completed several rounds of mitosis. The *sep G* mutant is unable to form septa but displays no defects in growth or nuclear division producing long

multinucleate hyphae at the restrictive temperture. The *sepG* gene has been cloned by complementation of the mutant with a chromosome specific library of *A. nidulans*. Sequencing and further characterization of the gene is underway. *sepG* is likely to be part of a signaling pathway mediating actin ring formation, the first event of septum formation.

342. Chemotactic signals trigger a calcium influx in zoospores and hyphae of *Phytophthora sojae*. Paul F. Morris, and Mary S. Connolly. Bowling Green State University, Biological Sciences, Bowling Green, Ohio, USA.

Both the motile zoospores, and germinating cysts of *Phytophthora sojae* respond chemotropically to nM levels of the isoflavonoids excreted from soybean roots. The role of calcium in the cellular response to a host signal was investigated by loading hyphal germlings in a chemotaxis chamber with Calcium Green -1. Cytoplasmic fluorescence of hyphal germlings increased in response to mechanical stimulation or the addition of daidzein to the media. The increase in fluorescence was transient and reversible. Once the chamber was rinsed free of daidzein and the cells were allowed to recover, a second fluorescent signal could be produced by the addition of daidzein. Confirmation that the increase in Calcium Green fluorescence was due to an influx of exogenous calcium demonstrated by x-ray microanalysis of individual encysted zoospores. Zoospores exposed to 10 mM calcium and daidzein at the time of encystment formed cysts that contained more calcium than zoospores exposed to only to calcium. The addition of exogenous calcium or daidzein to zoospores at the time of their encystment altered the development fate of cysts and the relative magnitude of calcium stores in the cells. Low levels of calcium reserves inhibited spore germination and enhanced the release of a zoospore from the cyst. High levels of calcium were associated with cyst germination. Isoflavones in the presence of at least 0.5 mM calcium also promoted cyst germination in cells fated to release a second zoospore, presumably by generating a transient increase in cytosolic calcium.

343. Mutants related to cyclic AMP cascade in *N. crassa*. Tadako Murayama. Kanto-Gakuin Univ., College of Engineering, Kanazawa-ku, Yokohama, Japan.

The wild type grew to be filamentous, whereas adenylyl cyclase mutant, *cr-1*, grew to be colonial on the solid medium. However, the wild type also grew to be colonial on the solid medium containing sorbose. The *cr-1* strain grew to be much smaller colony than the wild type on this medium. High number of large colonies appeared in single colonies from 2-weeks old conidia of *cr-1* mutant. The strains from these large colonies grew to be filamentous on the medium without sorbose, though they still showed some form of morphological abnormality. Genetic analysis showed that all 5 strains from the large colonies had original *cr-1* mutation and suppressor mutations. The suppressor mutants had the mutations in the different genes and showed different types of morphological abnormality. Some of the suppressor single mutants contained the increase amount of cyclic AMP (cAMP). These mutants did not or scarcely formed conidia and had elevated activity of trehalse. One of the suppressor mutants did not produce conidia and trehalase. Some of them showed abnormality in carotenoid formation. These results suggest that these suppresor mutant genes have some roles in the cyclic AMP cascade and transduce the signal related to the regulation of trehalase, carotenoid production, conidia formation.

344. Maize Ribosome-inactivating protein 1 has antifungal activity against *Aspergillus flavus* and *Aspergillus nidulans*. Kirsten Nielsen[1], Gary A. Payne[2], and Rebecca S. Boston[1]. [1]NC State University, Botany, Raleigh, NC, USA. [2]NC State University, Plant Pathology, Raleigh, NC, USA.

Seeds contain a variety of proteins that function primarily to provide storage reserves or defense functions against pests and pathogens. Maize ribosome-inactivating protein 1 (RIP1) is an abundant seed protein with a putative role in plant defense. RIP1 readily inactivates ribosomes from non-plant eukaryotes but has little activity against ribosomes from plant species. RIP1 was tested for antifungal activity in a microculture assay. Conidia were treated with RIP1 or control proteins and monitored for abnormal growth or morphology over time. Fungi from the genera *Aspergillus* exhibited a strong response to RIP1. *Aspergillus flavus*, a maize pathogen which produces aflatoxin B1, had an increase in hyphal branching after RIP1 protein treatment that was not observed in control protein treatments. Quantitative analysis confirmed that the branching phenotype was significant. The chitin binding dye Congo red was used to show that branched hyphae treated with RIP1 had only one actively growing hyphal tip whereas the few control hyphae with branching had multiple growing hypal tips. The genetically well characterized but non-pathogenic species *Aspergillus nidulans* was also tested in the microculture assay. A striking decrease in the number and growth of hyphae was observed when *A. nidulans* conidia were treated with RIP1 protein. The changes in growth of *A. flavus* and *A. nidulans* are consistent with maize RIP1 having antifungal activity.

345.Genetics of alkaloid biosynthesis in *Claviceps purpurea*: evidence for a gene cluster. Paul Tudzynski[1], Katja Hölter[1], Telmo Correia[1], Claudia Arntz[1], Nicolas Grammel[2] And Ulrich Keller [2]. [1]Institut f. Botanik, Westf. Wilh.-Universitaet, Muenster, Germany. [2]Max-Volmer-Inst. f. Biophys. Chemie and Biochemie, Techn. Universitaet. Berlin, Berlin, Germany

The production of ergot alkaloids by the grass pathogen *Claviceps purpurea* and related species is a well established biotechnological process. The biosynthetic pathway has been worked out in detail and several strains have been developed for production in submerged culture, yet there is still a deficit in detailed genetic analyses. Recently, we have cloned and characterized the genes for Dimethylallyltryptophansyntase (DMATS), catalizing the first step of the pathway, from two *C. purpurea* strains, a pathogenic field isolate (T5) producing alkaloids only *in planta* and a (mutant) submerse production strain (P1). A chromosome walking approach [1] showed that the DMATS-gene of strain P1 is part of an alkaloid gene cluster: one of the neighbouring genes encodes D-lysergylpeptide synthetase 1 (LPS1), a trimodular enzyme catalyzing a crucial step in the formation of peptide alkaloids [2].The existence of a gene cluster allows the identification of other genes involved in the pathway and therefore forms a basis for a detailed molecular analysis of this biotechnologically important biosynthetic pathway.[1] P. Tudzynski et al. 1998 Molec Gen Genet. in press [2] B. Riederer et al. 1996 J Biol Chem. 271: 27524-27530

346. Oxidative stress and senescence in *Podospora anserina*. H.D. Osiewacz, N. Averbeck, C. Borghouts, and E. Kimpel. Johann Wolfgang Goethe-Universität, Botanisches Institut, Frankfurt, Germany

Podospora anserina is an ascomycete with a limited lifespan. Lifespan is controlled by the environment and by mitochondrial and nuclear genetic traits. During the course of investigations to elucidate the molecular mechanisms involved in the control of lifespan, we cloned and characterized a nuclear gene that is able to complement the pleiotropic long-lived mutant grisea to wild-type characteristics. The cloned gene (termed Grisea) was found to code for a protein with significant identity to three yeast transcription factors. These are ACE1 and MAC1 of *Saccharomyces cerevisiae*, and AMT1 of the pathogenic yeast *Candida glabrata*. Most strikingly, the three yeast transcription factors control the expression of different genes belonging to the molecular apparatus that is involved in control of cellular copper homeostasis or is part of the defense system protecting the cell against oxidative stress. The detailed molecular characterization of GRISEA revealed that this fungal transcription factor is a functional homologue of MAC1. The most convincing evidence for this conclusion is the ability of the wild-type copy of grisea to complement the yeast mac1-1 deficiency mutant. Furthermore, a one hybrid system analysis revealed a modular structure of GRISEA that is similar to the structure of MAC1. Finally, the function of GRISEA becomes repressed by increased copper levels. A model will be presented that explains the phenotype of the long-lived *P. anserina* mutant as the result of an impaired copper homeostasis and by a reduction of oxidative stress. Acknowledgment: The experimental work was supported by a grant of the Deutsche Forschungsgemeinschaft.

347. Host- and biotope-specific populations of *Claviceps purpurea*. Sylvie Pazoutová, Marie Skvánová, Michaela Dolejsí, and Marek Linka. Czech Academy of Sciences, Institute of Microbiology, Prague, Czech Republic.

Claviceps purpurea occurs in Northern temperate regions and colonizes mainly grasses of pooid and arundinoid subfamily. Different authors tried to find the host specific populations or formae speciales in *C. purpurea* or chemoraces, producing different types of alkaloids. The genetical variability was assayed in over 70 isolates with defined locality and host plant using RAPD and EcoRI restriction polymorphism of the ITS1-5.8S-ITS2 region of rDNA. The type of alkaloid produced was assayed and the shape and dimensions of conidia and sclerotia were recorded, to enable the comparison with the groups postulated in older literature. Two groups were found, that shared distinct RAPD bands. The first group encompassed the isolates from field and open meadows, whereas the isolates from the second group were found in shady or wet habitats. Isolates of the second group are producing ergosine and ergocristine 1:1, the first group is more heterogenous. The average size of conidia in the second group is about 7-8 um, whereas in the first group, 5-6 um was mostly observed. The sclerotia of the second group tend to thin curved form. The second group combines isolates that were once denoted as *C. microcephala* and/or f. sp. *natans P. arundinaceae*. The host preferences of the sub-groups are not absolute, the biotop is more important. Without the use of RAPD or rDNA polymorphism, it is not possible to distinguish the sub-groups solely on the basis of morphological characters. That was probably the reason for the differences in specifity groups postulated by different authors.

348. Molecular phylogeny of Japanese *Amanita* species based on nucleotide sequences of the internal transcribed spacer region of nuclear ribosomal DNA. Takashi Oda, Chihiro Tanaka, and Mitsuya Tsuda. Kyoto University, Agriculture, Kyoto, Kyoto, Japan.

We constructed a molecular phylogenetic tree of forty specimens of Japanese *Amanita* species based on nucleotide sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. By comparing this phylogenetic tree with the classification systems of Bas (1969) and Singer (1986) which are based on morphological characters and have been widely accepted, we examined the phylogeny of Japanese *Amanita* species. Our results supported the taxonomical treatment in subgenus level of Bas (1969) and Singer (1986) who divided the genus *Amanita* into two subgenera *Amanita* and *Lepidella* based on the amyloidity of spores. However, in section level, we suggested that subgenus *Amanita* should be divided into three sections (*Amanita*, *Vaginatae* and *Caesareae*). Moreover, our results showed the necessity to rearrange the disordered assemblage of members in the subgenus *Lepidella*. As for three subspecies of *Amanita hemibapha* and three varieties of *Amanita vaginata*, it is necessary to grade up their taxonomical ranks from subspecies / variety to species. It appears that *Amanita pantherina* and *Amanita muscaria* are extremely related phylogenetically. The molecular biological technique was proven to be sensitive for detecting taxa that are difficult to detect by morphological characters.

349. The structure of the vacuolar ATPase in *Neurospora crassa*. Jack C. Reidling, Emilio Margolles-Clark, Karen Tenny, June Pounder, Emma Jean Bowman, and Barry Bowman. UCSC, Biology, Santa Cruz, Ca, USAThe filamentous fungus *Neurospora crassa* contains many small vacuoles. These organelles contain high concentrations of polyphosphates and basic amino acids such as arginine and ornithine. Because of their size and density the vacuoles can be separated from other organelles in the cell. The ATP-driven proton pump in the vacuolar membrane is a typical V-type ATPase. We have examined the size and structure of this enzyme by the technique of radiation inactivation and by electron microscopy. The vacuolar ATPase is a large and complex enzyme that appears to contain at least 13 different subunits. We have characterized the genes that encode 11 of these subunits, are currently characterizing a 12th, and are attempting to obtain a 13th. The ultimate goal of our laboratory has been to understand the function and structure of these subunits. One approach we are taking is to purify the enzyme to homogeneity for further studies.

350. Biological research on prospects of cultivation of the giant puffball (*Langermannia gigantea*). Imre Rimóczi and Jozsef Geml. University of Horticulture and Food Industry, Department of Botany, Budapest, Hungary.

Bringing giant puffball into cultivation is urged by its economical importance in many fields: it is an edible, tasty mushroom, the mass of mycelium can be used for feeding animals, furthermore it is a pharmacologically important species. Its requirements for soil types, the fenology and the environmental factors of fructification, and the coenological rules of its occurence have been examined for ten years in more than 81 habitats. More than 2,000 data, gained from soil components of 58 habitats, give a guide for preparing suitable substrate for the cultivation of *Langermannia gigantea*, and for choosing areas for extensive cultivation. The occurence of this species is not associated tightly with any type of vegetation, therefore the extensive cultivation is not affected seriously by certain countrysides. It needs shade in the initial period of fructification, but later become more resistant to direct sunlight. The *Langermannia gigantea* is exclusively saprophyte, even its facultative mikorrhiza connection is unlikely. Its spontaneous appearance in cultivated vinyards and orchards supports the possibility of its cultivation.

351. The Spitzenkorper of *Allomyces macrogynus* serves as a microtubule organizing center.Robert W. Roberson, and Dennis P. McDaniel. Arizona State University, Plant Biology, Tempe, AZ, USA.Elongating hyphae of higher fungi contain a well defined Spitzenkörper. The role(s) of the Spitzenkörper in fungal cell growth and morphogenesis have long been enigmatic. We have shown that hyphae of the zoosporic fungus *Allomyces macrogynus* (Chytridiomycota) contain a well defined Spitzenkörper and recent data confirmed its role as a microtubule organizing center (MTOC). Using indirect immunofluorescence methods and laser scanning confocal microscopy (LSCM), we have demonstrated that gamma-tubulin is a component of the Spitzenkörper in *A. macrogynus*. We also found that gamma-tubulin is associated with centrosomes. LSCM of the microtubule (MT) cytoskeleton under control conditions and upon recovery from MT depolymerization suggested that the *Allomyces* Spitzenkörper functions as a center for MT organization and nucleation. Further data will be presented elucidating the ultrastructural location of gamma-tubulin within the Spitzenkörper and *in vivo* dynamics of MT polymerization. That the Spitzenkörper of *Allomyces* regulates MT nucleation and distribution demonstrates its relevance in regulating intracellular motility and maintaining cytoplasmic organization during hyphal tip growth. There is presently no evidence that Spitzenkörper of higher fungi contain gamma-tubulin and their role as MTOCs remains speculative.

352. Isolation and characterisation of a *cot-1* homologue from *Aspergillus nidulans*. Mehran Safaie, and Geoffrey Turner. University of Sheffield, Molec Biol Biotechnol, Sheffield, South Yorkshire, UK.

Mycelial growth in filamentous fungi results from polarised growth and hyphal branching. In addition to interest in the fundamental processes controlling this form of growth, colonial morphology is important in industrial fermentations. In order investigate the genetic basis of hyphal branching and growth polarity in the genetic model *A. nidulans*, we are isolating genes by complementation of mutants affecting branching frequency (see poster by Memmott et al.) and by isolation of homologues of genes from yeast or filamentous fungi. The *cot-1*gene of *Neurospora crassa*, encoding a serine-threonine protein kinase (Yarden et al. 1992, EMBO J. 11, 2159) is one of a limited number of characterised genes known to affect polar growth and branching frequency. Degenerate oligonucleotides designed against *cot-1*, and homologues from *C. trifolii* and *S. cerevisiae* were used to amplify a fragment from *A. nidulans*. Sequence analysis revealed marked identity with *cot-1*. Using the PCR amplified fragment as a probe, 3 related cosmid clones, assigned to chromosome V, were isolated from the chromosome sorted genomic library. Although 2 were adjacent on the physical map (Prade et al. 1997, PNAS 94, 14564) a third was localised elsewhere on V, suggesting mislocalisation of one or more of the cosmids. Sequence analysis of the entire gene revealed high sequence identity at in the C-terminal region, but considerable divergence in the N-terminal region of the derived protein sequence. Attempts are underway to disrupt the gene, and alter the level of expression by promoter exchange.

353. The effects of some phenolic compounds on the growth and morphology of *Neurospora crassa*. Suraia Said[1], Juliano SimõesToledo[1], John Page[2], and Anthony J.F. Griffiths[2]. [1]Universidade de Sao Paulo, Ciências Farmacêuticas, Ribeirão Preto, São Paulo, Brazil. [2]UBC, Botany, Vancouver, BC, Canada.

After carbohydrates, phenolic compounds are the largest group of substances in wood. Phenolics are also present in the waste of many industries. Some natural phenolics like cinnamic acid are known as potent inhibitors of microbial germination. The effects of different phenolic compounds on growth and hyphal morphology of *N. crassa* were analysed, as well as some biochemical aspects of cultures growing in the presence of cinnamic acid. No effects on the size or morphology of the colonies were observed with most of the substances used. However, in the presence of ferulic or cinnamic acid the colonies were small and compact, their hyphae were more branched and there was an increase of precocious aerial hypha. Cinnamic acid (250 mg/L) reduced growth about 93% in the first 24 h of incubation and 56.3% after 144 h. In liquid cultures supplemented with cinnamic acid the fungus could grow after 48 h and at this time the concentration of cinnamic acid was 22% of the initial concentration. A malodorous compound was detectable in the cultures, identified as 1,2,3-trimethylbenzene. Activity of the enzyme aryl-aldehyde:NADP oxidoreductase was detected only in the culture growing in cinnamic acid presence. The results show that this natural phenolic changes the growth rate and the morphology of N. crassa, but after 48 h the fungus is able to induce at least one enzyme to degrade this inhibitor. Suppoted by: Natural Science and Engineering Council of Canada Grant 55695. Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brazil) Grant 201181/95-9. Fundação de Amparo a Pesquisa do Estado de São Paulo (Brazil) Grant 97/03824-0.

354. Isolation and analyses of *Neurospora crassa* genes encoding Rad50 and Rad52 homologues. Yoshiyuki Sakuraba, and Hirokazu Inoue. Saitama University, Faculty of Science, Urawa, Saitama, Japan.

DNA double-strand breaks (DBSs) induced by ionizing radiation or methylmethane sulfonate (MMS) are thought to be repaired by recombinational repair. In *Saccharomyces cerevisiae*, the RAD52 epistasis group genes are classified in this repair pathway. In *N. crassa*, some mutants defective in recombinational repair have been isolated and characterized. To further probe the mechanism of the recombinatinal repair pathway, we isolated the genes that encode Rad50p and Rad52p homologues in *N. crassa* using a PCR method.The DNA sequence of the PCR fragment of the $RAD50^{Nc}$ gene matched with the upstream sequence of the wc-2 gene which is located on the right arm of chromosome I. A *Kpn*I-*Kpn*I fragment which contains the $RAD50^{Nc}$ gene was subcloned from the cosmid including the *wc-2* gene (The cosmid was kindly given from Dr. G. Macino), and the DNA sequence was determined. The $RAD50^{Nc}$ protein deduced from the nucleotide sequence has 29.9% identity to Rad50p (*S. cerevisiae*) . The *uvs-6* mutation has been genetically mapped near the *wc-2* locus. Sensitivities to UV, MMS and histidine of the *uvs-6* mutant were complemented by transformation with the $RAD50^{Nc}$ gene fragment. This indicates that the *uvs-6* gene encodes the Rad50p homologue protein. We also cloned $RAD52^{Nc}$ gene from cDNA library and DNA sequence was determined. The $RAD52^{Nc}$ protein deduced from the nucleotide sequence has 27.6% identity to Rad52p (*S. cerevisiae*). RFLP mapping using a PCR fragment as a probe demonstrated that the $RAD52^{Nc}$ gene locates closed to the *ro-4* locus on the right arm of chromosome V. Both RAD50Nc and RAD52Nc mRNA could not detect in normal growth condition by Northern hybridization, but the transcription of these genes were induced after UV irradiation or MMS treatment.

355. Kinetic study of protein secretion in *Aspergillus niger*. Anne L. Santerre Henriksen[1], Jens Nielsen[1], and David Archer[2]. Technical University of Denmark, Biotechnology, Lyngby, Denmark.[2]Norwich Research Park, Food Research, Norwich, UK, United Kingdom.

Filamentous fungi are able to produce and secrete large amounts of proteins, but so far the mechanisms and kinetics of secretion have not been studied in details. In this study, pulse-chase experiments have been used since they allow quantitative study of protein production and secretion kinetics. Based on the experimental data the secretion of hen egg white lysozyme (HEWL) by *A.niger* has been modeled using first order kinetics.

356. Tagging genes and trapping promoters of the barley pathogen *Pyrenophora teres*. Wilhelm Schäfer, Frank Josef. Maier, and Anke Petra Lösch. University of Hamburg, Inst. of General Botany, Hamburg, Hamburg, Germany.

Pyrenophora teres (anamorph *Drechslera teres*) is the causal agent of barley net blotch, which is one of the most widely distributed foliar diseases of barley throughout the world. It was the most damaging pathogen of winter barley in Germany during the last two growing seasons. The interactions between the host plant and the pathogen are not understood. To make the fungus amenable to the methods of molecular biology, we started by developing a transformation system based on the resistance of transformed cells to the antibiotic hygromycinB. Based on this, transformation by restriction enzyme mediated transformation was established to identify genes by insertional mutagenesis. Vector configuration and restriction enzyme activity greatly influence the integration of the foreign DNA. Using optimized conditions, about 95% of all transformants showed single vector integration. The addition of a heat shock increases the number of transformants 2.5 fold, although the viability of the protoplasts decreases dramatically from 70% to 3%. Of several transformants, DNA flanking the integrated vector was cloned and sequenced. No deletions or rearrangements were detectable as compared to the genomic wild type sequence. To tag genes and simultaneously trap fungal promoters, we constructed a vector for REMI transformation which carries, after linearization, the promoterless reporter genes coding for ß-glucuronidase and luciferase at the ends. Therefore, promoter active genomic sequences can be tagged after vector integration irrespective of their direction of transcription. To test for virulence mutants, a petri dish assay with detached leaf sections was developed. In a second test, mutants with reduced virulence were spray inoculated on intact barley plants in the growth chamber. Tests for ß-glucuronidase and luciferase activity of fungal transformants in vitro and during plant infection were established. Out of 183 REMI transformants, 11 were clearly reduced in virulence, 94 showed promoter activity, with 12 plant inducible promoters.

357. A Neurospora linkage group VI specific sub-library. Thomas J. Schmidhauser. The University of Southwestern Louisiana, Biology, Lafayette, LA, USA.

We have used the chromosome assignments generated by the University of Georgia Neurospora genome project group to assemble a 222 clone sub-library of the Orbach/Sachs Neurospora genomic library representing cosmids with inserts specific to LGVI. The sub-library has been used to extend a chromosome walk from the *crosspathway control-1* locus to the *unknown-4* and *lysine-5* loci. Sib-selection is being used to isolate selectable markers. Results include the

isolation of a *cysteine-2*$^{+}$ cosmid and failure to identify a cosmid complementing the *unknown-23* mutation.

358. EST analysis of the entomopathogenic fungus *Metarhizium anisopliae*. Steven E. Screen, and Ray J. St. Leger. University of Maryland, Entomology, College Park, MD, USA.

The era of genomics has already begun. The technology for rapidly determining the entire catalogue of genes that constitute the repertoire of fungal physiological capacity, is now tried and tested. We aim to use this technology to sequence the genome of the most extensively studied entomopathogenic fungus *M. anisopliae*. The most efficient and cost effective means is first to catalogue the protein coding regions. At present the number of genes isolated from *Metarhizium* is very limited, and no genetic map exists. We are a long way behind comparable researchers studying *Aspergillus*, *Neurospora* and *Magnaporthe*. However, their wealth of sequence information and functional characterization will greatly facilitate the identification of homologous genes in *Metarhizium*. In addition and of more interest, genes which have no homologue in the non-pathogenic or plant-pathogenic fungi may be of great interest in studies on entomopathogenicity. Our pilot-scale EST sequencing project has already identified a number of interesting genes, including transcription factors, structural proteins, transmembrane proteins and intra- and extra-cellular enzymes.

359. *Aspergillus niger sagA*, a SEC4-like gene, is required for protein secretion but not for growth on glucose. B. Seiboth[1], P.J. Punt[2], C. Konetschny[1], A. Ram[2], CAMJJ van den Hondel[2], and CP Kubicek[1]. [1]TU Wien, Inst fur Biochem und Mikr, Wien, Austria. [2]TNO Nutrition and Food Research Institue, Dept Molecular Genetics, Zeist, The Netherlands.

Present knowledge on the secretory pathway is almost completely derived from *S. cerevisiae* and mammalian systems. In general, secretion of a protein involves its transport through a number of compartments (i.e.: ER, Golgi) mediated by small vesicles budding from a donor compartment and fusing to an acceptor compartment. Rab proteins form the largest branch of the Ras superfamily of GTPases. They are localized to the cytoplasmic face of organelles and vesicles and play an essential role in targeting and fusion of transport vesicles with their appropriate acceptor membranes. For a dissection of the Golgi to plasma membrane transport which in yeast is exclusively regulated by Sec4, the putative *A. niger* SEC4 homologue sagA was cloned. The *A. niger* sagA gene shows the typical features of a Rab-like protein, including conserved motifs for guanine- and phosphate/magnesium-binding. Two cysteines, putatively involved in membrane interaction through geranylgeranylation, are found at the C-terminus. The SAGA protein shows 70 %, 64 %, 61 %, and 56 % identity to *Schizosaccharomyces pombe* ypt2, *Candida albicans* Ryl1, human rab8, and *S. cerevisiae* Sec4, respectively These proteins belong to a branch of the rab family involved in Golgi to plasma membrane transport and are able to functionally complement a temperature sensitive sec4 mutant. Sec4 and its functional homologues are essential genes. However, a deletion of the endogenous sagA gene was not lethal in *A. niger*. The mutants showed differencies in colonial and hyphal morphology, combined with a delayed and reduced sporulation, but the same growht rate was observed when grown on glucose as carbon source. The amount of secreted protein of a deletion mutant on glucose was drastically reduced while not different on maltodextrine. We have further introduced several point mutations into sagA at positions leading in other GTPases to a thermosensitive growth

effect. *A. niger* strains were obtained which grew normally at 30°C but which were arrested in growth at 37°C (restrictive temperature). Their effect on growth and protein secretion will be shown.

360. Characterizing the Role of SEPA in *Aspergillus nidulans*. Kathryn E. Sharpless, Claire L. Pearson, and Steven D. Harris. Univ of Connecticut Health Center, Microbiology, Farmington, CT, USA.

Temperature-sensitive mutations in the *sepA* gene in *A. nidulans* cause defects in polarized morphogenesis and septation. SEPA is a member of the formin family of proteins, members of which are involved in actin microfilament organization. A previously characterized *sepA* disruption mutant (*sepA4DBm*) is able to undergo septation after a severe delay and is temperature-sensitive for growth. Another *sepA* disruptant has been constructed in which more of the gene, including the conserved FH1 and FH2 regions, has been deleted. This disruptant mutant, in contrast to *sepA4DBm*, is not temperature-sensitive for growth and fails to septate at any temperature. In addition to its defects in polarity and cytokinesis, *sepA* mutants have disorganized nuclei, suggesting that dynein function is compromised. Dynein, a microtubule motor, is required for proper nuclear distribution and mitotic spindle orientation in *A. nidulans*. We have found that mitotic spindles are misaligned in *sepA* mutants. Furthermore, double mutants containing a ts mutation in *nudA* (cytoplasmic dynein heavy chain) and compromised SEPA function (*nudA2; sepA4DBm*, and *nudA2; alcA::sepA* on glucose) display a synthetic growth defect. These findings suggest that SEPA and dynein share a similar function. To further characterize the role of SEPA in polarized growth and cytokinesis, the localization of a SEPA-GFP (green fluorescent peptide) fusion construct has been determined. SEPA-GFP localizes to the tips of growing hyphae. Additionally, SEPA-GFP localizes to a thin band coincident with septa. Localization of SEPA-GFP to septa may be cell-cycle regulated as the observation of SEPA-GFP at septa is much more rare than SEPA-GFP at the tips.

361. The beta-tubulin-encoding gene of *Microbotryum violaceum*: an unusually large number of introns makes it a useful tool for studies of host/pathogen co-evolution. Tie-Liu Shi, and Michael H. Perlin. University of Louisville, Department of Biology, Louisville, KY, USA.

M. violaceum is a heterothallic basidiomycete that infects over 200 flowering species of Caryophyllaceae. Its diploid teliospores are produced in the anthers of infected plants. Interestingly, isolates of the fungus obtained from one host species appear to be limited to that or to closely-related species. Such restricted host range defines pathovars of *M. violaceum* and molecular markers have been identified by our laboratory that correlate pathovars with their respective host species. In order to further examine such co-evolution, we sought additional genes whose intraspecific phylogenies might support the relationships thusfar identified. We sought to clone the beta-tubulin-encoding gene both for this purpose and to develop improved markers for transformation. Unfortunately, standard approaches used to clone genes for highly-conserved proteins such as beta-tubulin initially proved ineffective in this organism. No positive results were obtained by either hybridization using heterologous probes, or by the use of degenerate PCR primers which worked in other fungi. When the gene was finally cloned, the source of the difficulty was discovered: comparison of the cDNA with the genomic sequence

revealed 14 introns within the overall coding region for the nearly 3 kb gene. This represents the most introns for this gene reported for any organism and is twice the number of introns found in other fungal beta-tubulin-encoding genes. As an initial survey of the usefulness of this gene in intraspecific comparisons for this fungus, the respective genes were sequenced and compared from 10 sporidial strains (including one benomyl-resistant mutant and its progenitor); the strains comprised two distinct pathovars and were obtained originally from several geographic locations. Polymorphisms were observed, most often in introns, and predominantly in the first two-thirds of the gene, with near identity of all the genes examined after the 9th exon. Differences for the most part were correlated with pathovar designations, and, unlike previous studies in our lab with the gene for gamma-tubulin, were not limited to third positions of codons (i.e., conservative changes). The implications of this gene for evolutionary studies of introns and its usefulness in studies of co-evolution of this fungus with its many hosts will be discussed.

362. Deletion of a hydrophobin gene effects the integrity of cell wall of *Schizophyllum commune.* Hans J. Sietsma, Marianne Van Wetten, and Jos Wessels. University of Groningen, Plant Biology, Haren, Groningen, The Netherlands.

Hydrophobins are small proteins capable of forming insoluble aggregates at a hydrophobic / hydrophilic interface. They coat for instance the surface of aerial hypahe with a hydrophobic rodlet layer but submerged hyphae stay hydrophilic because these proteins are then secreted into the medium as insoluble monomeric compounds. By studying the effect of the deletion of the SC# gene coding for a hydrophobin in the basidiomycete *Schizophyllum commune* it was shown that the formation of aerial hyphae was severely hampered. But deletion of the SC3-gene also had a pronounced effect on the distribution of the major polysaccharides composing the hyphal wall. The amount of the water-soluble 1,3-beta-glucan increased dramatically at the cost of the alkali-insoluble glucan content. Also the mechanical strength of the wall seems to be diminished because the deleted strain was more vulnerable for osmotic shock. Replacement of the deleted SC3 gene by another hydrophobin structural gene, but with the regulator sequences of SC3, restored the wild phenotype. However addition of the sc3-protein to the medium had no effect on cell wall integrity. This indicates that sc3 only exercises its effect on cell wall integrity during synthesis and/or secretion. It has been shown before that the solubility of the 1,3-beta-glucan is effected by covalent linkage to the insoluble chitin. These results indicate that hydrophobins might influence this process.

363. Nitrogen recycling during phenylalanine metabolism in mycelium of *Lentinus lepideus.* Santokh Singh[1], Jin Woo Bok[1], Anthony J.F. Griffiths[1], Norman G. Lewis[2], and G. H. Neil Towers[1]. [1]University of British Columbia, Botany, Vancouver, BC, Canada. [2]Washington State Universi, Inst. Biol. Chemistry, Pullman, WA, USA.

Lentinus lepideus, a wood-decaying basidiomycete, displays high phenylalanine ammonia-lyase (PAL) activity and produces large amounts of methylated phenolic acids, especially *p*-methoxymethylcinnamate. The metabolic fate of the PAL-generated ammonium ion in *L. lepideus* was investigated. ^{15}N-L-Phenylalanine was administered to four-day old mycelium of *L. lepideus* in the dark. Analyses of the ^{15}N-labeled metabolites by ^{15}N-nuclear magnetic resonance spectroscopy indicate that this nitrogen is first incorporated into the amide moiety of L-glutamine and then into L-glutamate by the glutamine synthetase (GS)/glutamate synthase

(GOGAT) pathway. ^{15}N is also incorporated into gamma-aminobutyric acid (GABA) and alanine by a direct transamination at the level of phenylalanine. A new nitrogen recycling mechanism during phenylalanine metabolism in *L. lepideus* is proposed.

364. Characterisation of the cAMP-dependent protein kinase regulatory subunit gene from the fungus *Aspergillus niger*. Mojca Staudohar[1], Mojca Bencina[1], Henk Panneman[2], George J.G. Ruijter[2], Jaap Visser[2], and Matic Legisa[1]. [1]National Institute of Chemistry, Biotech. and Ind. Mycol., Ljubljana, Slovenia, Slovenia. [2]Wageningen Agricultural U, MGIM, Wageningen, Netherlands, Netherlands.

cAMP-dependent protein kinase (PKA) plays a crucial role in the regulation of metabolic pathways by means of enzyme phosphorylation. PKA is one of the essential mediators of the cAMP signalling pathway. We have recently isolated the gene (pkaR) encoding the regulatory subunit of cAMP-dependent protein kinase (PKAR) from the industrially important fungus *Aspergillus niger*. The pkaR has an open reading frame of 1233 bp and encodes a polypeptide of 411 amino acids with calculated molecular mass of 44527 Da which corresponds to the Mr determined by SDS-PAGE (Legisa & Bencina, 1994, FEMS Microbiol Lett 118, 327-334). An intron was detected upstream of start codon. Its length is 700 bp. The deduced amino acid sequence of PKAR of *A. niger* shows extensive homology with PKAR isolated from other eukaryotes. The protein contains two putative cAMP binding sites and putative PKA phosphorylation site. The cloned pkaR and cloned pkaR and pkaC (catalytic subunit) (Bencina et al. 1997, Microbiology 143, 1211-1220) were used for transformation of *A. niger*. Transformants overexpressing pkaR and transformants overexpressing pkaR and pkaC are phenotipically similar to wild type strain. An *A. niger* strain with disrupted pkaR gene was isolated as well which was confirmed by Southern and Northern analysis. The pkaR disruptant strain was phenotipically different from wild type with respect to sporulation that was abolished.

366. nuvF109, a DNA checkpoint gene affecting recovery from DNA damage in *Aspergillus nidulans*. Peter Strike, Jean Woods, and Gerard T. Gardner. University of Liverpool, Biological Sciences, Donnan Labs, Liverpool , UK.

The *nuv* mutants of *Aspergillus* comprise a collection of strains sensitive to a broad range of DNA damaging agents. Amongst them, the *nuvF102* mutant is abnormally sensitive to alkylating agents and 4NQO, but appears normal for mitotic and meiotic recombination. Unlike most of the other *nuv* mutants, it is also sensitive to hydroxyurea and this sensitivity can be supressed by mitotic inhibitors such as benomyl. We show that the mutant exhibits abnormal nuclear morphology following exposure to a variety of DNA damaging agents, and that this correlates with a premature resumption of post-damage DNA synthesis. In the mutant cell, both DNA synthesis and mitosis appear to be halted correctly by DNA damage. However, post-damage DNA synthesis is delayed much less than in wild-type, and the cell also enters mitosis early. The observation that the mutants are sensitive to HU suggests that recovery from a block to DNA replication is also aberrant, while the effects of benomyl indicate that the consequences of these aberrations may be overcome if mitosis is delayed. The *nuvF102* gene has been cloned and sequenced, and the gene product displays little homology to proteins of known function in the data bases. There is homology across a small region to the *Sta3* group of signal transduction / transcription activating proteins, and the protein also contains a large motif that is repeated in the

highly inducible yeast protein Ddr48. We show that *nimA* and *nimX* kinase activities appear unaffected in the mutant cells, and we propose a model in which *nuvF* plays a role in the *rad53* - *mec1* pathway to control both mitotic arrest and the resumption of DNA synthesis.

367. *In vivo* and molecular analysis of mitochondrial distribution in *Aspergillus nidulans.*
Rüdiger Suelmann, and Reinhard Fischer. Max-Planck-Institute for Terrestrial Microbiology, Biochemistry, D-35043 Marburg, Hessen, Germany.

Mitochondria provide the energy for growing hypha of aerobic fungi. Therefore, mitochondria have to grow, to divide and to distribute as hyphae elongate. To study their behavior *in vivo*, we have expressed GFP in mitochondria of *Aspergillus nidulans*. We found that these organelles are organized as an intracellular network of interconnected tubular structures. Fluorescence video-microscopy revealed that the pattern of mitochondria changes constantly, small organelles bud off, migrate some distance and fuse at other places. To get insights into the molecular basis of the movement, different strategies were applied. Comparison of the effect of microtubule-destabilizing agents to actin-destabilizing agents suggests that a functional actin cytoskeleton is required for mitochondrial movement and morphology. In the presence of cytochalasin A mitochondria loose their tubular structure. To finally prove actin-dependent mitochondrial movement, we are currently trying to establish an in vitro motility assay using actin filaments and isolated mitochondria. In addition, a mutant screen was performed and 25 strains were isolated with defects in mitochondrial distribution. One mutant will be studied in more detail.

368. Mutational analyses of the *NC-ras* gene in *Neurospora crassa*.
Mayumi Sugisaki[1], Sayoko Ito[2], Akio Toh-e[3], Yasushi Matsui[3], and Hirokazu Inoue[1]. [1]Saitama University, Regulation-Biology, Urawa, Saitama, Japan. [2]Inst. Res. and Innov., Biotechnology, Kashiwa, Chiba, Japan. [3]Tokyo University, Faculty of Science, Tokyo, Japan.

The *NC-ras* gene is one of the *ras* homologs in the filamentous fungus *Neuropora crassa*. In a previous report, we showed the strains overexpressing the *NC-ras* gene could not form aerial-hyphae, and the production of conidia was dramatically reduced. Also, they were female sterile. To further examine the function of the *NC-ras*, we carried out site directed mutagenesis of *NC-ras* gene; Glycine at the 17th amino acid was substituted by Valine (GTP-bound active form) and Lysine at 21st amino acid was substituted by Asparagine (GDP-bound dominant negative form). These genes were introduced into wild-type cells and expressed constitutively under the *lacc*promoter. Both mutations affected the hyphal growth rate, suggesting that the cycling between GTP- and GDP-bound form of NC-RAS is important for propagation. Moreover, the strain carrying the dominant negative form of the *NC-ras* gene was defective in aerial hyphal formation and production of conidia. Also they were female sterile. This phenotype was similar to the strains overexpressing the *NC-ras* gene. These data suggest that the activation of NC-RAS protein is necessary to the differentiation and sexual cycle progression of *N. crassa*.

369. Codon optimisation of a thermophile xylanase gene for the expression in *Trichoderma reesei*.
Valentino S. Te'o, Angela Czifersky, Peter L. Bergquist, and Helena Nevalainen. Macquarie University, Biological Sciences, Sydney, NSW, Australia.

T. reesei is currently developed as a production host for various heterologous proteins of eukaryotic and bacterial origin. The level of heterologous gene expression in the *Trichoderma* system depends on various factors such as strong promoters, gene copy number, site of integration, mRNA stability and translational efficiency. In this work, we address the effect of codon optimization to improve the yield of *Dictyoglomus thermophilum* xylanase B enzyme produced in *Trichoderma*. Codon usage between these organisms vary significantly: the overall AT content of the *xynB* gene is 61% whereas in a typical *T. reesei* cellulase gene it is under 40%. In addition, *D. thermophilum* prefers A or T at the third codon position whereas effectively expressed *T. reesei* genes have a strong bias against these nucleotides at the wobble position. We have reconstructed the 1014bp long *xynB* gene according to the codon preference of *Trichoderma* by PCR using twelve 85-107bp long oligonucleotides containing overlapping regions of 14-24 basepairs. Restriction enzyme sites were incorporated for directional cloning into the *T. reesei* expression vector. Initial primer extension was carried out in two lots each involving six oligonucleotides. The PCR products of these reactions together with outside primers were used to amplify the full length *xynB* gene. Initial clones contained some basepair changes and short deletions that have been corrected. The overall approach and expression of the synthetic *xynB* gene in *T. reesei* will be discussed.

370. Mutations in an abundant protein, associated with the vacuole, alters hyphal tip growth in *Neurospora crassa*. Karen Tenney, June Inez Pounder, and Barry J. Bowman. University of California, Biology, Santa Cruz, California, USA.

A very abundant 19 kDa protein, named *vac-5*, is associated with the vacuole in *Neurospora crassa*. We have characterized the gene which encodes this protein. A database search revealed homologous proteins only in two filamentous fungi, *Magnapothe grisea* and *Aspergillus nidulans*. The function of the protein is not known. Strains with null mutations in the *vac-5* gene were generated by repeat induced point mutations and the 19 kDa protein was not detectable in these mutant strains by Western blot analysis. Growth of these strains was slightly slower than wild type. The most striking phenotype of the mutants was that the hyphal tips lyse when grown on solid media containing sorbose, and form dark balloon-like structures. A fusion of the *vac-5* gene and the gene for a red-shifted variant of green fluorescent protein optimized for *Candida albicans* was transformed into a *vac-5*- strain. Expression of the *vac-5* and the GFP is being examined.

371. Symmetry of branching patterns in *Neurospora crassa*. Michael K. Watters, Aleksandra Virag, Jennifer Haynes, and Anthony J. F. Griffiths. Univ of British Columbia, Botany, Vancouver, BC, Canada.

Growth in Neurospora proceeds via the highly polarized extension of tips and the subdivision of these tips to form branches. While much attention has been devoted to tip growth, the mechanisms responsible for the decision to form a branch are unknown. Specifically, it is unclear if the decision to branch is made independently by each tip, or if branching is controlled centrally and triggered under the control of proximal regions of the colony. We address this question by comparing the lengths of branch intervals grown in parallel from a common origin. That is, the two intervals emerging from a common branch event. We find significant correlations between parallel intervals under nearly all circumstances tested. In contrast, we find no significant

correlation between branch intervals in tandem, where one follows the other along the hypha. We interpret this correlation to mean that the decision to form a branch at the tip is not independently controlled by the tip. It is instead determined, at least in part, by factors transmitted to the tip from proximal regions of the colony. The strength of this correlation appears to depend on the qualitative nature of the branches surveyed. Dichotomous/apical branching shows a high degree of correlation while lateral branching shows a weak but statistically significant correlation. This difference cannot be explained either by differences between the growth rates or distributions of lengths of branch intervals. Thus, the qualitative morphological difference between these two branch types appears to be responsible for the difference in symmetry.

372. Fundamental aspects of hyphal tip growth and branching in *Neurospora crassa*, revealed by response to temperature. Michael K. Watters, Christine Humphries, Ingrid deVries, and Anthony J. F. Griffiths. Univ. of British Columbia, Botany, Vancouver, BC, Canada.

Neurospora crassa increases its mass as a web of extending and branching hyphae. The mechanisms responsible for controlling hyphal extension and branching are still poorly understood. We have investigated these processes using temperature as a general probe. Tip growth is highly responsive to temperature, increasing linearly with temperature from 4° to 37°C. The distribution of branch intervals has been measured at different temperatures. However, over the same ten-fold range of temperature the branching pattern shows virtually no response. This is surprising since most models intrinsically link branching to tip growth. The responses of tip growth and branching to temperature shifts are highly predictable. If established hyphae are switched from growth at 25° to growth at 4°, a programmed series of changes occurs in their growth and branching patterns (we have dubbed these changes the "Cold Shock" response). There are three stages to this response. First there is an initial "Lag" phase of growth without branching. After this, the growing tips display a sudden burst of tightly spaced, dichotomous branches dubbed "Starbursting". The growing hyphae then enter a "Recovery" phase of the response, returning to normal branching frequencies and lateral branches. The Cold Shock response is unique in the strong, yet transient nature of its effect on branching frequency and character.

373. Rho proteins and growth control in the filamentous fungus *Ashbya gossypii*. Jurgen Wendland, and Philipp Knechtle. Universität Basel, Mikrobiologie, Basel, Switzerland.

We want to study regulatory components which control the polarised apical growth in filamentous fungi. Based on the applicability of powerful molecular genetic tools, e.g. PCR-based gene targeting, *A. gossypii* was chosen as a model organism. Polarized cell growth is viewed as a complex process in which proteins that are responsible for the dynamic organization of the actin cytoskeleton play an important role. The actin cytoskeleton is required to direct secretory vesicles containing eg. cell wall compounds and plasma membrane to sites of growth. In *S. cerevisiae* several Rho proteins govern the yeast cell through the distinct processes of bud-site selection (BUD1/RSR1), bud formation (CDC42), and bud growth (RHO1-4). Therefore, the isolation and characterization of RHO-genes in *A. gossypii* has promised to yield important insights into polarized hyphal growth.To this end we have isolated and characterized several RHO genes. Deletions of these genes and their knock-out phenotypes range from essential to

non-essential for cell viability. In a second step we have begun to characterize genes for *A. gossypii* proteins whose homologues in other organisms are known to interact with Rho proteins. These proteins fall into three classes. First, the Guanine nucleotide Exchange Factors (GEFs) like CDC24p which in yeast acts on CDC42p, second, the GTPase Activating Proteins (GAPs) like BEM2p and BEM3p which in *S. cerevisiae* act on RHO1p and CDC42p, respectively, and third effectors of activated Rho proteins like the *S. cerevisiae* BOI1p/BOI2p. Assembly of this and other data will provide first steps toward understanding the informational network exerting growth control in the filamentous fungus *A. gossypii*.

374. Analysis of septins in *Aspergillus nidulans*. Patrick J. Westfall. University of Georgia, Botany, Athens, GA, USA.

The need of a dividing cell to partition its contents evenly into two daughter cells is faced by all organisms. It is essentially a two-part process; first a cell must decide where it is going to divide; then it has to distribute the cytoplasmic and nuclear contents appropriately between the two new cells. A family of proteins, called septins, is thought to be essential to the cell division process by marking the cleavage plane, and by serving as a scaffold for the attachment of other proteins that act in cytokinesis. Septins were first described as a series of 10nm rings formed at the base of the daughter cell in the yeast *Saccharomyces cerevisiae*. Since their initial discovery, homologues to septins have been found in other species including fruit flies, mice and humans. They seem to be important developmental proteins, yet very little work has been done with them in filamentous fungi. Three septin homologues have been found in *Aspergillus nidulans* using degenerate PCR. The current project focuses on characterization of these septins in *A. nidulans* using both localization studies and the generation of null alleles. To identify its cellular location, antibodies are being raised to aspB (*Aspergillus septin* B) and fusions with green fluorescent protein are being built. Null alleles of aspB have already been reported, and result in a lethal phenotype. Construction of a null allele by homologous recombination of a marker gene at the aspA locus is currently in progress. In addition, fusion proteins of aspA will be constructed for antibody production, purification, and localization of the gene product.

375. The arg-13 gene of *Neurospora crassa* encodes a putative arginine carrier. Sean William Wilmot and R. L. Weiss . UCLA, Chemistry & Biochemistry, Los Angeles, CA, USA.

In the arginine biosynthetic pathway of *Neurospora crassa*, all of the genes have been assigned a function except for the *arg-13* gene product. Based on sequence homology, ARG13, has the characteristic features of a mitochondrial transport protein. Growth studies indicate that *arg-13* mutants are blocked before ornithine synthesis. It was suggested that ARG13 could be a mitochondrial ornithine or citruilline carrier. However, experiments in which the *arg-13* mutation was put in a background containing cytosolic ornithine transcarbamylase (a mitochondrial enzyme involved in ornithine biosynthesis) did not restore arginine prototrophy. When the *arg-13* mutation was put in a background containing feedback resistant acetylglutamate synthase (a mitochondrial enzyme catalyzing the first committed step of arginine biosynthesis) prototrophy was restored. Transport assays showed that overexpressed ARG13 specifically transports arginine. Therefore, ARG13, may function to transport arginine from the cytosol to the mitochondria in order to facilitate feedback inhibition.

376. Identification of determinants for specificity at the het-c locus in *N. crassa* Jennifer Wu, and Louise N. Glass. University of British Columbia, Biotechnology Laboratory, Vancouver, BC, Canada

In *Neurospora crassa*, at least eleven *het* loci including the mating type locus have been identified controlling heterokaryon formation. One of the most genetically and molecularly characterized locus, *het-c*, was shown to encode three alleles *het-c*^{OR}, *het-c*^{PA} and *het-c*^{GR}. The three *het-c* alleles encode similar polypeptides that contain a hydrophobic sequence, a coiled-coiled lucine rich domain and glycine-rich carboxyl terminal. A 34 - 48 amino acid polymorphic region, which is dissimilar in HET-c^{OR}, HET-c^{PA} and HET-c^{GR} by both amino acid differences and the pattern of insertion (or deletion), was found controlling allelic specificity. To discriminate that either the amino acid sequences of the *het-c* specificity domain are critical for determining specificity or the spatial organization as affected by pattern of insertion/deletion is the most important factor, we have constructed chimeras between specificity domain from 29 different isolates that display amino acid and insertion/deletion variants and a number of artificial het-c specificity domains that contain both amino acid combinations and insertion/deletion motifs. The chimeric alleles were introduced into Neurospora crassa *het-c*^{OR}, *het-c*^{PA} and *het-c*^{GR} strains and the phenotype of transformants were monitored by growth inhibition and cell death. Our results suggest that amino acid sequences within the region may modulate incompatibility, but the spatial characteristics within the specificity domain contribute substantially to the vegetative incompatibility phenotype.

377. Genetic analysis of full and partial revertants of two osmotic-sensitive, fungicide-resistant strains of *Neurospora crassa*. Mikako Yamauchi[1], Regan M. Challinor[1], Benjamin W. Conner[2], C. Brian Odom[2], Wayne A. Krissinger[1], and Sara N. Bennett[1]. [1]Georgia Southern University, Biology, Statesboro, GA, USA [2]Wingate University, Biology, Wingate, NC, USA.

The Vinclozolin-resistant mutant of *N. crassa*, KT-27, isolated in the Georgia Southern Neurospora Genetics Laboratory, Statesboro, GA, following UV irradiation, was found to be osmotic-sensitive and allelic to *os-1*, consistent with previously reported work of Grindle (1984. Trans. Br. Mycol. Soc. 82:635). UV irradiation and selection on complete medium containing 6% NaCl and sorbose were used to obtain revertants of KT-27 and of os-1 (M-16). A trp-1 marker was included in the strains to assure that putative revertant progeny were not wild type contaminants. From KT-27 and from *os-1*, both complete revertants (no longer osmotic sensitive or resistant to Vinclozolin) and partial revertants (no longer osmotic sensitive but still resistant to Vinclozolin) were obtained. These results indicate that osmotic sensitivity and Vinclozolin resistance might be under the control of different portions of the product of the *os-1* locus. Schumacher, *et al.* have reported that the *os-1* locus encodes a putative histidine kinase [1997. Curr Microbiol 34(6);340-347]. PCR primers generated from their *os-1* sequence have been used to further analyze these revertant alleles.

378. Recombination in the histidine-3 region of Neurospora. Patricia Jane Yeadon, and David E. Catcheside. Flinders University, Biological Sciences, Adelaide, South Australia, Australia.

Recombination stimulated by the polymorphic recombination hotspot cog is regulated by the unlinked gene *rec-2*. *cog* is flanked by *his-3* and *lpl* coding sequences. DNA sequence in the

region flanking *cog* is highly variable in Lindegren 25a and St Lawrence 74A, the strains carrying cog^{La} and cog^{Ea}, diverging 3.4% between *his-3* and *cog*. Sequences responsible for the difference between phenotypes of $cog^{La}$and cog^{Ea} have been located with respect to sequence variations about 3.5kb distal of *his-3*. The 10bp sequence is close to the 5' end of *lpl* and includes two sequence variations, either or both of which may result in the increase in recombination in crosses including cog^{La}. These sequence variations alone appear to be responsible for both the increase in conversion within *his-3* and crossing over between *his-3* and *ad-3*. The sequence heterology has also been used to investigate the molecular outcome of recombination events that generated histidine prototrophs from a cross between two different *his-3* mutants. Most prototrophs appear to result from conversion. Conversion appears to be initiated at cog unless $rec\text{-}2^{+}$ is present, in which case initiation is from the 5' end of *his-3*. Conversion tracts are up to 6 kb and 40% are interrupted. 30% of conversion events have an apparently associated crossover, a similar result to that obtained at yeast hotspots. Analysis of conversion tracts in histidine prototrophs from crosses including the translocation mutant TM429 suggests that conversion can occur on either side of the translocation breakpoint.

379. Suppressor analysis of a *Neurospora crassa* apolar growth mutant, *mcb*. Yi Zhou, In Hyung Lee, Michael Plamann. University of Missouri-Kansas City, Biological Sciences , Kansas City, MO, USA.

The conditional *Neurospora crassa* mutant *mcb* shows a loss of growth polarity at restrictive temperature and has been shown to be defective in the regulatory subunit of cAMP-dependent protein kinase (PKA). To understand how the PKA pathway regulates hyphal growth polarity, we screened for suppressors of *mcb*. From 17 independent screens, 13 suppressors were identified that result in a "*cr-1*-like" phenotype which is characterized by hyperconidiation and reduced radial growth rate of colonies. Addition of cAMP did not rescued growth of any of the 13 *cr-1*-like suppressor mutants, but transformation of the gene encoding the catalytic subunits of PKA from *Magnaporthe grisea* into two of *cr-1*-like mutants resulted in complementation. These results suggest that the *cr-1*-like mutants are defective in the catalytic subunit of PKA. An additional suppressor was identified that partially restores growth polarity to mcb, and this mutant was designated *som1* (suppressor of *mcb*). Cloning and sequencing of the som1 gene revealed that it encodes putative protein containing a glutamine-rich region in the C-terminus and with a short region having high sequence identity (27/29 a.a.) with a corresponding segment of a hypothetical protein of *Schizosaccharomyces pombe*.

Unclassified abstracts

Abstract numbers > 380

Unclassified abstracts

380. Osmotin, an antifungal plant defense protein, induces apoptosis-like cell death in yeast.Barbara Damsz, Meena L. Narasimhan, Jose I. Ibeas, Paul M. Hasegawa and Ray A.

Bressan. Center for Plant Environmental Stress Physiology . Purdue University. West Lafayette, IN.

Osmotin is a tobacco pathogenesis-related, protein (PR-5) that is antifungal. It is implicated in plant defense against invading fungi. Cell death induced in the unicellular Ascomycete, *Saccharomyces cerevisiae*, by osmotin exhibits several features of apoptosis. First, cells can be rescued after brief osmotin treatments but not after prolonged treatments. Cytosol shrinkage, an early marker of apoptosis, was observed in cells after brief treatments, with greater than 50% of the cells exhibiting cytosol shrinkage after prolonged treatment when most cells had lost viability. Plasma membrane blebbing, unusual mitochondrial morphology (enlarged, with swollen inner membrane) cytoplasmic blebbing and apoptotic bodies were observed in the osmotin-treated cells that were losing viability. Nuclear fragmentation in 14-25% of the cells was observed in this population by DAPI staining. Chromatin fragmentation was also detected using terminal nucleotidyl transferase. Osmotin-induced cell death was clearly different from necrosis that was induced by poly-L-lysine in low ionic strength medium.

381. G proteins mediate the fungal cytotoxicity of osmotin, a plant defense protein. Maria A. Coca, Meena L. Narasimhan, Dae-Jin Yun, Paul M. Hasegawa and Ray A. Bressan. Center for Plant Environmental Stress Physiology . Purdue University. West Lafayette, IN.

Osmotin is a pathogenesis-related protein[1], that has in vitro and in vivo antifungal activity against a broad range of fungi, including several plant pathogens[2]. Tobacco osmotin induces rapid cell death in some strains of *Saccharomyces cerevisiae*[3]. We have shown that the cytotoxic effect of osmotin in yeast cells is mediated by a heterotrimeric G protein and a MAP kinase based signaling pathway[4]. We report here that G proteins are also required for the cytotoxic action of osmotin in *Aspergillus nidulans*. Osmotin induces spore lysis, inhibits spore germination and inhibits hyphal tip elongation of *A. nidulans*. Deletion of the *flbA* gene, which encodes an *A. nidulans* RGS (for regulator of G protein signaling) protein[5], results in osmotin resistance. Also, a dominant interfering mutation in the *fadA* gene, that encodes the subunit of a heterotrimeric G protein, results in osmotin resistance. Moreover, the addition of the guanidine nucleotide GDPS, that locks G-proteins in a GDP-bound inactive form inhibits the cytotoxic effect of osmotin on *A. nidulans* spores. The G proteins regulate osmotin sensitivity by altering the porosity of the cell wall. These results suggest that the mode of osmotin action elucidated in *S. cerevisiae* can be applied to filamentous fungi.1. Yun DJ, Bressan RA, Hasegawa PM. (1997). Plant antifungal proteins. In Plant Breeding Reviews, J. Janick, ed. (New York:John Wiley & Sons, INc.), 14, 39-88.2. Abad LR, et al. . (1996). Plant Sci. 118, 11-23.3. Yun DJ, et al. (1997). Proc. Natl. Acad. Sci. USA 94, 7082-7087.4. Yun DJ, et al. (1998). Molecular Cell 1, 807-817.5. Lee BN and Adams TH. (1994). Mol. Microbiol., 14, 323-334.6. Yu JH, Wieser J and Adams TH. (1996). EMBO J. (15),19, 5184-5190.

382. A genetic analysis of *H. capsulatum* pathogenesis. Anita Sil. Department of Microbiology and Immunology, University of California San Francisco, San Francisco, CA.

Our goal is to identify fungal genes that are required for the pathogenesis of *Histoplasma capsulatum*. This organism grows in hyphal or conidial forms in the soil; hyphal fragments or microconidia are inhaled by the host. At 37 C, the cells undergo a morphogenetic switch and

grow as a budding yeast form which parasitizes macrophages, multiplying within their phagolysosomes. How *H. capsulatum* is able to escape killing by macrophages and colonize the phagolysosome, an intracellular niche that is normally hostile to microbes, is a mystery. The recent development of molecular genetic tools now makes it possible to use genetics and molecular biology to dissect the mechanism of interaction between *H. capsulatum* and the host cell.We are using molecular genetic methodology to identify *H. capsulatum* genes that are necessary for the organism to parasitize macrophages. We are employing a previously developed cell culture assay in which *H. capsulatum* cells are co-cultured with a monolayer of mouse macrophages (Eissenberg et al., *Infect Immun*, 1991. **59**(5)). *H. capsulatum* is quickly internalized by the macrophages and lyses the macrophage monolayer after 3-5 days. EMS mutagenesis will be used to generate a bank of mutant yeasts; we will then use the monolayer lysis assay to identify mutant strains that fail to lyse macrophages. Microscopic assays will be used to sort the mutants into the following classes: those that affect adherence of the fungus to macrophages, entry into macrophages, evasion of killing by macrophages, replication within macrophages, and lysis of macrophages. Genes that are defective in the mutant strains will be cloned by complementation of the lysis phenotype in the monolayer assay. The gene products so identified will be analyzed using sequence comparison, expression pattern, and sub-cellular localization. We believe that molecular genetics, which has been key to guiding our understanding of biology in other organisms, will shed light on the pathogenesis of this fascinating fungus

383. The hydrophobin cryparin strengthens cell walls of the ascomycete *Cryphonectria parasitica*. P. Kazmierczak, D.H. Kim, P.M. McCabe, and N.K. Van Alfen. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132.

During stationary growth of the ascomycete *Cryphonectria parasitica* in liquid culture up to 1% of the dry weight of the fungus is the Class II hydrophobin cryparin. The amino acid sequence of cryparin is remarkably similar to that of the hydrophobin produced by *Ophiostoma ulmi* (53% of the amino acids are conserved) . To better understand why this type of hydrophobin would be so highly conserved in these fungi, the gene encoding cryparin was deleted and the consequent phenotype characterized. As observed for most hydrophobins, the lack of cryparin resulted in a wettable aerial hyphae phenotype. Since aerial hyphae play no known role in the biology of this fungus, other phenotypic changes were also sought. Antibody detection methods have demonstrated that when the fungus grows on its natural substrate, chestnut trees, the hydrophobin is found only in fruiting body walls. We found no evidence that cryparin plays a role in virulence of the fungus, sexual sporulation, or asexual sporulation in culture. We have found that the hyphal cell walls are reduced in strength without cryparin and that the strength can be restored by exogenous addition of cryparin. We postulate that cryparin plays an important role in the eruption of pycnidia in stroma of infected trees.

384. Perturbation of cryparin secretion by the Cryphonectria hypovirus. P. M. McCabe and N. K. Van Alfen. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas. 77843-2132

Strains of the chestnut blight fungus, *Cryphonectria parasitica,* infected with a dsRNA virus, CHV1, show a reduction in pigmentation, conidial formation, sexual reproduction and virulence.

Virus infection also results in the accumulation of small membranous vesicles which are many fold more abundant in the infected strains than the uninfected ones. Viral replication in vitro is correlated with this vesicle fraction and the putative CHV1 polymerase was present in this fraction. A number of host genes have been shown to be differentially regulated as a result of viral infection. We have characterised three secreted proteins that are down-regulated by the virus: a laccase, a hydrophobin and a mating type specific pheromone. All contain signal peptides followed by a pro region with recognition sequences for cleavage by a protease similar to Kex2p.One of these proteins, the hydrophobin cryparin, is present as a cargo protein in the vesicle fraction on which CHV1 replicates. Using pulse-chase methods to study the secretion of cryparin we have found that the virus is utilising a host secretory system for replication, causing an accumulation of vesicles and the cargo protein cryparin, with a resultant decrease in the rate transport of the cargo protein.

385. Mixing Signals: Schizophyllum pheromones and receptors initiate pheromone response in Saccharomyces, providing a means to study receptor/ligand interaction.
Thomas J. Fowler, Susan M. Desimone, Michael F. Mitton, Janet Kurjan and Carlene A. Raper, Dept of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT USA

The homobasidiomycete *Schizophyllum commune* encodes about eighteen receptors and possibly more than fifty pheromones among the eighteen variants of its Ba and Bb mating-type loci. These seven-transmembrane-domain receptors and compatible lipopeptide pheromone ligands trigger multiple downstream events during mating. We asked whether Schizophyllum receptors and pheromones could substitute for their yeast counterparts to initiate the Saccharomyces cerevisiae pheromone response pathway, and if so, was the receptor/ligand specificity of Schizophyllum maintained in the heterologous yeast system. Genes encoding these Schizophyllum molecules were separately introduced via plasmids into different strains of yeast. Their expression and the ability of their products to interact was assayed through several pheromone-inducible events in yeast: transcriptional induction of a pheromone-responsive gene, cell-cycle arrest, mating projection display, and mating. Four molecules - Schizophyllum receptors Bbr1 and Bbr2, and pheromones Bbp1(1) and Bbp2(4) - were successfully expressed in yeast. Of the four possible combinations of these pheromones and receptors, only the two naturally compatible Schizophyllum pairs initiated downstream events of yeast pheromone response. Further investigations showed that the compatible pair Bbp2(4)/Bbr1 signals through a pathway similar to that activated by yeast pheromones and receptors. Interestingly, Schizophyllum pheromone Bbp2(4) processing and secretion does not require many of the proteins used in biogenesis of the yeast lipopeptide a-factor, although the Schizophyllum pheromone does appear to require farnesylation. We are now in a position to exploit the natural variation of pheromones and receptors that exists in Schizophyllum in order to understand the recognition process of these molecules. We can also take advantage of the excellent genetic and biochemical tools available for yeast to introduce changes in both the receptors and the pheromone ligands in order to better understand how they work.

386. Molecular analysis of the cellular reaction of *Fusarium sambucinum* to defence compounds of potato.
K. Loser, J. Wessels, P. Becker and K.-M. Weltring. Westf. Wilhelms-Universität, Inst. für Botanik, 48149 Münster, Germany.

Fusarium sambucinum (teleomorph *Gibberella pulicaris*) is a causal agent of potato dry rot. The fungus enters the tubers via wounds where it has to cope with phytoanticipins like the saponins -chaconine and -solanine, and phytoalexins such as the sesquiterpenes rishitin and lubimin. *F. sambucinum* is able to detoxify all of these plant defence compounds. To learn more about the cellular reaction of the fungus to rishitin, apart from detoxification, we set out to identify genes specifically expressed in response to rishitin exposure. By differential cDNA screening we isolated several rishitin induced (rin) genes which showed increased expression in response to the phytoalexin. Sequence comparison of the isolated genes with known genes revealed homology, among others, to a polyubiquitin and a HMG14/17 gene as well as to multi-facilitator-superfamiliy (MFS) proteins of the multi-drug-resistance type found in yeast. Based on the deduced function of the identified genes a new model of the cellular reaction of *F. sambucinum* to rishitin is proposed. The model is supplemeted by our investigations of the metabolism of the phytoanticipins -chaconine and -solanine. We have purified and characterized the enzyme -chaconinase, which on one hand is inducible by other saponins like -solanine and -tomatine, but on the other hand is highly specific for the removal of the 1,2-bound rhamnose of -chaconine. We are currently in the process of isolating the corresponding gene to study its regulation and to perform knock-out experiments to evaulate the importance of saponin metabolism for pathogenicity of *F. sambucinum* on potatoes.

387. Translation Elongation Factor Three: a unique target for anti-fungal drugs. Andrew Hopkins, Dina Shayevich, Jennifer Luebke-Wheeler and Elizabeth Rute. Alverno College, Milwaukee, Wisconsin.

Translation Elongation Factor Three (EF-3) was originally identified in *Saccharomyces cerevisiae.* It has been shown to be absolutely required for translation in *S. cerevisiae* and *Candida albicans* in addition to the two translation elongation factors required for protein synthesis in other eukaryotes: EF-1 & EF-2. The requirement for this factor appears to be uniquely limited to the fungal kingdom. We have identified homologues of EF-3 in two pathogenic fungi which infect immuno-compromised patients: *Aspergillus fumigatus* and *Histoplasma capsulatum*. We have sequenced approximately 80% of the *A. fumigatus gene*. It is most similar to the previously sequenced *Candida albicans* gene. The unique requirement for EF-3 presents a very attractive target for anti-fungal drugs. Determination of the structure and function of EF-3 will facilitate the design of drugs targeted at this essential factor for protein synthesis in fungi. We recently identified a homologue of EF-3 in the thermophilic fungus *Thermomyces lanuginosus*. We are in the process of sequencing the gene for the EF-3 protein in this fungus, with a view to obtaining adequate quantities for crystallization and 3-dimensional structure determination.

388. Maintenance of Iron Homeostasis in *Ustilago maydis*. Sally A. Leong,[1,2] W. M. Yuan,[2] and L. Kearney[2.] [1]USDA, ARS Plant Disease Resistance Research Unit, University of Wisconsin, Madison, WI USA. [2] Department of Plant Pathology, University of Wisconsin, Madison, WI 53706

Iron present in loosely bound or unchelated forms within cells can act catalytically to produce DNA damaging and lipid peroxidizing oxygen radicals. Living cells regulate the transport and storage of iron to minimize free radical damage. When this regulation is disrupted, the

consequences on cellular growth and survival are dramatic. Mutations in *fur*, a gene regulating high affinity iron uptake in *Escherichia coli*, lead to 8-fold elevated levels of intracellular iron (Keyer and Imlay, 1996) and increased rates of cell death and mutagenesis during aerobic growth (Keyer and Imlay, 1996; Touati *et al.*, 1995). Likewise, an *AFT1*^{up} mutation in yeast leads to deregulated iron uptake and reduced cell growth in iron-replete medium (Yamaguchi-Iwai *et al.*, 1995). In both systems, the effects of iron overload are aggravated by mutations in DNA repair systems demonstrating that DNA damage is prevalent under these conditions (Touati et al., 1995; Philpott *et al.*, 1996). In humans, hemachromatosis leds to iron loading in the liver, heart and pancreas, resulting in cirrhosis of the liver, pancreatic fibrosis and cardiac dysfunction, as well as increased risk of hepatocellular carcinoma (Crawford et al., 1996). Moreover, an iron-unresponsive mutant of the IRP protein, which normally controls iron uptake and storage at the cellular level, results in diminished cell survival (DeRusso et al., 1995). Despite the wealth of information on the negative impact of iron overload in cells, surprising little is known about the molecular basis of iron-mediated, transcriptional control of iron uptake in eucaryotes where transcription occurs on a chromatin template. In response to iron starvation, the basidiomycete fungus *Ustilago maydis* produces two cyclic hydroxamate siderophores ferrichrome and ferrichrome A. Three genes required for siderophore biosynthesis and regulation have been characterized: *sid1* encodes ornithine-N^5-oxygenase, the first enzyme in the ferrichrome biosynthetic pathway; *sid2* encodes a putative peptide synthetase required for ferrichrome biosynthesis; and *urbs1* encodes a transcription repressor that interacts via its C-terminal finger domain with GATA sequences in the *sid1* (Leong and Winkelmann, 1998). Our working hypothesis is that iron modulates siderophore gene expression at one or more levels. Iron may act as a corepressor of genes regulated by Urbs1 and as a physiological effector that indirectly affects expression of siderophore genes by altering the structure and/or cellular location of Urbs1. As with Fur, the procaryotic analog of Urbs1, iron may directly activate Urbs1 in DNA binding. Urbs1 may repress expression of *sid1* through positioning of nucleosomes and/or by formation of a DNA loop in the *sid1* upstream region. Consistent with this hypothesis, discrete and iron-dependent, nuclease hypersensitivity sites have been identified in the *sid1/sid2* intervening promoter region. To investigate the role of additional genes in iron-mediated regulation of iron homeostasis, we are developing an iron-dependent screen for auxotrophy. Progress on the development of this screening method will be presented.

389. Overexpression of the penicillin biosynthetic genes in *Penicillium chrysogenum*. M.A. van den Berg, D.J. Hillenga and R.A.L. Bovenberg. Gist-brocades, IPPD/PDD. Delft, The Netherlands.

To analyse the effect of overproduction of the penicillin-biosynthetic genes in the fungus *Penicillium chrysogenum*, two strategies were applied. The strain Wisconsin 54-1255 strain was transformed with DNA-fragments containing the three penicillin biosynthetic genes. Transformations were done using four combinations: *pcbAB, pcbAB+pcbC-penDE, pcbAB-pcbC-penDE* en *pcbC-penDE*. The *amdS* selection was applied. Several strains with a substantially improved penicillin production were obtained. With the second approach we want to modulate the amount of delta-(L-alpha-aminoadipyl)-L-cysteinyl-D-valine synthetase (ACVS), the first enzyme in the penicillin pathway. To obtain differential expression levels of the gene, the autonomous promoter of *pcbAB*, the gene encoding ACVS, should be replaced by several other promoters. As a host we used a *P. chrysogenum* strain in which the complete

pcbAB promoter and a small part of the ORF (5' end) were removed. As selection marker the *Tn5ble* gene, encoding phleomycin resistance, and the *cytC* terminator were cloned in the same orientation as the *pcbAB* gene; behind the *pcbAB* promoter and just in front of the ORF. Two unique restriction sites just in front of the ATG were introduced for easy cloning of heterologous promoters. So far we obtained *Penicillium* transformants with *P. chrysogenum pgkA* and *niaD* promoters replacing the *pcbAB* promoter, which are currently investigated.

390. Characterization of the Penicillium *paf* gene promoter for gene expression in *Penicillum chrysogenum*. R. Kerkman, A.W.H. Vollebregt, R.A.L. Bovenberg. Gist-brocades, IPPD/PDD, Delft, The Netherlands.

In contrast to *Aspergillus*, only few *Penicillium* promoters have been characterised so far. In order to isolate and analyse strong promoters for gene expression in *Penicillium* we examined the relative strength of the promoters of the *Penicillium pcbC* and *paf* genes in *P. chrysogenum*. The promotor of *pcbC*, is one of most extensively investigated promoters and is considered to be a strong promotor. The *paf* gene, which encodes a 12-kDa protein with potential antifungal activity, is one of the most abundant proteins present in the culture medium (Vollebregt, ECFG2 abstract B48). To analyse the *paf* gene promotor in more detail integration vectors were constructed containing various parts/fragments of the promotor region fused to the *Streptomyces clavuligerus cefE* gene encoding expandase which can be used as a reporter gene. In the presence of suitable side chains e.g. adipate *Penicillium* transformants expressing expandase are able to convert penicillins to cephalosporins, which can be measured by using bio-assays and shake flask fermentations.Transformants were obtained by co-transformation using the *Aspergillus amdS* gene as a selectable marker. Selected transformants were tested for promoter activity by performing shake flask experiments and measuring formation of adipoyl- 7-ADCA. The results which will be presented indicate that the *paf* gene promotor is a strong/suitable promotor for heterologous gene expression in *P. chrysogenum*.

391. The *Aspergillus nidulans* spermidine synthase gene, spdA, is required for fungal differentiation.Doralinda Guzman-de-Peña[1], Yuan Jin[2] and Nancy Keller[2]. [1]Laboratory of Mycotoxins. Unidad Irapuato CINVESTAV-IPN México, [2]Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132.

The *Aspergillus nidulans spdA* gene, encoding for a putative spermidine synthase, was cloned, sequenced, and localized on chromosome VIII. The deduced amino acid sequence has a high similarity with *Saccharomyces cerevisiae* spermidine synthase encoded by the *spE3* gene. In both yeast and the filamentous fungus *Neurospora crassa*, spermidine and other polyamines are required for fungal differentiation processes. To investigate the function of *SpdA* on Aspergillus development, we constructed a *spdA* null mutant by replacing a portion of the coding region with the *argB* gene. The DspdA strain, which accumulates the biosynthetic precursor of spermidine,putrescine, has an absolute requirement of spermidine for growth, sporulation and sterigmatocystin production. Wildtype growth and development could not be recovered in the DspdA strain by a high concentration (3mM) of either putrescine or spermine but was partiallyrecovered by a high concentration (3mM) of spermidine.

392. Apoptosis of meiotic mutants of *Coprinus cinereus.*Benjamin C. Lu and Ursula Kuees, Department of Molecular Biology and Genetics, University of Guelph, Guelph, Canada, and Institute of Mikrobiologie, ETH Zurich, Switzerland.

Meiotic mutants of *Coprinus cinereus* were induced by the Restriction Enzyme Mediated transformation of the AmutBmut strain. These Remi mutants produced white caps with few basidiospore tetrads. This poster presents studies of meiotic proccesses leading to the formation of white caps as a consequence of apoptosis. Cytologically, the white-capped strains can be divided into two major groups: one that assembles perfect synaptonemal complelxes (SC) during meiotic prophase-I, and one that fails to do so. Both groups grogressed through meiotic prophase-I and entered meiotic metaphase-I. Those that assembled the SCs showed chromosome condensation and congregation, and those that did not assemble the SCs showed scatteredcondensed chromosomes. After a brief arrest at this stage, the basidia entered the path of apoptosis. Apoptosis is restricted to basidia which underwent DNA degradation and cellular disintegration, while the rest of fruiting cap continued to develop, showing cap expansion, stipe elongation, and the final stage of autolysis normally found in fruiting bodies of this species.

393. A new database supporting the collection at the Fungal Genetics Stock Center. Kevin McCluskey and Chris D. Kunce. FGSC, Department of Microbiology, University of Kansas Medical Center.

The FGSC has had a variety of databases throughout its life. The original database was the collection of deposit sheets that accompanied each strain as it was added to the collection. Key information was added to each sheet and was extracted by hand as the catalog was prepared. As the number of strains grew, this became overwhelming and when the FGSC moved to the University of Kansas, an electronic database was developed. The catalog was prepared electronically as well. With the advent of widespread use of the internet, the tables in this database were made available through a text-search capacity. While this is very useful, there is a great deal of information that is not readily accessible either to the FGSC staff or to clients. With the assistance of the Univ of Kansas Info-Tech staff, we have developed a relational database that allows more information to be presented to the staff and ultimately to users via the FGSC web-site. The on-line plasmid and bibliography searches are examples the features that are possible. The data model and future developments will be presented.

394. Virulence of *Puccinia recondita* f. sp. *tritici* on Lr26-resistance wheat: distribution and genetic originPardes E., Manistersky J., Ben Yehuda P., Kosman I., Anikster Y., and Sharon A. Institute of Cereal Crop Improvement, Tel Aviv University, Tel Aviv, Israel.

Leaf rust of wheat caused by *Puccinia recondita* f. sp. *tritici* is among the most widespread diseases of wheat. Fast distribution of the pathogen over long distances and development of epidemics, cause sever crop losses in wheat around the world. The sexual stage of the fungus takes place on *Thalictrum apeciosissimum* which had been eradicated in most wheat growing regions and thus in nature, *P. recondita* f. sp. *tritici* does not reproduce sexually. Despite the lack of a sexual stage, leaf rust epidemics are common around the world and build up of rust populations with the ability to infect resistant wheat varieties occur whenever a new resistance gene is introduced into cultivated wheat. We studied the genetic mechanisms that control the

development of isolates virulent on wheat with the Lr26 resistance gene. Sixty isolates representing rust populations from Israel, Europe and the USA were characterized using molecular and phytopathological markers. Phylogenetic analyses showed, that isolates virulent on Lr26 had been evolved independently in several places around the world. The data suggest that Lr26-virulent isolates existed in low proportions in rust populations and their level increased following the introduction and extended use of wheat varieties with the Lr26 resistance gene. Our data also shed new light on the migration of rust. Two separate rust population were identified in Israel suggesting two different sources from which rust arrives in this region. The Israeli populations were found unrelated to the European population ruling out the current model that suggests transfer of wheat leaf rust between the middle east and Europe. Large differences were found between the results obtained using RAPD and phytopathological markers. The differences between the two methods suggest that the phenotypic markers may not be suitable for predicting the source of the rust isolates since they are markedly affected by the cultivars grown in the destination area.

395. CA^R 100 – A *Neurospora crassa* mutant strain with a novel branching pattern

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Tip growth and branching of hyphae have received considerable attention because of their importance in fungal development. Nevertheless, the mechanism of branching initiation is still not known. Previous research suggests that migration and incorporation of vesicles, changes in calcium concentration and redistribution of a cortical actin meshwork occur at branching sites. To test the involvement of actin in the initiation of hyphal branches in *Neurospora crassa*, conidia of the Oak-Ridge wild type strain 74-OR23-1A were irradiated with UV light and screened for new strains resistant to cytochalasin A, an F-actin antagonist. A strain named CA^R 100 (cytochalasin A resistant) with a single gene mutation was isolated from this screen. Resistance of this strain to cytochalasin A is possibly a result of a change in the structure and function of an actin, or an actin associated protein. The branching pattern of CA^R 100 is predominantly dichotomous and different from the lateral branching pattern of the wild type strain from which it derived. The presence of a differing branching pattern in CA^R 100 together with the resistance to cytochalasin A supports the possible involvement of actin in branching. A reversible cold sensitivity detected in CA^R 100 stands in favour of this involvement. Wild type strains have also showed a potential to change their branching pattern from lateral to dichotomous under low temperature conditions. Dichotomous branching was reported earlier in some strains, e.g. *peak,* but the mechanism was not investigated. The colony morphology of CA^R 100 is abnormal. Growth from a conidium is similar to the wild type strain in the initial period, except for a mainly dichotomous branching pattern. After 18-28 hours the distance between branches rapidly decreases together with copious growth of aerial hyphae bearing numerous conidia. This stage is followed by a reduction in the abundance of aerial hyphae and a constant increase of the dense highly branched mycelium. The growth rate and biomass increase are slower than in the wild type. On the cytological level CA^R 100 hyphae show small protrusions of the cell wall. Similar protrusions are present in the granular mutants, but the cause has not been investigated. cAMP and Ca^{2+} do not suppress the CA^R 100 phenotype. The CA^R 100 mutant strain gives a new possibility for investigating genes with a function in branching and tip growth.

396. Participation of NO and NO-Synthase in light-enhanced conidiation of *Neurospora crassa* and sporangiophorogenesis of *Phycomyces blakesleeanus.*

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We investigated the involvement of flavins, pterins and NO/NO-Synthase (NOS) in enhanced formation of conidia in *Neurospora crassa* and of sporangiophores in *Phycomyces blakesleannus*. *Neurospora crassa* albino mutant *al-2, bd*, grown on nitrate medium, shows light-promoted conidiation. A photoreceptor for the reaction is the FAD of nitrate reductase, which also contains molybdopterin (NR) (Klemm, E. and H. Ninnemann 1979 Photochem Photobiol 29:629; Ninnemann, H. 1991 J Photochem Photobiol B Biol 9:189; Ninnemann, H. 1991 J Plant Physiol 137:677). Additional pterins were shown by us in extracts of *Neurospora*, *Phycomyces* and other fungi and algae, including tetrahydrobiopterin (BH_4) (Maier, J. and H. Ninnemann 1995 Photochem Photobiol 61:43). Inhibitors of pterin biosynthesis (e.g., 2,4-diamino-6-hydroxypyrimidine (DAHP) = against GTP-cyclohydrolase I) prevented light-promoted conidiation in *Neurospora* as well as light-enhanced formation of macrosporangiophores in *Phycomyces*. We assume that - beside the flavin of NR - a pterin acts as a photoreceptor or is part of the signal transduction chain. The NO-donor SNP (sodium nitroprusside) inhibited photoconidiation in *Neurospora* and formation of microsporangiophores in *Phycomyces*; NO enhanced formation of macrosporangiophores. Inhibitors of NOS like L-NA or L-NAME (N^G-nitro-L-arginine and its methyl ester), but not D-NAME, had the opposite effect. In animals NO activates soluble guanylate cyclase producing a burst of cGMP. In *Neurospora* blue light depletes cGMP whereas in *Phycomyces* blue light activates production of cGMP in accordance with the observed light-regulated activities of NOS in both fungi. ^{3}H-Citrulline production from ^{3}H-arginine could be demonstrated in *Phycomyces* (1-10 pmol min^{-1} mg^{-1}) and in *Neurospora* (10-100 pmol min^{-1} mg^{-1}) (Ninnemann, H. and J. Maier 1996 Photochem Photobiol 61: 393). Light lowers the NOS activity in *Neurospora* and activates NOS in *Phycomyces*. NO was also detected after its reaction with ozone by chemiluminescence. However, the amount of NO was 10 to 100 times lower than the production of citrulline. In *Neurospora* the high activity of nitrite reductase will consume the nitrite produced from NO.