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Abstract

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Abstracts of Talks and Posters

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Talks: Signal Transduction in Yeasts, Fungi and Plants

Developmental signal pathways in Dictyostelium

Rick Firtel. *University of California, San Diego*
The cellular slime mold Dictyostelium discoideum grows as single-celled, mononucleated amoebae. When starved, the amoebae initiate a multicellular developmental program that results in the production of a mature fruiting body comprised of a stalk with a mass of spores on top. Approximately 4 hrs after removal or exhaustion of the food source, cells within the population produce and secrete extracellular cAMP. This interacts with G protein-coupled receptors that leads to chemotactic aggregation of ~10(5) cells to form a multicellular organism, activation of adenylyl cyclase and relay of the cAMP signal, and the activation of gene expression. Aggregation and several aspects of multicellular development have been shown to be controlled through several classes of serpentine receptors coupled to multiple heterotrimeric G proteins. Recent results have suggested that some of the responses that are regulated by cAMP receptors are G protein-independent, suggesting novel mechanisms by which the same ligand can elicit different developmental responses. Genes encoding eight distinct Ga protein subunits have been cloned and disrupted by homologous recombination. The function of some of these and the novel G protein-independent pathways will be discussed.

Signal transduction in Saccharomyces cerevisiae and Ustilago maydis

Flora Banuett and Ira Herskowitz. *Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143*
Cells respond to external stimuli by transducing these signals from surface to nucleus where gene activation then results in a variety of physiological responses. The MAPKs (mitogen activated kinases) and their upstream regulatory kinases are key players in signal transduction. They constitute a functional module consisting of the MAPK, its activator (MEK), and an activator of MEK (MEKK). S.-cerevisiae has several different MAPKs, each involved in a different pathway. The most intensively studied is the pheromone response pathway. Pheromones produced by haploid a or alpha cells bind to membrane receptors causing dissociation of a trimeric G protein into Galpha and Gbeta gamma. The latter activates the kinase cascade in an unknown fashion, which may involve Ste20 (another kinase), and Ste5 (a scaffold for the MAPK module). Ste20 is proposed to activate Ste11 (MEKK), which in turn activates Ste7 (MEK), which then activates Fus3/Kss1 (MAPK). These MAPKs then activate the transcriptional activator Ste12, which regulates expression of genes for cell fusion, cell cycle arrest, and morphological changes. Recent work indicates that pseudohyphal growth requires several components of this pathway. U. maydis codes for peptide pheromones and receptors that play a role in mating and filamentous growth. A MEK homolog (Fuz7) was identified necessary for the pheromone response and for tumor induction, which may reflect response to a plant signal.

Involvement of leucine-rich-repeat and protein kinases in disease resistance signal transduction in tomato

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Eleven tomato mutants were isolated with altered response to the bacterial phytopathogen Pseudomonas syringae. Five lines carried mutations at Pto, a member of a clustered gene family predicted to encode serine/threonine protein kinases. Six lines carried mutations a second locus termed Prf and showed loss of sensitivity to the insecticide Fenthion as well as disease susceptibility. In contrast, pto mutants retain Fenthion sensitivity. Tight linkage between the Prf and Pto loci allowed cloning of candidate genomic DNA for the Prf locus. Within a 200-kbp contig spanning the Pto locus, a fragment was identified which detected a 1-kbp deletion in a prf mutant line. Sequencing this DNA revealed a gene predicted to encode a protein with nucleotide triphoshate binding (P-loop) and leucine-rich-repeat motifs, as contained in signalling proteins from a wide range of eukaryotes. To rapidly study members of Pto gene family, an assay for transient expression of these genes was developed based on infection of tomato leaves with recombinant derivatives of the Potato Virus X. Using this assay a Pto family member, designated Fen, was identified that confers Fenthion sensitivity. The predicted protein product of the Fen gene shows 80% amino acid identity to Pto and autophoshorylates in vitro, as does Pto. Therefore, tomato contains distinct kinases (Fen and Pto) specific for transduction of Fenthion and pathogen elicitor signals, and the Prf protein which functions in both signal transduction pathways.

Mechanism and function of the oxidative burst in plant defense

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Treatment of bean or soybean cells with fungal elicitor causes a rapid insolubulization of pre-existing (hydroxy) proline-rich structural proteins in the plant cell wall. This insolubilization, which involves H2O2 - mediated oxidative cross-linking, is initiated within 2-5 min, and is complete within 10-20 min, and hence precedes the expression of transcription-dependent defenses. Oxidative cross-linking makes the cell wall refractory to digestion by microbial protoplasting enzymes and is strictly dependent on gene-for-gene mediated incompatibility in an isogenic setting. Thus stimulus-dependent oxidative cross-linking of wall proteins likely has an important function in the initial stages of plant defense. Cross-linking is substrate controlled by the rapid generation of H2O2 at the cell surface. We show that oxidative burst H2O2 also acts as a signal to trigger other key aspects of the hypersensitive responses deployed in the early stages of an incompatible interaction. Emerging evidence indicates that elicitor or pathogen stimulation of H2O2 production in plant cells bears a striking mechanistic and functional resemblance to the cytochrome b- mediated plasma membrane oxidase involved in the oxidative burst during macrophage activation.

G protein-linked signal transduction and fungal virulance: virus-mediated or

transgenic suppression of a G protein a subunit and attenuation of fungal virulence

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Strains of the chestnut blight fungus Cryphonectria parasitica harboring RNA viruses of the genus Hypovirus exhibit significantly reduced levels of virulence (hypovirulence). The accumulation of a heterotrimeric GTP-binding protein a subunit of the Gi class, designated CPC-1, was found to be reduced in hypovirus-containing C. parasitica strains. Transgenic co-suppression, a phenomenon frequently observed in transgenic plants, reduced CPC-l accumulation in virus-free fungal strains. Significantly, the resulting transgenic fungal strains were also hypovirulent. These results indicate a crucial role for G protein-linked signal transduction in fungal pathogenesis and suggest a molecular basis for virus-mediated hypovirulence. Subsequent differential mRNA display analysis revealed a battery of potential reporter genes that are identically regulated in hypovirus infected and in the transgenic CPC-1 suppressed strains. Representative cDNA clones obtained from that analysis are currently being employed to dissect this pathogenesis-related, G protein-linked signaling pathway.

cAMP dependent protein kinase catalytic subunit (cpkA) gene is required for appressorium formation in Magnaporthe grisea

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Successful infection of rice by the fungal pathogen Magnaporthe grisea depends upon the formation of a dome shaped, highly melanized infection structure, an appressorium. The differentiation of this unique, specialized cell from the tip of an emerging germ tube is a response to environmental stimuli. The exogenous and endogenous signalling mechanisms involved in surface recognition and the transfer of this information into the cell leading to infection related morphogenic events remain to be elucidated. We have shown that cyclic AMP, a second messenger involved in signal transduction systems, regulates appressorium formation. In other systems, the primary target for cAMP is cAMP dependent protein kinase. Activation of this kinase directly or indirectly results in specific developmental changes. To elucidate the mechanism of cAMP action in M. grisea, we have isolated, sequenced and disrupted the single gene encoding the catalytic subunit of cAMP dependent kinase (cpkA). Strains lacking the cpkA gene appear unaffected in their ability to grow and reproduce sexually and asexually. However, they are unable to form appressoria on rice or in the presence of cAMP and are non pathogenic. This is the first direct evidence that fungal pathogenesis is mediated via cAMP dependent protein kinase.

Talks: Gene Expression and Genome Structure I

The facB88 translocation creates a fusion of two regulatory genes which mediates superactivation of the amdS gene of Aspergillus nidulans

Rachael L. Murphy, Meryl A. Davis and Michael J. Hynes, Department of Genetics, University of Melbourne, Parkville 3052, Victoria, Australia.
The acetamidase gene (amdS) of Aspergillus nidulans is subject to complex transcriptional regulation. The facB gene product, which contains a Zn(II)2Cys6 DNA binding motif, mediates acetate induction of amdS and genes required for acetate metabolism via binding to the 5' regions of these genes. The facB88 reciprocal translocation results in very high level, constitutive expression (superactivation) of amdS. The translocation breakpoints lie within facB on chromosome VIII and a previously unidentified gene on chromosome IV, designated amdX. Only one of the hybrid genes created by the translocation, 5'facB-3'amdX, functions to superactivate amdS. The sequence of this hybrid gene revealed that the DNA binding domain of facB is fused to another DNA binding motif, consisting of two C2H2 zinc fingers, encoded by amdX. AmdX binds in vitro to sequences 5' of amdS that are known binding sites for the CreA and AmdA C2H2 zinc finger proteins. Both DNA binding domains are essential for maximal function of 5'facB-3'amdX, as are both the FacB and AmdX binding sites 5' of amdS. Thus the AmdX DNA binding domain contributes significantly to amdS regulation in the facB88 mutant. amdX has been cloned and sequenced. Gene disruption indicated that amdX is a non-essential gene. The phenotype of this loss of function mutant is slightly but consistently weaker growth on acetamide than wild type strains. Our results are consistent with a minor positive role for native amdX in the regulation of amdS expression.

The Aspergillus pacC zinc finger transcription factor mediates regulation of both acid- and alkaline-expressed genes by ambient pH

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Many microbes encounter large ambient pH variations in their natural environments. Microorganisms capable of growing over a wide pH range require efficient pH homeostasis and a means of ensuring that activities undertaken beyond pH homeostasis boundaries are appropriate to ambient pH. Aspergillus nidulans is able to grow over a range of approximately eight pH units. The formal genetics and physiology of its regulatory system for controlling syntheses of secreted enzymes, permeases and exported metabolites in response to ambient pH have been described. This system enables, inter alia, the secretion of alkaline phosphatase in alkaline environments and acid phosphatase in acidic environments, as well as a considerable elevation in penicillin biosynthesis at alkaline pH. pH regulation of gene expression in A. nidulans is mediated by pacC whose 678 residue derived protein contains three putative Cys2His2 zinc fingers. Ten pacCc mutations mimicking growth alkaline pH remove between 100 and 214 C-terminal residues, including a highly acidic region containing an acidic-glutamine repeat. Nine pacCc mutations mimicking acidic growth conditions remove between 299 and 505 C-terminal residues. Deletion of the entire pacC coding region mimics acidity but leads additionally to poor growth conidiation. A PacC fusion protein binds a hexanucleotide core consensus sequence. At alkaline ambient pH, PacC activates transcription of alkaline-expressed genes (including pacC itself) and represses transcription of acid-expressed genes. pacCc mutations obviate the need for pH signal transduction.
Initiation of conidiophore development in Aspergillus nidulans

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Aspergillus nidulans conidiophore formation can initiate as a programmed part of the lifecycle or in response to nutrient deprivation. In both cases, activation of the conidiation pathway ultimately leads to the expression of the complex brlA developmental regulatory locus which in turn results in activation of other genes required for conidiophore formation. Many developmental mutants blocked prior to activation of brlA expression have morphological abnormalities described as "fluffy". These mutants are characterized by an unrestricted proliferation of aerial hyphae resulting in formation of large cotton-like colonies that, unlike wild type, are able to grow into other colonies. One typical fluffy mutant results from mutation in the fluG gene. Strains containing a deletion of fluG are completely aconidial and no brlA expression is detectable during growth on rich medium. However, fluG mutants conidiate when grown on minimal medium suggesting that fluG null mutants maintain the ability to develop in response to nutrient limitation but have lost programmed developmental initiation. fluG mutant strains also conidiate when grown in contact with wild type colonies or with strains carrying different developmental mutations (e.g. brlA) suggesting that fluG mutants are deficient in production of a conidiation signal. We have identified five additional genetic loci, designated flbA, flbB, flbC, flbD, and flbE, that when mutated result in decreased brlA expression and a fluffy phenotype. We have shown that the predicted flbD product is related to mammalian myb proteins and that flbC encodes a protein with two putative C2H2 zinc fingers suggesting that these genes likely encode transcription factors. The flbA gene is predicted to encode a protein with significant similarity to the Saccharomyces cerevisiae SST2 product which is required for adaptation to mating pheromones. We have shown that overexpression of flbA, flbC, flbD, or fluG in vegetative hyphae can activate conidiophore development. These results have led us to propose that each of these genes functions in the programmed initiation of development and that flbA functions in mediating the response to the fluG-conidiation signal through a mechanism that requires the activity of the flbC and flbD DNA binding proteins.

The nitrogen regulatory circuit of Neurospora

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The expression of many genes which encode nitrogen catabolic enzymes is highly regulated by nitrogen repression and pathway-specific induction. Transcription of nit-3, which encodes nitrate reductase, is controlled by NIT2, a positive-acting global nitrogen regulatory protein, and by NIT4, a pathway specific factor which mediates nitrate induction. Three NIT2 and two NIT4 DNA binding sites occur in the nit-3 promoter. NIT4 binds DNA as a homodimeric protein, and recognizes an 8 bp palindromic sequence 5'TCCGCGGA and closely related sequences. NMR is a putative negative-acting regulatory protein that may act in nitrogen repression; nmr mutants are largely insensitive to repression by glutamine or ammonium ion. A specific NIT2-NMR protein-protein interaction has been detected with the yeast 2-hybrid system in vivo and with GST-fusion studies in vitro. NMR binds to two regions of the NIT2 protein, one of which is a short a-helical motif within the DNA binding domain.

Isolation of white collar-1, a central regulator of the blue light response in Neurospora

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The filamentous fungus, Neurospora crassa, is responsive to blue light irradiation, through the activation of specific genetic programs like: induction of mycelial carotenogenesis, promotion of conidia and protoperitecia development, phototropism of peritecial beaks and inhibition or shift of the circadian rhythm of conidiation. Two blind mutants, called white collar 1 and 2 (wc-1 and wc-2), defective in all the blue light-induced phenomena are known. The pleiotropic phenotype of these white collar mutants strongly suggests that they are involved in signal transduction. The blue light transduction pathway has never been dissected at molecular level, only recently a putative blue light photoreceptor has been described in Arabidopsis thaliana. We report here the isolation by chromosome walking and mutant complementation of the white collar-1 gene, a further key piece of the blue light puzzle. The wc-1 deduced product is a protein with a predicted molecular weight of 125 kDa, characterized by a single class 4 Zn finger and a polyglutamine stretch. The wc-1 Zn finger is highly homologous to the finger domains of vertebrate GATA factors, of fungal regulator proteins like Nit- 2, AreA, GLN3, DAL80, and of plant NTL1. The wc-1 Zn finger domain expressed in E. coli is able to bind in vitro to the promoter region of the blue light regulated Neurospora albino-3 gene, which contains two GATA sequences. Wc-1 gene expression is self-regulated and induced, at the transcription level, by blue light irradiation.

Talks: Sexual and Asexual Reproduction

Sexual reproduction in ascomycetes: roles of the N. crassa mating type polypeptides

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The N. crassa mating type idiomorphs encode polypeptides that play crucial roles in vegetative incompatibility, mating, and formation of ascogenous hyphae. RIP experiments also suggest a role for the mating type genes within the ascogenous hyphae. The sole product of the mt a idiomorph, MT a-1, is a sequence-specific DNA binding protein of the HMG box family. The presumed regulatory targets of MT a-1 include genes necessary for mating pheromone biosynthesis and response. MT a-1 DNA binding activity is not necessary for vegetative incompatibility function, but it is necessary for mating functions. The mt A idiomorph encodes at least three products. One of these products, MT A-3, is also a member of the HMG box family. MT A-3 produced in E. coli binds DNA fragments that are also bound by MT a-1. The interactions of the mating type polypeptides with DNA and with each other as well as the biochemical and biological consequences of such interactions are under investigation.

Genes involved in vegetative incompatibility reaction in Podospora anserina

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The three genes, het-c, het-e and het-d, involved in the two nonallelic. incompatibility het- c/het-e and het-c/het-d systems have been cloned. HET-C protein is similar to a glycolipid transfer protein. A strain containing a disrupted het-c locus is affected in ascospores production. A het-e gene encodes a 1356-amino acid protein which exhibits two domains. In the N-part of the protein, there is a P-loop motif characteristic of GTP binding protein. In the C-part of the protein, there are ten direct repeats of a motif of 42 amino aids which are characteristic of beta-transducin like protein. Mutation in the P-loop motif results in a loss of the incompatibility phenotype. It has been shown in P. anserina that mutation in some genes named mod genes results in the suppression of a barrage reaction. Moreover, resulting strains have defects in secondary ramification and reproductive female organ production. On the basis of the wild type phenotype restoration of a modD mutant strain, a gene has been cloned. It encodes a polypeplide which has homology with the N. crassa adenylate cyclase. But, by RFLP, the modD locus and the adenylate cyclase locus are distinct. Transcriptional and/or translational regulation of genetic expression and enhancement of the proteolytic activity had been shown in cells undergoing cell death associated to incompatibility. Two genes under transcriptional regulation have been cloned. Study of their expression is in progress. The cloned gene papA, encoding an aspartylprotease, has been disrupted.

Mating type regulation of sexual reproduction in Coprinus

Lorna Casselton, University of Oxford, Oxford, UK
Mating compatibility in the basidiomycete Coprinus cinereus is determined by multiallelic genes at two complex loci termed A and B. These genes regulate a developmental programme that converts the asexual monokaryon into a fertile dikaryon. It is estimated that there are some 160 versions of A and 79 versions of B which would generate more than 12000 cross compatible mating types in nature. We have a fascinating biological problem in trying to resolve how such large numbers of A and B specificities are derived and how it is that so many versions can interact to trigger the same developmental pathway. The A locus contains several genes that encode multiple versions of two dissimilar homeodomain proteins. Specific combinatorial interactions between these proteins promotes the A-regulated part of the pathway; we are interested in why certain pairs of proteins can heterodimerize but others cannot. The B locus also contains several genes of different classes; preliminary data indicate that these play a role in signal transduction. Rare mutations that result in self-compatibility arise within the A and B loci and molecular analysis of these provides novel insights into the function of the genes.

The multispecific B mating-type genes of Schizophyllum commune encode complexes of putative pheromones and pheromone receptors

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Molecular genetic analyses of the B alpha and B beta mating-type loci have produced

evidence that a multispecific system of pheromones and pheromone receptors govern self/nonself recognition and sexual development in the Homobasidiomycete Schizophyllum commune. Two of the nine known specificities at the B alpha locus (alpha 1 and alpha 3) and two of the nine known specificities at the linked B beta locus (beta 1 and beta 2) have been cloned and are being examined. A synthesis of data to date, from DNA sequencing, nucleic acid hybridizations, and functional tests via DNA-mediated transformation experiments, suggests that B alpha 1 and B beta 1 each contains several genes encoding pheromones and at least one gene encoding a pheromone receptor similar to those known in Ustilago maydis and Saccharomyces cerevisiae. Each of these loci spans approximately 8 kb of genomic DNA and includes multiple elements capable of differentially activating B-regulated development in strains representing the other eight specificities at the relevant locus. None of these elements can elicit a response in strains carrying the self B mating-type specificity. This is the first evidence for a multispecific system of pheromones and pheromone receptors governing mating in a fungus. Comparison of pheromone receptor genes in Schizophyllum commune Jurgen Wendland, Jorg Hegner and Erika Kothe, University of Marburg, Germany The mating type locus Ba1 of S. commune contains a pheromone receptor and putative pheromone genes. The pheromone receptor Bar1 shows homology to other pheromone receptors such as Ste2 and Ste3 of Saccharomyces cerevisiae and pral and pra2 of Ustilago maydis. It also contains sites conserved among other G protein-linked receptors of the seven transmembrane domain family. The fact,that the mating system in S. commune provides nine different allelic specificities at the mating type locus B-alpha makes it a model system to investigate ligand interaction between different allelic specificities of pheromones and receptors. For S. commune multiple isolates for each mating specificity exist. In order to obtain evidence for functional domains, different alleles for the BAR1 gene will be compared. Southern hybridization experiments could show that strains sharing the specificity B-alpha1 exhibit strong hybridization signals. This indicates higher homology of the alleles of BAR1, all sharing the mating specificity B-alpha1, as compared to strains with other mating specificities, which also hybridize, but to a lesser extent.

Regulatory interactions that control multicellular development during Aspergillus sporulation

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Multicellular development in the filamentous fungus Aspergillus nidulans requires that spatiotemporal regulation of cell-specific gene expression be coupled to alterations in cell cycle regulation. A genetic hierarchy consisting of five essential regulatory genes controls the transition from multinucleate filamentous growth to uninucleate budding growth, the assembly of a multicellular reproductive structure (conidiophore) and the production of dormant conidia (spores). Complex regulatory interactions provide a mechanism for establishing overlapping gradients in these transcription factors which, in turn, coordinately activate hundreds of conidiation-specific genes. One class of genes (brlA, abaA, wetA) establishes a linear regulatory pathway that directs formation of the terminally differentiated conidia. A second class modifies these events to allow

multicellular development. The modifier gene medusa (medA) is required for commitment to the developmental program. MedAp physically interacts with BrlAp, functioning as a co-activator of a subset of BrlAp target genes that includes abaA. The stunted (stuA) gene encodes a member of a family of transcription factors that control cell cycle progression and fungal development. Overexpression of StuAp blocks the ability of BrlAp to drive terminal differentiation and promotes a pseudohyphal growth pattern. During conidiation, cell-specific morphology is established by a balance between BrlAp, AbaAp and MedAp driving terminal differentiaton and StuAp driving uninuclear cell divisions by regulating expression of brlA, a subset of BrlAp target genes and possibly cell cycle regulators.

The first non-mammalian homologue of the PAFl gene (Zellweger syndrome) discovered as a gene involved in caryogamy in Podospora anserina

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The fusion of haploid nuclei (caryogamy) is a vital part of the sexual cycle. To date, in fungi, this process has been well studied only in S. cerevisiae. Caryogamy in filamentous ascomycetes is more complex than in unicellular yeasts. It does not occur immnediately after fertilization but it is a deferred stage occuring in specialized cells. The car1 mutants of P. anserina, completely defective for caryogamy, have been isolated during a systematic search for sporulation deficient mutants. The car1 gene was cloned by complementation. Surprisingly, this gene encodes a protein that shows similarity to the mammalian PAF1 protein (Zellweger syndrome). Altogether, the molecular, physiological (inability for the car1 mutants to grow on oleic acid), genetical (revertants analysis) and ultrastuctural approaches (immunofluorescence and electron microscopical analyses) gave evidence that the P. anserina car1 protein is actually a peroxisomal protein. Therefore, this study shows that peroxisomes are required at a specific stage of sexual development, at least in P. anserina, and that a functional homologue of the PAF1 gene is present in a lower eucaryote. The data will be discussed in terms of the possible functional role of peroxisomes in the caryogamy process or, more likely, in the cell determination or differentiation programs.

Control of DNA replication and mitotic checkpoint function by the Aspergillus nidulans nimO gene

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We are investigating the role of the Aspergillus nimO gene in DNA replication and mitotic checkpoint function. Flow cytometric and cytological analyses showed that when conidia of a temperature sensitive lethal nimO18 mutant were germinated at the restrictive temperature (44 C), they were unable to replicate their DNA, but 35-40% of nuclei nevertheless progressed into mitosis and arrested with condensed chromatin. These results identify nimO as a candidate regulator of G1/S or S phase, and further suggest that this gene influences the checkpoint that prevents mitosis (M) from occurring until DNA synthesis has been completed. Sequencing of a nimO cDNA revealed a predicted protein of 72.8 kd that shares 29% identity with DBF4, a budding yeast G1/S regulator which

associates with origins of replication and with the CDC7 kinase in order to initiate DNA synthesis. nimO and DBF4 are especially strongly conserved in a C-terminal domain containing a single, novel motif which bears strong resemblance to Cys2-His2 zinc fingers. A deletion of this 60 bp motif from a nimO gene fused with the inducible alcA gene promoter rescued the ts-lethality of nimO18 for growth, but not conidiation, when high-level expression was induced by ethanol. On glycerol, which permits low basal expression, the strains grew poorly and were likewise unable to conidiate. Thus, the putative zinc finger appears non-essential for mycelial growth when the variant gene is highly expressed, but may be necessary for the accelerated cell cycling that must occur during conidiation. We are continuing to examine the function of this motif and to test nimO function through gene disruption.

Talks: Fungal Cell Biology and Morphogenesis

Phosphates and other genes required for completion of nuclear division in Aspergillus

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The ultimate purpose of mitosis is to separate replicated DNA precisely into identical, and viable daughter nuclei. A subset of temperature sensitive cell cycle mutants are unable to complete the later stages of mitosis. These include alleles of bimG, various hfa genes and several nim genes. Mutations in all of these genes lead to the inability, at restrictive temperature, to separate daughter nuclei. Molecular analysis revealed that bimG encodes a regulatory protein phosphatase very similar both structurally and functionally to mammalian type 1 phosphatase. A single nucleotide change, responsible for the mutant phenotype, alters the 5' splice site of the second intron and leads to a temperature sensitive defect in splicing. Overexpression of the mutant gene from palcA produces a dominant lethal phenotype supporting the idea that aberrant splicing leads to the production of a toxic peptide which interferes with normal phosphatase function. To identify gene products which might interact with the phosphatase, extra-genic suppressors have been identified in three different genes, sugA, B & C. Mutations in these genes cause defective nuclear separation when grown at low temperature. Other genes involved in late mitosis include the nimU gene. nimU encodes a very basic novel protein, whose amino acid sequence suggests the potential for direct binding to DNA. The nimU24 mutation is partly epistatic to the bimE7 mutation (which causes DNA to become highly condensed) suggesting that NIMU may be required for normal chromosome condensation.

Nuclear positioning in Aspergillus nidulans

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Organellar movement is an important feature of all eukaryotic cells. Vesicles,

chloroplasts, mitochondria and nuclei migrate within the cytoplasm. Rapid, long distance nuclear migration has been observed in filamentous fungi for more than 60 years. However, the understanding of the enzymatic and molecular mechanisms driving nuclei are still at the beginning. In Aspergillus nidulans nuclei migrate along microtubules, probably driven by the microtubule dependent motor protein dynein. Besides a "basic translocation machinery" auxiliary components are required for triggering the movement and its coordination with other cellular functions. As one of the latter components apsA (anucleate primary sterigmata) was characterized. ApsA is a coiled coil protein required for nuclear positioning in hyphae and also in conidiophores, where it is needed for conidia production. The 180 kD protein was detected in crude protein extracts of hyphae with polyclonal antibodies rose against a part of ApsA expressed in E. coli. In a mutational screening of the apsA- strain (no conidia) a partially suppressed, conidiating strain was discovered. Formal genetics suggested that this suppression was due to a single mutated gene, extragenic to apsA. In a wild type (apsA+) background, the mutated suppressor gene had no obvious effect on conidiation, but a slight inhibitory effect on hyphal growth rate, and sexual spore formation was completely prohibited. Molecular characterization of the suppressor gene might shed some light on the function of apsA and the suppressor gene in the nuclear migration and positioning process.

Breaking the mold: cytokinesis in Aspergillus nidulans

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Fungal cells are divided by crosswalls termed septa. We are determining the order of events in septum formation. These events include mechanisms that coordinate septum formation with cell growth and nuclear division; positioning a site in the cortex for septum formation; constricting of the protoplasm; recruitment of cell wall biosynthetic capacity to a site distal from the growing point (the hyphal tip), and assembly of the septal pore complex. Thus, septum formation represents a complex process of cellular morphogenesis. Physiological and cytological studies have demonstrate that nuclear positioning plays a critical role in determining the placement of the fungal septum site. The capacity to initiate septum formation requires nuclear division. Once the fungal septation site is established, septation can occur independently of nuclear division. Actin is recruited to the site of the incipient septum. Confocal microscopy demonstrates that actin structures at this site closely resemble the animal cell contractile ring. Genetic analysis has defined a pathway for septation; four genes act before site establishment, and four genes act after site establishment. The failure of the A. nidulans cells to establish a site for septum formation results in a cessation of growth, nuclear division and eventual cell death.

Genetic dissection of cytoplasmic dynein in Neurospora

Mike Plamann, Kenneth Bruno, Peter Minke, and John Tinsley. Department of Biology, Texas A&M University, College Station, TX
Cytoplasmic dynein is a multisubunit, microtubule-dependent mechanochemical enzyme that has been proposed to function in a variety of intracellular movements, including

minus-end-directed transport of organelles. A second protein complex referred to as the Glued or dynactin complex is required for cytoplasmic dynein to mediate efficient microtubule-dependent transport of organelles in vitro. We have determined that mutants defective in cytoplasmic dynein or the associated Glued/dynactin complex can act as partial suppressors of a Neurospora crassa protein kinase mutant (cot-1). These mutants, defined as ropy, display curled hyphal growth and abnormal nuclear distribution. We have shown that ro-1 encodes the heavy chain of cytoplasmic dynein and ro-4 encodes the actin-related protein Arp1, which is the most abundant subunit of the Glued/dynactin complex. In addition, we have isolated and characterized additional ro genes and determined that ro- 3 encodes the largest subunit of the Glued/dynactin complex. We have now isolated >2000 ro mutants in an effort to identify all genes encoding subunits or specific regulators of cytoplasmic dynein. Complementation analysis of ro mutants has identified approximately 20 complementation groups. Many of the ro mutants show complex genetic interactions with examples of unlinked noncomplementation and allelic complementation being common.

asm-1: a gene involved in formation of protoperithecia and ascospore maturation in Neurospora crassa

Rodolfo Aramayo, Yoav Peleg and Robert L. Metzenberg. University of Wisconsin, Madison.

To identify novel, key regulatory genes of sexual development in Neurospora crassa, we explored the possibility that regulatory genes known to affect sexual development in Aspergillus nidulans may be evolutionary conserved and play similar roles in N. crassa. Among the classical developmental mutants of conidiophore development in A. nidulans, two genes, stuA (stunted A) and medA (Medusa A), are the only ones whose loss of function mutations completely block sexual development in this organism. Using stuA as heterologous probe in low stringency hybridizations we have cloned asm-1+ (ascus maturation 1) from Neurospora crassa. asm-1 was mapped to linkage group VR, near al-3, using RFLP analysis. We determined by Northern and Western blot analysis that both its message and its protein product are very abundant and constitutively expressed in both low and high nitrogen media. Loss of function asm-1 mutants (obtained by gene replacement) present multiple phenotypes that affect both the asexual and sexual phases of the life cycle. During asexual development asm-1 loss of function mutants lack aerial mycelia and conidiate rather close to the agar surface. During sexual development the mutant strain is unable to form protoperithecia and is therefore female sterile. As a male, in heterozygous crosses, the mutant presents a dominant phenotype. This dominant phenotype is characterized by the production of 99.9% of very small, white, and inviable ascospores. The rare, viable black ascospores that have been tested have always proven to be asm-1+, except for a single heterokaryotic segregant which must have come through meiosis as a heterozygous disomic. The loss of function mutation can be complemented when the asm- 1 chromosomal region is integrated at the his-3 locus. The resulting strain presents a normal vegetative phenotype as well as protoperithecia formation. The asm-1 loss of function phenotype presents formally similar characteristics to those of other ascus dominant (zygote dominant) mutations like Banana (Ban), Perforated ascus (Pfr), Indurated ascus (Iasc), and Round ascospores (R). Possible modes of action and

regulation of asm-1 during sexual development were discussed.

The nudF and nudC genes are required for nuclear migration in Aspergillus nidulans

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The nudF gene was identified as an extra copy suppressor of the temperature sensitive (ts) nudC3 mutation that blocks mycelial and conidial nuclear migration in Aspergillus nidulans. NudF encodes a protein similar to the beta-transducin (WD-40) family of heteromeric G-protein subunits and has a molecular weight of 49 kDa. In sucrose gradients the NUDF protein has an S value greater than that of albumin (68 kDa), suggesting that it is sedimenting as a subunit of a protein complex. Ts mutations in nudF lead to failure of nuclear migration and an almost complete disappearance of the protein. The ts nudC3 mutation also causes a disappearance of NUDF protein at restrictive temperature. Nine different extragenic suppressors of nudC3 restore the depleted NUDF protein toward normal levels and allow growth at restrictive temperature. We conclude that maintainance of a normal intracellular level of NUDF protein is required for nuclear migration to occur.

Talks: Plant and Animal Fungal Pathogenesis

Cloning of the Candida albicans genes encoding virulence factors is the essential first step in the molecular genetic dissection of their role in pathogenesis

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Adherence and extracellular phospholipase production have been identified as putative virulence factors. To determine the role of these two factors in candidal pathogenicity we have cloned two genes from C. albicans that encode an adhesin and an extracellular phospholipase respectively. Saccharomyces cerevisiae was transformed with genomic library from C. albicans and the transformed cells were screened for adherent and phospholipase producing clones, respectively. Adherent clones of the transformed S. cerevisiae were selected by repetitive passages over human umbilical vein endothelial cells. One clone was identified that exhibited 50-fold greater adherence to endothelial cells compared to control organisms transformed with an empty plasmid. This clone was found to contain candidal gene with a 1737 bp open reading frame. The predicted amino acid sequence had significant homology with both transcription factors and structural genes. Immunoprecipitation of an epitope- tagged fusion protein from this gene identified a 65-67 kd protein. Experiments are in progress to determine whether the gene product localizes to the nucleus or to the cell surface in S. cerevisiae. Also mutants of C. albicans in which both alleles of the adherence gene have been deleted are being constructed. Clones expressing phospholipase were identified on egg yolk agar medium. The phospholipase gene was localized to a 2.5 kb XhoI-PstI fragment. Sequencing of this

fragment revealed a single open reading frame 1055 bp in length. The putative protein encoded by the open frame is 352 amino acids in length with a predicted molecular weight of 37,904 Daltons. The predicted protein contains an hydrophobic amino terminus consistent with the secreted nature of the phospholipase. Five N-glycosylation consensus sites are present at the amino acid positions 114, 202, 300, 305, and 334. No amino acid sequence similarity was found with other proteins, including other phospholipases. However, the predicted protein contains the sequence (97)GYSGG(101) which matches the G-X-S-X-G motif characteristic of extracellular lipase from C. cylindracea. This same motif has a significant homology with a lipid-binding site of lipases from human, pig, dog, rat, and mouse. The cloned phospholipase gene was confirmed to be a structural gene and not a regulatory one by gene dosage.

Genetic control of race specificity in Cochliobolus heterostrophus

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Pathogen populations are inherently unstable, frequently giving rise to races with altered virulence or host specificity. New forms of the distantly related corn pathogens, C. heterostrophus and Mycosphaerella zeae-maydis appeared in 1970, distinguished by their specificity to corn containing Texas male sterile (T) cytoplasm. In both cases high virulence is conditioned by production of similar polyketide toxins which bind to a protein unique to T cytoplasm corn. Thus, race change is marked by production of a distinctive secondary metabolite: T-toxin in C. heterostrophus and PM- toxin in M. zeae-maydis. Our goal is to investigate race changes by comparing the genetic mechanisms underlying toxin production by these fungi. Although it is known that new pathogenic races can result from nucleotide substitutions, complex genome rearrangements can also accompany a change in race. We know that T-toxin production by C. heterostrophus is associated with a reciprocal translocation, a large insertion, and repeated, AT-rich DNA, features not yet detected in M. zeae-maydis. Moreover, at least two unlinked loci are required for T-toxin production by C. heterostrophus; one encodes a polyketide synthase which has no close homolog in M. zeae-maydis, or in nontoxin producing strains of C. heterostrophus. Induced Tox- mutants of both fungi have drastically reduced virulence, indicating that both toxins play prominent roles in pathogenesis.

The search for virulence associated genes in Cryptococcus neoformans

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The search for virulence associated genes in Cryptococcus neoformans began soon after the discovery of the heterothallic life-cycle of the fungus. Since heterothallism provided the means of recombinational analysis, the first trait of C. neoformans that we have focused on was diphenol oxidase activity. The rationale for this approach was that C. neoformans is the only undisputed pathogen within the genus Cryptococcus and the only species among more than 20 members of the genus that expresses diphenol oxidase activity in addition to good growth at 37 C. The Mel+ and Mel- siblings of opposite mating types were crossed and the progeny of Mel+ and Mel- were tested in a mouse

model. As we expected, the Mel+ progeny killed mice much faster than Mel- isolates. The enzyme diphenol oxidase was purified and the analysis of the amino acid sequence of the enzyme indicated that it is a laccase. The gene CNLAC1 encoding this laccase in C. neoformans was recently cloned and characterized in our laboratory. Work is underway to molecularly prove that the CNLAC1 gene is associated with virulence in C. neoformans. The second factor we have focused was mating type. Although laboratory analysis shows alpha and a mating types to be alleles, and alpha and a progeny grows equally well, most clinical isolates are of alpha type. We suspected that alpha type may be more virulent for mice. An isogenic set with identical karyotype, DNA finger-print pattern, Mel phenotype and growth characteristics at both 30 and 37 C were constructed and their progeny of alpha and a type were tested for virulence in mice. As expected, the alpha type parent as well as the alpha type progeny killed more mice in a shorter period of time than did a type strains. The gene has been cloned by J. Edman and is being characterized in his laboratory. Although every member of the genus Cryptococcus produces a large extracellular polysaccharide capsule, there is evidence that the capsule of C. neoformans is clearly associated with virulence. We have focused on capsule formation and cloned 2 (CAP59 and CAP64) of the 6 loci proposed to be associated with capsule formation according to classical mutational analysis. Although the biochemical function of these gene products is unknown, both CAP59 and CAP64 genes are found to be essential for the virulence in mice. Work is underway to identify all the genes involved in capsule formation.

Avirulence genes and pathogenicity genes of the tomato pathogen Cladosporium fulvum

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The interaction between C. fulvum and tomato has a gene-for-gene basis. Here we report on the race-specific elicitors encoded by the avirulence genes Avr9 and Avr4. The AVR9 elicitor consists of 28 amino acids and induces a hypersensitive response (HR) in Cf9 tomato genotypes. (1)H NMR studies revealed that the AVR9 elicitor is a compact sandwich-like molecule consisting of 3 antiparallel beta-sheets. By in vitro mutagenesis we have produced mutant peptides with the same, higher or lower HR-inducing activities than the wild type AVR9 elicitor. The (125)I-AVR9 peptide binds to membranes of different tomato genotypes. A positive correlation exists between affinity of (125)I-AVR9 to membranes and HR-inducing activity. However, it is yet unclear whether the cloned Cf9 resistance gene encodes a receptor for the AVR9 elicitor. The mature AVR4 elicitor is a 106 amino acid peptide. All strains avirulent on Cf4 genotypes contain an identical Avr4 gene, while virulent strains contain alleles with single basepair changes in the coding region, or a frameshift mutation (in one case). Peptides encoded by virulent Avr4 alleles appear to be very unstable. Disruption of the putative pathogenicity genes ecp1 and ecp2 affect the sporulation of C. fulvum on susceptible tomato plants.

Saponin detoxification by plant pathogenic fungi

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Saponins occur in many plant species, and because of their antifungal properties they have been implicated as pre-formed determinants of resistance to fungal attack. Some fungi produce enzymes which remove sugars from saponins, to give molecules which are less fungitoxic. Mutants of the cereal-infecting fungus Gaeumannomyces graminis var. avenae which do not produce the saponin glucosyl hydrolase avenacinase can no longer infect the saponin-containing host oats (but are still fully pathogenic to wheat, which does not contain saponins) (see accompanying poster by Bowyer et al). Southern blots using avenacinase cDNA as a probe revealed cross-hybridising DNA sequences in a number of other phytopathogenic fungi, suggesting that enzymes related to avenacinase may be widespread. We have demonstrated for one of these fungi (the tomato pathogen, Septoria lycopersici), that the cross-hybridising DNA in this fungus also encodes a saponin detoxifying enzyme (in this case tomatinase). While avenacinase and tomatinase are clearly related, the relative activities of these enzymes towards avenacin and tomatine reflect the host specificity of the fungi from which they originate. Structure/function analysis of these two highly conserved saponin glucosyl hydrolases should allow us to identify the regions of the enzymes which are important for activity and for substrate specificity, and to design inhibitors of enzyme action which may have significance for crop protection strategies. The occurrence of DNA sequences which hybridise to avenacinase cDNA in genomic DNA of other phytopathogenic fungi suggests that saponin-saponinase combinations may be more important in determining host range than has previously been appreciated.

Genetic improvement and the molecular basis of fungal pathogenesis

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Entomopathogenic fungi represent an untapped reservoir of pesticidal genes for the production of advanced engineered pestcides; an important consideration given that the lack of "useful" pesticidal genes for transfer has been a major constraint in the implementation of biotechnology in crop protection We are assembling a bank of pathogenicity related genes from Metarhizium anisopliae and Beauveria bassiana which could be used to transform other fungi, bacteria, or viruses to create novel combinations of insect specificity, or to produce transgenic plants with improved resistance to insect pests.To perform these studies, specific vectors are being constructed which facilitate strain construction to enhance virulence using constitutive and regulatory promoter regions for expression of homologous and heterologous genes. The potential for this approach has been demonstrated by transferring the gene for the Prl protease from M. anisopliae to Aschersonia aleyrodis, which consequently became a pathogen of late instar whitefly. We have developed a direct strategy for engineering enhanced virulence in M. anisopliae by constitutive expression of some of the many, normally inducible anti-insect proteins. Our initial candidates for this approach have been genes encoding cuticle-degrading enzymes and toxins, since the active agents are encoded by single genes and have been shown to be active in vitro against insects. Constitutive expression of Prl was

obtained by transforming M. anisopliae with cDNA for Prl behind the Neurospora crassa cross pathway control promoter. Transgenic strains continued to produce Prl in the haemocoel of Manduca sexta caterpillars following penetration of the cuticle causing extensive melanization in the body cavity and cessation of feeding 30-40 hr earlier than controls infected with wild type. These studies provide the first conclusive demonstration of the utility of heterologous gene expression in molecular analysis of the insect-fungus interaction, and for strain construction of improved mycoinsecticides.

Characterisation of the pathogenicity gene MPG1 from the rice blast fungus Magnaporthe grisea

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Magnaporthe grisea infects its host by producing a specialized cell known as an appressorium. This cell works by adhering tightly to the leaf surface and generating high internal turgor which is translated into the mechanical force necessary to break the underlying plant cuticle. Recently, we identified a gene known as MPG1 which appears to play an important role in the elaboration of appressoria. The gene was identified as a fungal transcript produced abundantly in planta. Temporal analysis revealed that MPG1 was highly expressed as soon as 18h after inoculation of rice seedlings and was also expressed during disease symptom expression 72-96h later. A directed gene replacement showed that MPG1 is required for efficient appressorial development and mpg1-mutants therefore showed a reduced pathogenicity phenotype. MPG1 appears to encode a hydrophobin-like protein with homology to the Sc3, Sc1 and Sc4 genes from Schizophyllum commune, the rodA gene from Aspergillus nidulans and the eas gene from N. crassa. Consistent with this, M. grisea mpg1 mutants show an 'easily wettable' phenotype showing that cell surface hydrophobicity of aerial hyphae is reduced. Hydrophobins are unusual proteins which are known to be produced during aerial growth of fungi. Hydrophobins appear to undergo self-assembly into high molecular weight amphipathic complexes when they reach interfaces between liquids and air. Such physical characteristics would predict a number of potential roles for MPG1p in appressorial development and pathogenesis. It is, for example, conceivable that MPG1p acts either as a structural component of the appressorium, or as an adhesion protein. Its secretion and potential incorporation into cell wall complexes may therefore be a rate limiting step in the transduction of the inductive signals required for appressorial morphogenesis. In order to test these hypotheses a number of strategies have been adopted to purify and characterise MPG1p and to determine its precise role in the pathogenesis of M. grisea. Progress in these areas will be discussed.

Talks: Gene Expression and Genome Structure II

In vitro reconstruction of fungal chromosomes and genomes. I. A physical map of the entire Aspergillus nidulans genome

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Physical maps of fungal genomes provide new research strategies for a wide range of fundamental biological problems, for engineering the production of new pharmaceuticals, and for understanding the cause of fungal diseases in plants and animals. The physical maps reported here represent the in vitro reconstruction of entire chromosomes from recombinant DNA libraries and provide useful tools to clone genes, determine genomic structure, and study genome evolution. We report a 29 kb resolution physical map of the entire 31 Mb genome of Aspergillus nidulans reconstructed from a 5134 clone cosmid library using 648 probes. The maps are the result of a novel two-way ordering process in which overlapping clones (redundant) and non-overlapping probes (tiles) are ordered to span the entire genome. The physical map is composed of eight matrices with clones down the rows and probes across the columns, one for each chromosome. The redundant order of clones contains 4550 anchored clones (89% of the cosmid library) into 132 contigs with an average of 17 contigs per chromosome. The compressed map (providing a minimum tiling of all 8 chromosomes) is reduced 5-fold in redundancy and contains 1085 clones. By integrating the physical and genetic maps with chromosome and clone hybridization data, we found that repeated DNA sequences are non-randomly distributed along chromosomes in a way reminiscent of heterochromatic banding patterns on cytological maps in other eukaryotes.

Interaction between specific induction and carbon catabolite repression in the ethanol regulon in A. nidulans

B atrice Felenbok, Cristina Panozzo, Sabine Fillinger, Martine Mathieu and Veronique Capuano, Universit Paris-Sud, Orsay

A. nidulans is able to use a wide variety of compounds as sole carbon sources. These pathways have in common the involvement of two transcriptiona1 regulatory circuits. The first one is specific induction mediated by trans-activators which usually belong to the zinc binuclear cluster family (C6 class). The second is carbon catabolite repression, controlled by thw wide domain repressoor CreA, which contains two zinc fingers of the C2H2 class. In the ethanol utilization paathway, specific induction proceeds by the binding of the AlcR activator to a number of specific targets localized to the alcR promoter itself, the alcR gene being positively autoregulated, and to the promoters of structural genes under its control e.g. alcA. Two types of targets, direct and invert repeats containing the same consensus core, are both necessary for full transcriptional induction. The high strength of the alcA promoter results from the synergistic transciptional activation by AlcR mediated by the multiple binding sites The carbon catabolite repressor, CreA, exerts a double transcriptional repression, on the trans-acting gene alcR and independently on structural genes. A great number of CreA binding sites have been identified in the cis-acting regions of the alcR and alcA genes. The disruption of different functional CreA targets all result in superinduced and derepressed alcR and alcA expression. Those which overlap or are in very close proximity to AlcR targets account for a direct competition between AlcR and CreA for the same cis-acting region. The interplay between these two regulatory circuits regulates the expression of the ethanol

regulon genes under all physiological conditions.

Promoter analysis of the Neurospora crassa circadian clock-controlled ccg-2 (eas) gene

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The N. crassa ccg-2 gene encoding a fungal hydrophobin is transcriptionally regulated by the circadian clock. In addition, ccg-2 is positively regulated by light, and transcripts accumulate during asexual development. To sort out the basis of this complex regulation, deletion analysis of the ccg-2 promoter was carried out to localize the cis-acting elements mediating clock, light, and developmental control. A distinct positive clock element was localized to within a 45 nt region, just upstream of the TATA box. Using an unregulated promoter/reporter system we show that this element is necessary and sufficient for conferring clock regulation on the ccg-2 gene. We are currently using this element as a probe in gel-mobility shift assays to identify trans-acting clock factors.

The relationship between DNA methylation and transcription in Neurospora crassa

Michael Rountree and Eric Selker. University of Oregon.
A correlation between DNA methylation and lack of gene activity has been observed in animal, plant, and fungal systems. Our understanding of the relationship between methylation and transcription remains limited, however. Exploration of this relationship in Neurospora is facilitated by RIP (repeat induced point mutation), which frequently results in methylation of the mutated sequences and by a mutation (dim-2) that appears to prevent all DNA methylation. Using dim-2 and the methylation inhibitor 5-azacytidine, we found that the methylation associated with RIPed genes, and their surrounding sequences, results in a reduction (or abolishment) of stable transcripts. Results of our study also revealed that mutations by RIP can affect the length of transcripts produced from an affected gene. In addition, we determined that the methylation associated with the tandemly repeated rDNA in Neurospora does not affect rRNA levels, probably because the methylation is confined to the non-transcribed spacer regions. We are currently exploring the mechanism by which DNA methylation reduces transcript accumulation. In separate experiments to examine the possible effect of transcription on methylation, we found that placement of an intact promoter in or next to a methylated region does not prevent methylation. Methylation of a region was reduced by placing it upstream of the am gene, however. We are dissecting the upstream am region to determine the cause of this effect.

Substrate specificity and transcriptional regulation of purine permeases in Aspergillus nidulans

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There are three purine permeases in Aspergillus nidulans. One, encoded by the uapA gene, is specific for uric acid and xanthine uptake, a second, encoded by the azgA gene, is specific for hypoxanthine, adenine and guanine, while a third, encoded by the uapC

gene, is a broad specificity permease able to incorporate all purines into the cell. Early genetical data established that uapA and uapC are under the control of the specific transcription factor UaY, mediating uric acid induction, and of the GATA-like factor AreA, mediating nitrogen metabolite repression. The azgA gene is neither under UaY or AreA control. UaY mediates uric acid induction of at least nine genes of the purine assimilation pathway. There are striking similarities between UaY and the Saccharomyces cerevisiae protein PPR1, involved in the regulation of pyrimidine biosynthesis. Both proteins bind to 5'CGG-6X-CCG sequences. uapA and uapC have been cloned and sequenced. The binding sites for UaY and AreA on the uapA and uapC promoters have been established by gel retardation and footprinting techniques. An allele of areA, which changes a conserved leucine to a valine residue in the DNA binding domain, reduces drastically and specifically the expression of uapA and uapC. Suppressors specific for either gene have been isolated, characterised and sequenced. These provide a quite detailed picture of the structure of the uapA and uapC promoters. The uapA and uapC open reading frames reveal typical membrane proteins of superimposable hydropathicity profiles and show a 58% identity. The construction of hybrid proteins and mutagenesis of selected hydrophilic regions is been used to determine the residues involved in substrate recognition.

Mechanism of coordinate regulation of ribosomal genes by nutrition and growth in Neurospora

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In Neurospora crassa, transcription of the large (40S) ribsomal RNA genes by RNA polymerase I and of the ribosomal protein genes by RNA polymerase II are coordinately regulated by the carbon supply. Transcription of the 5S rRNA genes by RNA polymerase III however is largely insensitive to regulation by carbon or growth. We have characterized in detail sequences required for transcription and carbon regulation of the ribosomal protein gene crp-2. The crp-2 promoter contains at least six specific elements within 250 bp of the transcription startpoint. None are similar to UASrpg sequences required for transcription of yeast ribosomal protein genes. Two elements (CGrepeat and Dde box) occur together as a pair twice in the crp-2 promoter. Variations of these elements occur in all 5 ribosomal protein gene promoters examined, and in most cases, the two elements occur as a pair close to -70 with conserved spacing. This pair of elements also occurs with the same spacing within a functional domain of the 40S rRNA promoter, suggesting that the pair is repsonsible for coordinating rRNA and r protein gene expression. The CGrepeat/Dde box pair also corresponds to the region responsible for carbon regulation of crp-2 The CG-repeat binds the Aspergillus carbon catabolite repressor creA. Therefore we have isolated cre-1, the Neurospora homolog of creA, and are investigating whether it is responsible for coordinate regulation of the r-protein and rRNA genes by carbon, mediated via the CGrepeat/Dde box pair.

Regulation of sulfur metabolism in Neurospora crassa

John V. Paietta, Wright State University.

The sulfur regulatory system of N. crassa is composed of a group of highly regulated structural genes (e.g., arylsulfatase, ars-1+) which are under coordinate control of the cys-3+ positive and scon+ (sulfur controller) negative regulatory genes. The CYS3 regulator is a bZIP DNA-binding protein that is necessary and sufficient to induce sulfur structural gene expression. Experiments modifying the dimerization specificity of CYS3, through leucine zipper alterations, demonstrate that CYS3 functions in vivo as a homodimer. The cys-3+ gene is also subject to autoregulation. In vitro studies have defined CYS3 binding sites on the structural and regulatory genes in the system. In vivo studies with the ars-1+ promoter have confirmed the importance of the putative CYS3 binding sites. The ars-1+ promoter constructs included deletions and site-directed alterations to control elements which were assayed following integration at a defined chromosomal site. In addition, a heterologous promoter construct has been used to demonstrate the minimum sequence necessary for CYS3 controlled expression. The scon-2+ negative regulatory gene has been characterized and encodes a beta-transducin homolog with six WD-40 repeats. SCON2 potentially defines a new subclass of WD proteins. Using band shift and Northern blot analysis a control loop has been identified between cys-3+ and scon-2+.

Posters I: Gene Expression/Genome Structure

1. Aspergillus niger cytochrome P450 reductase (cprA)

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To improve activity of cytochrome P450 enzymes in Aspergillus niger we have cloned and overexpressed the cytochrome P450 reductase encoding gene (cprA) from A. niger. The gene was overexpressed in strains having multiple copies of two fungal cytochrome P450 genes. The highly substrate inducible A. niger bphA gene encodes a cytochrome P450 involved in the conversion of benzoate to 4-OH-benzoate, the P. italicum P45014dm gene encodes lanosterol 14(alpha) demethylase. Transformants having multiple copies of both the P450 gene and the cprA gene had highly increased enzymatic activities as compared to transformants which had an identical cytochrome P450 gene copy number but only the wildtype cprA gene. This clearly shows that cprA expression levels can be limiting in cytochrome P450 overexpressing filamentous fungi. Induction of expression of the cprA gene by a number of generally used important inducers of mammalian cytochrome P450s was studied. None of these well known inducers had an effect on the expression levels of the cprA gene. However, the use of benzoic acid, the inducer of the highly regulated cytochrome P450 bphA gene, resulted in a 2-4 fold induction of CPR activity. This results clearly indicate the presence of co-induction of the two components of this P450 enzyme system. At present we are investigating the DNA elements involved in this co-regulation event.

2. Cloning of the ornithine transaminase gene from Aspergillus nidulans

Agnieszka Dzikowska, Marta Wisniewska and Piotr Weglenski. Department of Genetics,

Warsaw University.
In Aspergillus nidulans arginine can serve as a source of proline. It depends on the presence and inducibility of arginine catabolic enzymes, arginase and OTAse, which are coded by agaA and otaA genes, respectively. Both agaA and otaA genes are subject to nitrogen and carbon catabolite repression. In A. nidulans these two kinds of general control are mediated by AreA and CreA gene products. The otaA gene has been cloned by transformation of the A. nidulans with cosmid gene library. The gene contains two putative introns and codes for a 453aa long protein. The protein shows a high degree of homology to other known ornithine transaminases. There is evidence that otaA gene is controlled at the transcriptional level.

3. Gene expression studies in Acremonium chrysogenum: promoter analysis using reporter gene systems

Renate Radzio, Jorg Nosek, Stefan Loseke & Ulrich Kuck. Lehrstuhl fur Allgemeine Botanik, Ruhr- Universitat Bochum, 44780 Bochum, Germany
Acremonium chrysogenum (syn. Cephalosporium acremonium) is the most important industrial producer of the beta-lactam antibiotic cephalosporin C. The pcbAB/pcbC and cefEF/cefG genes, encoding key enzymes of the cephalosporin C biosynthetic pathway, are organized in clusters in this fungal genome [1]. A detailed promoter analysis is required to understand the molecular mechanisms controlling cephalosporin C gene expression. The relative promoter strength of the above mentioned genes was initially evaluated by fusing the intergenic regions with reporter genes. We have developed a system that allows the simultaneous detection of promoter strength from two divergently orientated genes in Acremonium chrysogenum. This system is based on transcriptional fusions of pcbAB/pcbC and cefEF/cefG intergenic regions with the bacterial reporter genes lacZ and gusA. Quantitative tests of reporter gene expression revealed that the pcbC promoter expression is significantly stronger than the pcbAB promoter expression [2]. For this reason the pcbC promoter was chosen for a detailed deletion analysis. Similarly, the cefEF/cefG promoter region was also investigated in expression studies using chimeric reporter gene constructs. In addition, the reporter gene system described above is suitable for the investigation of unknown promoter sequences and we have developed a rapid test system for the detection of functional DNA sequences mediating promoter activity. [l] Smith DJ, Burnham MKR, Bull JH, Hodgson JE, Ward JM, Browne P, Brown J, Barton B, Earl AJ, Turner G (1990) EMBO J 9:741-747 and [2] Menne S, Walz M, Kuck U (1994) Appl Microbiol Biotechnol (in press).

4. Inviability among Aspergillus nidulans transformants containing multiple copies of the selectable marker pyr-4

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Transformation of a haploid Aspergillus nidulans pyrG auxotrophic strain (1-85) with a vector containing the heterologous Neurospora crassa pyr4 gene (pGM32) resulted in strong growing transformants that could be classified into two groups based on morphology. The minority were morphologically very similar to the parental strain,

easily purified and mitotically stable. The majority (10 times more frequent) appeared to be mitotically unstable and were subsequently shown to be heterokaryons that could not be resolved into homokaryons. The ratio of the two types of transformants depended on several parameters such as the protoplast preparation, addition of spermidine to the transformation mix, the conformation of the transforming vector and the concentration of DNA in the transformation mix. Genetic analysis of a random sample of both types of transformants indicated the difference was due to the number of copies of the integrated vector. The heterokaryons were shown to have a high copy number in a small proportion of the nuclei while the homokaryons had a low copy number in all nuclei. This suggests that multiple copies of pyr-4 are lethal. These results were obtained as part of a study investigating the effect of various parameters on the frequency of gene targeting in filamentous fungi using Aspergillus nidulans as a model organism. The well characterised loci of niaD and amdS were targeted using the pGM32 vector containing an internal fragment of the targeted gene. Disruption could be screened phenotypically by loss of function. Parameters investigated include (a) length of homologous DNA in the disruption cassette and (b) transcriptional status (on/off) of the targeted gene.

5. Disruption of hapC, the Aspergillus nidulans homologue of the Saccharomyces cerevisiae HAP3 gene, is lethal

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DNA binding factors that recognise the core sequence CCAAT have been found in many eukaryotic systems and regulate a wide range of processes, including respiration and immunoglobulin production. The 5' regulatory region of the amdS gene of Aspergillus nidulans contains a CCAAT sequence which is required for setting the basal level of amdS transcription. Mobility shift studies have identified a factor in A. nidulans nuclear extracts which binds to this CCAAT sequence. The gene(s) encoding this factor have yet to be identified. In Saccharomyces cerevisiae the HAP3 gene encodes one component of a multisubunit complex which binds CCAAT sequences. Genes with homology to HAP3 have been isolated from a number of species. A search of the Genbank database has found an A. nidulans sequence with significant homology to the HAP3 gene adjacent to the previously cloned regulatory gene amdR. Sequencing of the remainder of this region has revealed a gene with extensive homology to HAP3. This gene has been named hapC. hapC cDNA clones have been isolated and sequencing has revealed three introns in the coding region of hapC and one in the 5' untranslated region. Disruption of hapC is lethal in haploids and this lethality can be reversed by providing a complete copy of the hapC gene in trans. This finding indicates that hapC is an essential gene in A. nidulans. The function of hapC in A. nidulans and its possible role in the regulation of the amdS gene is being investigated.

6. Activity of N-acetylglutamate synthase requires N-acetylglutamate kinase polypeptide in Neurospora

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The arg-14 locus of Neurospora crassa encodes acetylglutamate synthase (AGS), which catalyzes the first step of arginine biosynthesis. AGS activity is also defective in some of arg-6 mutant strains. The arg-6 gene produces two mitochondrial enzymes, acetylglutamate kinase (AGK) and acetylglutamyl-phosphate reductase (AGPR), the second and the third enzymes of the same pathway. We have reexamined the three reported complementation groups of arg-6, A (AGK-, AGPR+), B (AGK+, AGPR-), and NC (AGK-, AGPR-). We found AGS+ members in both group A (K- R+ S+) and B (K+ R- S+), and identified two new AGS- groups, A' (K- R+ S-) and B' (K+R- S-). We also found a new AB group (K+ R+ S-) which complements group A, B, and arg-14, but not groups A', B', or NC. Existence of the extended groups indicates that arg-6 gene products play roles in AGS activity, although neither AGK nor AGPR enzyme activity is necessary for AGS enzyme activity. Transformation of a NC (K- R- S-) strain with the cloned arg-6+ gene restored AGS activity as well as AGK and AGPR activity. Transformation of an A' strain with pAKH4 that produces only the AGK polypeptide restored AGS activity. Thus, the AGK protein can activate AGS. Evidence for physical contact between AGK and AGS using the yeast two-hybrid system were described.

7. Conversion of Fusarium oxysporum pro-trypsin to active trypsin in Aspergillus oryzae by co-expression with a Fusarium metalloprotease

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Proteases are often encoded as zymogens containing an N-terminal polypeptide pro-region that results in an in active form of the enzyme. A secreted trypsin from Fusarium oxysporum contains a five amino acid pro-peptide that is proteolytically processed to yield the active enzyme in Fusarium. When the gene encoding this pro-trypsin is expressed in the heterologous host, Aspergillus oryzae, processing of the pro-region is inefficient resulting in a mixture of pro-trypsin and active enzyme. We have purified a secreted metalloprotease from F. oxysporum based on its ability to process pro-trypsin to active enzyme in vitro. The corresponding gene has been cloned, and co- expressed along with the Fusarium pro-trypsin gene in the host, A. oryzae. The addition of the metalloprotease gene resulted in a 3.5-fold increase in the production of active trypsin. We conclude that the production of an enzyme activity from a heterologous host organism can be significantly enhanced by the co-expression of the pro-enzyme along with a "maturase" activity that has also been derived from the same source organism.

8. Extracellular polysaccharidases of Neurospora

Alan Radford, Paul Stone, Fawzi Taleb and J. Howard Parish, University of Leeds.
Enzymatic studies have shown glucoamylase and a cellulase complex in Neurospora. Two genes, gla-1 (glucoamylase) and cbh-1 (cellobiohydrolase) have been cloned and sequenced. There is close sequence homology between these and equivalents from other filamentous fungi including the ascomycete-like deuteromycetes Trichoderma, Humicola, and the basidiomycete Phanetochaete. Constructs containing both gla-1 and cbh-1 have been made and transformed back into Neurospora, using hygromycin as the selectable resistance marker. Representative transformants have subsequently been crossed to an

Oak Ridge wild-type, and meiotic progeny isolated. These progeny have been screened for RIP derivatives, by screening halo sizes on media containing starch (subsequently stained with iodine in potassium iodide) for gla-1 and carboxymethyl-cellulose (stained with Congo Red) for cbh-1. RIPed gla-1 and cbh-1 progeny have both been obtained. They have reduced or absent halos, poor growth on starch or cellulose respectively, and altered DNA sequence as detected by Southern blotting and analysis of restriction fragments. Representative gla-1 and cbh-1 mutants have been deposited in FGSC.

9. Molecular cloning of an initiation factor kinase (eIF-2alpha-kinase) of Neurospora crassa

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Phosphorylation of eIF-2alpha, the a subunit of the eukaryotic initiation factor 2, is one of the best characterized mechanism of translational control in eukaryotic cells. Dependent on the organism eIF-2alpha kinases are activated by distinct stimuli, e. g. hemin deficiency, double-stranded RNA or amino acid starvation. We identified a Neurospora eIF-2alpha kinase sequence via homology with known eIF-2alpha kinases especially that of GCN2 in Saccharomyces cerevisiae. GCN2 is an essential control element in the mechanism of general control of amino acid biosyntheses. The Neurospora sequence contains - like GCN2 - in close proximity to the kinase domain a second domain with homology to histidyl-tRNA synthetases. According to the working model of A. Hinnebusch and collaborators the synthetase interacts with uncharged tRNAs as the signal for amino acid limitation which in turn activates the kinase. We intend to disrupt the gene to examine its function in Neurospora crassa.

10. Restriction endonuclease analysis of mitochondrlal DNA of Metarhizium anisopliae strains

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The species Metarhizium anisopliae contains two recognized varieties, M. anisopliae var. anisopliae and M. anisopliae var. majus, which mainly differ in spore size. M. anisopliae var. anisopliae isolated from various geographical locations is currently being used for the biological control of insects. Mitochondrial DNAs from strains A4, A19, AL, E6, E9 and MT of M. anisopliae var. anisopliae, collected from several Brazilian States, were isolated and characterized by restriction-endonucleases. The banding pattern obtained was the same for all strains investigated, indicating that their mtDNA have the same molecular sizes (about 36 Kb) and the same restriction sites for the enzymes used. Mitochondrial DNA from a strain of M. anisopliae var. majus showed an electrophoretic pattern that differed markedly from that of var. anisopliae. Thus, the restriction pattern of mtDNA may serve as a specific marker in the identification of var. anisopliae or of some subpopulation of this variety. Financial Support: FAPESP, CAPES and CNPq.

11. Acid phosphatase occurs in multiple active forms in the mold Neurospora crassa

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Acid phosphatase excreted by both pregC (a constitutive mutant for Pi-repressible phosphatases) and wild-type strains of the mold Neurospora crassa grown at pH 5.4 consists of multiple active forms, as demonstrated by isoelectric focusing (IEF). The enzyme components were visualized by activity staining using alpha-naphthylphosphate in the presence of Fast Garnet GBC salt and found to be predominantly acidic proteins with isoelectric points in the range of pH 4.0 to 7.2. In addition, the population of active isoforms excreted by pregC was altered as a function of time of growth and variations in extracellular phosphate concentration, suggesting that this constitutive mutant is still sensing Pi levels. Financial support: FAPESP, CNPq, CAPES and IDB-USP

12. Expression of yeast citrate synthase gene in Aspergillus nidulans

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The coding region of the CIT1 gene for mitochondrial citrate synthase (CSl) from Saccharomyces cerevisiae was amplified by PCR and cloned into an Aspergillus nidulans expression vector, pAL4, downstream of the alcohol dehydrogenase (alcA) promoter to yield pALCSl. In addition, deletion of the N-terminal mitochondrial targeting signal from the CIT1 gene was performed by PCR and the mutant gene (CIT1delta) was cloned as the wild type CIT1 gene, yielding pALCSldelta. After transforming into A. nidulans, both constructs gave stable transformants that were phenotypically stable for several mitotic divisions. Southern blot analysis of the DNA from the transformants showed that both the CIT1 and CIT1 genes were successfully integrated into the chromosomes of A. nidulans. Western blot analysis and enzyme assay for citrate synthase activity proved that both the integrated genes are effectively expressed by the alcA promoter induced by ethanol or threonine.

13. Identification and characterization of alternative oxidase gene and its upstream regulatory sequence in Neurospora

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Mitochondria of Neurospora, like certain other fungi, algae, protists and most plants, contain a cyanide- insensitive alternative pathway of respiration in addition to the standard electron transport chain. In Neurospora, the alternative oxidase is only active (or induced) when electron flow through the cytochrome chain is restricted. Thus the regulation of the alternative oxidase gene(s) is of particular interest as it may provide a model system for studying the mechanism of communication between mitochondria and nucleus. By using degenerate PCR primers, derived from the most conserved regions of alternative oxidase proteins from other species, we have isolated and sequenced both genomic and cDNA for the alternative oxidase of Neurospora and have mapped the transcription start site. We have also shown that the sequence of the Neurospora protein is homologous to that of other species and the induction of its expression is at the transcriptional level. Mutants deficient in the alternative oxidase contain mutations causing amino acid substitutions in the coding sequence of the alternative oxidase gene.

We are currently attempting to identify the regulatory element(s) responsible for the induction of alternative oxidase.

14. Reverse transcriptase activity of an intron-encoded polypeptide of Podospora anserina

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A number of group II introns from eukaryotic organelles and prokaryotes contain open reading frames for polypeptides with homology to reverse transcriptases (RTs). We have used a yeast system to express intronic RT- ORFs from eukaryotic organelles (1). This include the mitochondrial coxI intron il from the fungus Podospora anserina, the plastid petD intron from the alga Scenedesmus obliquus and the mitochondrial RTL gene from the alga Chlamydomonas reinhardtii (2). The ORFs were fused with the TYA ORF from the yeast transposon Ty to produce virus-like particles in the recipient yeast strains with detectable amounts of RT-like polypeptides. Analysis of the heterologous gene products revealed biochemical evidence that the P. anserina coxI intron encodes an RNA-directed DNA polymerase with properties typically found for RTs of mobile elements. The direct biochemical evidence of an intron-encoded RT supports the idea that RTs are involved in intron mobility.
(1) Muller et al. (1987), Mol Gen Genet 207:421-429
(2) Fassbender et al. (1994), EMBO J 13:2075-2083.

15. The cloning and characterization of the Neurospora crassa catalase gene, cat-1

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The filamentous fungus Neurospora crassa is believed to possess at least three biochemically and genetically distinct catalases (Cat-1, Cat-2, Cat-3), which are expressed differentially during development. An effort to clone the Neurospora crassa genes was undertaken using a labeled fragment from the Aspergillus niger catalase-R (catR) gene to probe the Orbach-Sachs cosmid library of Neurospora crassa. Overlapping cosmids were identified as having sequences that hybridized to the catR gene. A 7.7 kb KpnI fragment containing the putative catalase gene was subcloned into pUC-18 and sequenced. Analysis of this sequence revealed a strong similarity with catR and other catalase genes. This fragment was found to contain the entire catalase structural gene as well extensive upstream and downstream regions. Multicent (RFLP) mapping has tentatively identified this new catalase gene as cat-1 (LG IIIR). We are attempting to confirm this by examining mRNA and enzyme levels in N. crassa cells at different developmental stages. Similarities in the structure and amino acid sequences of catalases of N. crassa, A. niger, and E. coli suggest the existence of a separate, and previously unrecognized, catalase sub-family.

16. Structure, inheritance, and transcriptional effects of Pce1, an insertional mutation of Phanerochaete chrysosporium lignin peroxidase gene lipI

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An insertional mutation within a lignin peroxidase allele of Phanerochaete chrysosporium strain BKM-F-1767 has been characterized. The 1747 bp element, Pce1, lies immediately adjacent to the fourth intron of lipI2. Southern blots reveal the presence of Pce1-homologous sequences in other P. chrysosporium strains. Transposon-like features include inverted terminal repeats and a dinucleotide (TA) target duplication. Atypical of transposons, Pce1 is present at very low copy numbers (1-5), and conserved transposase motifs (Doak et al 1994 Proc. Nat. Acad. Sci. 91:942-946) are lacking. The mutation transcriptionally inactivates lipI2, and it is inherited in a 1:1 Mendelian fashion among haploid progeny. Thus, Pce1 is a transposon-like element which may play a significant role in generating ligninolytic variation in P. chrysosporium.

17. Cloning and molecular characterization of the genes encoding extracellular phenoloxidases from Myceliophthora thermophila and Scytalidium thermophilum

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Genomic DNA fragments encoding extracellular phenoloxidases (laccases) have been isolated from two thermophilic fungi, Myceliophthora thermophila and Scytalidium thermophilum. By DNA and peptide sequencing techniques we have deduced the primary structure of these two genes and their polypeptide products. The gene encoding M. thermophila laccase is composed of seven exons (246, 79, 12, 70, 973, 69 and 411 nt) interrupted by six introns (85, 84, 102, 72, 147, and 93 nt). The coding region, excluding intervening sequences, is rich in guanosine and cytosine residues (65.5% G + C) and encodes a preproenzyme of 620 aa (22 aa signal peptide, 25 aa propeptide, mature laccase comprising 573 aa). The gene encoding S. thermophilum laccase is also GC-rich (60.8% G + C) and comprises five exons (243, 91, 70, 1054, and 390 nt) punctuated by four small introns (63, 58, 55, and 65 nt). Like the Myceliophthora enzyme, Scytalidium laccase appears to be synthesized as a preproenzyme (616 aa) with a 21 aa signal peptide and a propeptide of 24 aa. The deduced amino acid sequences of both laccases are approximately 60% identical to Neurospora crassa laccase. The sequence similarity is highest in regions that correspond to the four histidines and one cysteine which are involved in formation of the trinuclear copper cluster at the active site of the enzyme.

18. Cloning of the cpc-1/GCN4 homolog of Aspergillus niger

Christoph Wanke, Sabine Eckert, and Gerhard H. Braus, Friedrich-Alexander-University, Dept. of Biochemistry, Staudtstr. 5, D-91059 Erlangen, Germany.
We have cloned the Aspergillus niger gene encoding the transcriptional activator protein responsible for the general control ("cross-pathway control") of amino acid biosynthesis. The cDNA clone regulated by a heterologous yeast promoter was able to complement a S. cerevisiae gcn4 mutant strain. The cDNA clone was used as probe to clone the corresponding chromosomal locus which we have named gcnA. The nucleotide sequence of both clones revealed significant similarity to the yeast GCN4 and the N. crassa cpc-1 gene. The gcnA protein contains an apparently degenerate leucine zipper motif with only

a single leucine residue. Gene disruption experiments and the study of gene regulation are on their way.

19. Polyamines regulate the stability of ornithine decarboxylase mRNA in Neurospora crassa

Mariya Broun, Martin A. Hoyt, and Rowland H. Davis. University of California, Irvine.
Ornithine decarboxylase (ODC) initiates the synthesis of polyamines and is encoded by spe-1 gene. ODC is highly regulated by polyamines, but in contrast to other organisms, the variation of the synthetic rate of ODC in Neurospora crassa (5 to 7-fold) is correlated with a similar variation in the abundance of spe-1 mRNA. This correlation suggested that spe-1 mRNA abundance was transcriptionally controlled. Run-on transcription experiments, however, showed that the transcription rate of spe-1 mRNA does not vary in response to the polyamines. The stability of spe-1 messenger was then measured by using the transcriptional inhibitor thiolutin. The stability of spe-1 mRNA was different in the presence and the absence of polyamines, and the difference accounts adequately for the difference in the steady-state levels of spe-1 mRNA in the two conditions. The stabilization of spe-1 mRNA in the absence of polyamines was accompanied by an increase in the size of spe-1 mRNA polysomes. Decreasing the translational elongation rate with low concentrations of cycloheximide, leading to larger spe-1 mRNA polysomes, also led to a substantial increase of spe-1 mRNA abundance. This fact suggests the participation of protein synthesis in polyamine-mediated regulation of spe-1 mRNA turnover. (Supported by NIH Grant GM35120 to R. H. D.)

20. Removal of the 5' untranslated leader increases the abundance and ribosomal loading of spe-1 mRNA in Neurospora crassa

Martin A. Hoyt, Mariya Broun, Laura J. Williams, and Rowland H. Davis, University of California, Irvine.
The spe-1 gene of Neurospora crassa encodes ornithine decarboxylase (ODC), a key regulatory enzyme in the synthesis of polyamines (putrescine, spermidine and spermine). Starvation for polyamines leads to concomitant increases in ODC activity and spe-1 mRNA abundance. The increase in spe-1 mRNA abundance in this condition is due to stabilization of spe-1 mRNA. The increase in spe-1 mRNA abundance is correlated with an increase in the size of spe-1 mRNA polysomes. The spe-1 mRNA has an unusually long (535 nucleotides) untranslated leader with no obvious secondary structure or open reading frame. The removal of nucleotides +9 to +486 of the leader also leads to an increase in spe-1 mRNA abundance and an increase in polysome size. Deletions show that this effect is localized to the first 315 nucleotides of the leader. However, substantial regulation of spe-1 mRNA turnover by polyamines remains in constructs lacking the leader. We now wish to know the extent to which the increases in spe-1 mRNA abundance following polyamine starvation and following removal of the spe-1 mRNA leader share mechanistic features, such as protection by ribosomes, and in what way these increases arise from distinct mechanisms. (Supported by NIH Grant GM35120 to R. H. D.)

21. Transformation of Neurospora crassa with the Aspergillus nidulans creA gene

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The qa gene in N. crassa is subject to carbon catabolite repression in wild type. These studies have involved transformation with a plasmid which contains the creA gene which is involved in carbon catabolite repression in A. nidulans and the qa-2 gene from N. crassa as a selectable marker. Transformants were selected in a qa-2 mutant background. These transformants were crossed to met-7 (a very tightly linked gene to the qa cluster) to obtain homokaryotic isolates with duplicated copies of the qa-2 gene and the creA gene and its N. crassa homologues. Inactivation (RIPing) of the qa-2 gene was detected in tetrads when two isolates carrying the transformed DNA were crossed. These strains no longer had catabolic dehydroquinase activity as determined by enzyme assays. DNA from these tetrad isolates was digested with NdeII and Sau3A and the Southern blot was probed with a 32P labeled creA gene. These results indicated that the A. nidulans creA gene had been inactivated (methylated). Northern blot analyses of RNA's from these tetrad isolates grown on dextrose plus quinic acid (carbon repression conditions) and quinic acid (induction conditions) indicated that qa gene expression was still carbon repressed. These studies indicated that the mutant creA gene had no affect on relieving carbon repression of the qa gene cluster.

22. Hsp80 of Neurospora crassa as a molecular chaperone of cellular protein folding

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The heat shock response of N. crassa is characterized by the rapid and transient production of a number of heat shock proteins (HSPs) along with the cessation of synthesis of a fraction of the normal cellular proteins. The most abundant stress protein, HSP80, is synthesized at high levels on exposure to heat shock or carbon source starvation. NAD-specific glutamate dehydrogenase of N. crassa is also induced by carbon-catabolite repression but gdh gene expression is switched off during heat shock. During growth under carbon starvation conditions, however, both gdh and hsp80 genes are expressed. To assess the role of HSP80 as a chaperone of cellular protein folding, denaturation/renaturation of NAD-GDH was investigated in vitro, in the presence and absence of HSP80. Purified preparations of HSP80 and GDH were employed in conjunction with guanidine hydrochloride as a protein denaturant. Our experiments show that the presence of HSP80 reduces the rate of GDH denaturation. In addition, renaturation of denatured enzyme is enhanced by HSP80, suggesting the capacity of this stress protein to act as a molecular chaperone.

23. Regulation of genes for amino acid biosynthesis in Magnaporthe grisea

Wei-Chiang Shen and Daniel Ebbole. Texas A&M University
Magnaporthe grisea is the causal agent of rice blast disease. To understand how this organism is able to infect plants it is important to determine which aspects of the biology of M. grisea differ from non-pathogenic fungi. We expect conservation of mechanisms for regulation of genes of primary metabolism in pathogenic and non-pathogenic fungi. However, data supporting this assumption are lacking. To examine control of amino acid

biosynthesis, we have cloned the M. grisea homolog of Neurospora crassa arg-2. Control of N. crassa arg-2 expression is complex, the gene is transcriptionally regulated by cross-pathway control and translationally regulated by a cis-acting upstream open reading frame. We provide evidence that transcriptional and translational regulation of the M. grisea gene is likely identical to the regulation of the arg-2 gene of N. crassa.

24. Analysis of the Neurospora con-10 promoter

Kwangwon Lee and Daniel Ebbole. Texas A&M University.
As an approach to understanding the regulation of gene expression in filamentous fungi, we are studying the promotor region of con-10 gene, a gene that is expressed preferentially during spore formation in Neurospora. Initial studies showed that there are at least four segments in the promotor that are involved in expression of con-10. Using site-directed mutagenesis, we have further defined the sequences that are involved in con-10 expression. The expression of con-10 is regulated not only by developmental signals such as conidiation, but also by environmental signals such as light. It appears that there are factors both of repression and of activation involved in con-10 expression. Our initial questions are 1) What are the promotor elements involved in repression and activation of con- 10? 2) Do different induction cues use common regulatory components? 3) Do developmental and environmental signalling pathways influence each other?

25. Isolation of the fluffy and fluffyoid genes of Neurospora crassa

Lori Bailey and Daniel Ebbole. Texas A&M University.
Macroconidiation in Neurospora begins with the production of aerial hyphae from mycelia. The aerial hyphae undergo apical budding to form minor and major constriction chains. Septation of the procondial chain is followed by maturation and release of free conidia. Fluffy, a Neurospora conidiation mutant isolated in 1933 by Carl Lindegren, is blocked at the minor constriction stage. Fluffy does not form free conidia under the most permissive conditions for development. Fluffyoid, another conidiation mutant, is blocked at an earlier stage than fluffy. However, fluffyoid does produce abundant proconidial chains under carbon starvation. We have begun chromosome walking experiments to isolate the fluffy and fluffyoid genes.

26. Molecular dissection of NIT4 reveals multiple functional domains and a novel leucine- rich activation motif

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NIT4 is pathway-specific transcriptional activator of nitrate assimilation in Neurospora crassa. NIT4 positively regulates nit3 and nit6 genes specifying nitrate reductase and nitrite reductase, respectively. To understand the mechanism by which NIT4 acts, we took several approaches to dissect the functional domains of the NIT4 protein. By fusing different NIT4 regions to the GAL4 DNA-binding domain, three transcriptional activation domains were identified by their ability to activate transcription in yeast. These three acitivation domains of NIT4 are scattered within the C-terminal 400 residues and

are both structurally and functionally separable from the DNA-binding domain. A novel 28 residue leucine-rich activation domain was identified at the C-terminus of NIT4, which alone showed strong transcription activation potency. However, NIT4's full activity requires all three activation domains. A dimerization region of NIT4 was identified within residues 109-144. NIT4 homodimer formation was confirmed both by chemical crosslinking in vitro and with the yeast two- hybrid system in vivo. The importance of the internal region of NIT4 was shown by deletions which caused complete loss of function. Previous experiments have suggested that the NIT3 protein autogenouly controls its expression by binding to the NIT4 protein. A possible physical interaction between the NIT4 and NIT3 proteins was tested by yeast two-hybrid system as well as by the GST fusion. Neither experimental approach revealed any evidence for NIT4-NIT3 protein-protein interaction.

27. The frequency locus encodes a central component of the circadian clock, the level of which is rapidly reset by light

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Based on its genetics, the frequency (frq) locus has been proposed as a key component in the cellular oscillator generating circadian rhythmicity (Dunlap, Ann. Rev. Physiol. 55:683-728, 1993). Several testable predictions have been made regarding the regulation of genuine components (state variables) of the clock, and frq encodes a factor satisfying all of these criteria (Aronson, Johnson, Loros, and Dunlap, SCIENCE 263:1578-1584, 1994). frq encodes a central component of a molecular feedback loop in which the product of frq depresses the level of its own transcript, resulting in a daily oscillation in the level of this frq transcript. Rhythmic frq mRNA expression is essential for overt circadian rhythmicity: constitutively elevated expression of FRQ-encoding RNA in a frq+ background results in arrhythmicity, and no level of constitutive expression is capable of rescuing normal rhythmicity in frq loss-of-function mutants. Step reductions in frq transcript levels at any time in such constitutively elevated strains sets the clock to a unique and predicted phase. Recent data (Merrow and Dunlap, EMBO J. 13:2257-2266, 1994) also show phylogenetic conservation of frq structure and function. Finally, it is now clear that light acts rapidly to increase the level of transcript(s) arising from frq, consistent with a model in which elevation of the level of frq transcript(s) in the cell is the initial clock-specific event involved in resetting of the clock by light. These data support a model where the Neurospora circadian clock consists of a negative feedback loop in which the product of the frq gene regulates the level of the transcript(s) of the frq gene.

28. The ccg-1 gene of Neurospora displays multiple levels of regulation including the clock, development, light and heat shock

Jennifer J. Loros, Kristin M. Lindgren, Norman Garceau, and Jay C. Dunlap. Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755
A circadian biological clock controls several aspects of growth and development in the ascomycete fungus Neurospora crassa, including the timing of the initiation of

conidiogenesis. This property has allowed genetic and molecular techniques to be used to study the circadian clock itself from two converging angles. One approach has been to examine the pathways whereby clocks act to control cellular metabolism and behavior. Initial efforts targeted the isolation of genes whose transcript levels are controlled by the clock. One of the genes, designated clock controlled gene-1 (ccg-1), identified by a subtractive hybridization procedure (Loros et al, Science 243:385-388, 1989) was shown by nuclear run-on analysis (Loros and Dunlap, Mol. Cell. Biol. 11:558-563, 1991) to have clock regulated transcription as the primary point of regulation. ccg-1 is a highly abundant gene, with a 535 nt mature transcript encoding a 71 aa peptide. Sequence analysis of ccg-1 genomic and cDNA clones showed it to be identical to a Neurospora gene, glucose repressible gene-1 (grg-1) (McNally & Free, Curr Genet 14:545-551, 1988). In collaborative work with Giuseppina Arpaia and Giusseppe Macino, University of Rome, we have found ccg-1 gene expression to be blue-light photo-inducible (MGG in press, 1995). Disruption of the ccg-1 locus produces no detectable phenotype and does not effect normal operation of the clock. We have shown by both transcript and protein analysis that ccg-1 is turned on early in development and is regulated by the aconidial-2 (acon-2) locus which, when mutated, blocks conidiation completely. This locus acts early in the developmental pathway at the decision point of commitment to development where aerial hyphal cells are forming constrictions on the way to budding off conidia. ccg-1 is now the earliest gene known to be turned on at the point of commitment in the Neurospora developmental pathway. In collaborative work with Stephen Free, State University of New York at Buffalo, Western analysis of the CCG-1 protein indicates CCG-1 to be present in undifferentiated hyphae in a time-of-day specific manner. Immunocytochemical localization shows extensive, non-nuclear staining of CCG-1 in the cytoplasm of aerial hyphae and conidiospores. Additionally, we demonstrate that ccg-1 is heat-shock inducible but shows limited similarity at the structural and sequence level to the diverse class of evolutionarily conserved small heat-shock and stress proteins. Deletion analysis of upstream ccg-1 sequences suggests that the clock regulatory elements lie near the start site of transcription and are distinct and separate from sequences conferring glucose and developmental regulation.

29. Promoter analysis of the Neurospora crassa circadian clock-controlled ccg-2 (eas) gene

Deborah Bell-Pedersen, Jay C. Dunlap, and Jennifer J. Loros. Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755

The N. crassa ccg-2 gene encoding a fungal hydrophobin is transcriptionally regulated by the circadian clock. In addition, ccg-2 is positively regulated by light, and transcripts accumulate during asexual development. To sort out the basis of this complex regulation, deletion analysis of the ccg-2 promoter was carried out to localize the cis-acting elements mediating clock, light, and developmental control. A distinct positive clock element was localized to within a 45 nt region, just upstream of the TATA box. Using an unregulated promoter/reporter system we show that this element is necessary and sufficient for conferring clock regulation on the ccg-2 gene. We are currently using this element as a probe in gel-mobility shift assays to identify trans-acting clock factors.

30. Output and input: clock control, heat-shock and photo-blind mutants in Neurospora

Jennifer J. Loros, Kristin M. Lindgren, Norman Garceau, and Jay C. Dunlap. Dept. of Biochemistry, Dartmouth Medical School.

A circadian biological clock controls several aspects of growth and development in the ascomycete fungus Neurospora crassa, including the timing of the initiation of conidiogenesis. This property has allowed genetic and molecular techniques to be used to study the circadian clock itself from two converging angles. One approach has been to examine the pathways whereby clocks act to control cellular metabolism and behavior. Initial efforts targeted the isolation of genes whose transcript levels are controlled by the clock. One of the genes, designated clock controlled gene-1 (ccg-1), identified by a subtractive hybridization procedure (Loros et al, Science 243:385-388, 1989) was shown by nuclear run-on analysis (Loros and Dunlap, Mol. Cell. Biol. 11:558-563, 1991) to have clock regulated transcription as the primary point of regulation. ccg-1 is a highly abundant gene, with a 535 nt mature transcript encoding a 71 aa. peptide. Sequence analysis of ccg-1 genomic and cDNA clones showed it to be identical to a Neurospora gene, glucose repressible gene-1 (grg-1) (McNally & Free, Curr Genet 14:545-551, 1988). In collaborative work with Giuseppina Arpaia and Giusseppe Macino, University of Rome, we have found ccg-1 gene expression to be blue-light photo-inducible (MGG in press, 1995). Disruption of the ccg-1 locus produces no detectable phenotype and does not effect normal operation of the clock. We have shown by both transcript and protein analysis that ccg-1 is turned on early in development and is regulated by the aconidial-2 (acon-2) locus which, when mutated, blocks conidiation completely. This locus acts early in the developmental pathway at the decision point of commitment to development where aerial hyphal cells are forming constrictions on the way to budding off conidia. ccg-1 is now the earliest gene known to be turned on at the point of commitment in the Neurospora developmental pathway. In collaborative work with Stephen Free, State University of New York at Buffalo, Western analysis of the CCG-1 protein indicates CCG-1 to be present in undifferentiated hyphae in a time-of-day specific manner. Immunocytochemical localization shows extensive, non-nuclear staining of CCG-1 in the cytoplasm of aerial hyphae and conidiospores. Additionally, we demonstrate that ccg-1 is heat-shock inducible but shows limited similarity at the structural and sequence level to the diverse class of evolutionarily conserved small heat-shock and stress proteins. Deletion analysis of upstream ccg-1 sequences suggests that the clock regulatory elements lie near the start site of transcription and are distinct and separate from sequences conferring glucose and developmental regulation.

31. Expression of cloned genes in Penicillium chrysogenum

S. Graessle (1), H. Haas (1), B. Redl (1), E. Friedlin (2), H. Kuernsteiner (2) and G. Stoeffler (1). (1) Dept. of Microbiology, University of Innsbruck, Medical School, A-6020 Innsbruck, Austria, (2) Biochemie GmbH, A-6250 Kundl, Austria

The ability of industrial strains of certain filamentous fungi to secrete large quantities of protein continues to elicit interest in their potential for large scale homologous and heterologous protein production. Some, like Aspergillus and Neurospora, have been

extensively used as model organisms for diverse transformation and expression systems. Although Penicillium chrysogenum is of great industrial importance, only a few attempts were made to use this organism for homologous or heterologous gene expression. One reason is, that in contrast to Aspergilllus no suitable promoter system was available until now. We have recently cloned the gene encoding a secreted phosphate- repressible acid phosphatase (phoA) of P. chrysogenum, which has all the features required as a basis for an efficient expression system. To develop a regulated secretion system for proteins in Penicillium, a fungal xylanase gene (xylP) and the gene encoding the human lipocalin 1 (LCN1) as model genes were fused to the phoA promoter and signal sequence. Synthesis of recombinant XylP and LCN1 in positive transformants carrying the corresponding constructs was regulated by inorganic phosphate (Pi) present in the medium, so that induction of the heterologous protein expression could be attained by lowering Pi concentration.

32. NRE, the major nitrogen regulatory protein of Penicillium chrysogenum, interacts with the promoter regions of nitrate assimilation and penicillin biosynthetic gene cluster

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Nitrogen metabolite repression is a wide-domain regulatory system, which operates to ensure that a constant supply of nitrogen is readily available for growth in response to variable or rapidly changing environments. In Aspergillus nidulans and Neurospora crassa, the global nitrogen repression/derepression is mediated by the major positive control genes areA and nit-2, respectively. Because of the central position of nitrogen regulation and its effects upon the synthesis of secondary metabolites, we elected to investigate this regulatory mechanism in the filamentous fungus P. chrysogenum, a well known producer of the beta-lactam antibiotic penicillin. In a first step, the areA/nit-2 homologous gene from P. chrysogenum, nre, was isolated and shown to complement nit-2- mutants of N. crassa. nre encodes a protein of 862 amino acids and contains a single Cys2/Cys2-type zinc finger with an adjacent basic region. The overall amino acid sequences of AREA, NIT-2 and NRE show only 30% identity, but they display 98% identity in their zinc finger motif. We have expressed the putative DNA-binding region of NRE as a fusion protein with E. coli beta-galactosidase. Mobility shift assays and missing contact probing experiments indicated that NRE binds in a sequence-specific fashion to the intergenic promoter regions of the Penicillium nitrate assimilation and penicillin biosynthetic gene clusters.

33. In vitro reconstruction of fungal chromosomes and genomes. I. A physical map of the entire Aspergillus nidulans genome

R.A. Prade, K. Kochut, J. Griffith, R. DiGiorgio, W.E. Timberlake, and J. Arnold, University of Georgia, Athens.

Physical maps of fungal genomes provide new research strategies for a wide range of fundamental biological problems, for engineering the production of new pharmaceuticals,

and for understanding the cause of fungal diseases in plants and animals. The physical maps reported here represent the in vitro reconstruction of entire chromosomes from recombinant DNA libraries and provide useful tools to clone genes, determine genomic structure, and study genome evolution. We report a 29 kb resolution physical map of the entire 31 Mb genome of Aspergillus nidulans reconstructed from a 5134 clone cosmid library using 648 probes. The maps are the result of a novel two-way ordering process in which overlapping clones (redundant) and non-overlapping probes (tiles) are ordered to span the entire genome. The physical map is composed of eight matrices with clones down the rows and probes across the columns, one for each chromosome. The redundant order of clones contains 4550 anchored clones (89% of the cosmid library) into 132 contigs with an average of 17 contigs per chromosome. The compressed map (providing a minimum tiling of all 8 chromosomes) is reduced 5-fold in redundancy and contains 1085 clones. By integrating the physical and genetic maps with chromosome and clone hybridization data, we found that repeated DNA sequences are non-randomly distributed along chromosomes in a way reminiscent of heterochromatic banding patterns on cytological maps in other eukaryotes.

34. In vitro reconstruction of fungal chromosomes and genomes. II. Assessing the statistical reliability of physical maps by bootstrap resampling

J. Griffith, R.A. Prade, K. Kochut, R. DiGiorgio, Y. Wang, W.E. Timberlake, and J. Arnold, University of Georgia, Athens.

We provide a methodology for quickly ordering clones in a genomic library into a physical map and for applying a statistical tool known as the bootstrap to assess the statistical reliability of a clonal ordering. Each clone is assigned a binary fingerprint by one of a variety of experimental approaches to physical mapping. For example, the binary fingerprints might be generated by hybridizing a panel of m probes to a library of n clones. The resulting n x m binary data matrix, X, is input to a program, ODS_BOOTSTRAP, which utilizes the similarity in binary fingerprints of clones to construct a physical map. Under this particular implementation of bootstrap resampling, the m probes (or columns of the data matrix) are sampled randomly with replacement in the computer to generate a new n x m matrix, X*, from which a second physical map is constructed. The resampling process is repeated 100 or more times to generate 100 or more X* matrices. The resulting 100 or more physical maps are compared with the original physical map based on the original data matrix X by counting how often links in the original physical map reappear. These counts yield confidence measures for each link on the physical map. The procedure is illustrated with the physical map of Aspergillus nidulans chromosome IV.

35. In vitro reconstruction of fungal chromosomes and genomes. III. ODSTM, software and user interface for physical mapping

R. DiGiorgio, K. Kochut, R.A. Prade, J. Griffith, W.E. Timberlake, and J. Arnold, University of Georgia, Athens.

The ODS package provides computational and graphical tools for reconstructing physical maps. The software is appropriate for those engaged in chromosome walking, STS

content mapping, and contig mapping by a variety of experimental approaches. The objective of the ODS software package is to couple physical mapping algorithms (e.g. Genetics 132:591-601 and PNAS 91:11094-11098) with a friendly user interface so that the software can be easily used by laboratory scientists. This work was based on the DNA fragment ordering problem described in Ordering DNA Sequences, CABIOS 9:215- 219. ODS orders DNA fragments based on the similarity of their fingerprints assigned to DNA fragments in a library by one of several experimental approaches. The algorithm has been used to map the entire Schizosaccharamyces pombe genome (PNAS 9:4461-4465), the entire Aspergillus nidulans genome, and a region of human chromosome 9 (Cytogenetics and Cell Genetics 64:120). The ODS software displays the maps graphically in a variety of formats and prints graphical displays of summary statistics to check mapping progress, the quality of a physical map, and whether or not underlying models of physical mapping are fitting the data. More information can be obtained by email to ODS@BSCR.UGA.EDU, by phone to (706) 542-9359, or by mail to Robyn Ansley, Genetics Department, Life Sciences Building, University of Georgia, Athens, GA 30602 USA.

36. In vitro reconstruction of fungal chromosomes and genomes. IV. A fungal Genome database (FGDB)

K. Kochut, R.A. Prade, J. Griffith, R. DiGiorgio, W.E. Timberlake, and J. Arnold, University of Georgia, Athens.

An object-oriented Fungal Genome Database (FGDB) system is being developed at the University of Georgia. (See Kochut, K.J., J. Arnold, J.A. Miller, and W.D. Potter (1993). "Design of an object- oriented database for reverse genetics." pp. 234-242. First International Conference on Intelligent Systems for Molecular Biology. AAAI Press. Menlo Park, CA). The goal of this project is to provide a software system to support reverse genetics in fungi. FGDB currently contains physical and genetic maps of all 8 Aspergillus nidulans chromosomes. FGDB is based on an object-oriented database management system, ONTOS. The system includes a user-friendly X-windows based interface. Unique features of the system in comparison to other genome database systems are the: (1) ability to create maps; (2) ability to edit maps; and (3) tools to integrate genetic and physical maps. Contig maps can be automatically created and then manually edited and fine-tuned within FGDB. In order to verify and to assist in the editing of contig maps, the user can integrate the genetic and contig maps. Currently, FGDB supports connections over the mnetwork via an X-Windows interface.

Posters I: Signal Transduction

41. Calcineurin, a calmodulin-dependent protein phosphatase is essential for hyphal elongation and involved in the action of cylosporin A and FK506 in Neurospora crassa

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We investigated the function of calcineurin in N. crassa through the inductible antisense expression of the catalytic subunit. Three independent transformants were found to grow at wild-type rates on normal medium. However on induction medium at 37 C they displayed an "oscillating- growth phenotype". This was characterized by cycles of growth followed by several hours of arrest: after 20 hours of growth extensive hyphal branching and conidiation marked the end of each growth cycle. The mechanism involved is under investigation. The severity of the phenotype was temperature dependent, at 29 C the transformants exhibited hardly any growth once induction became effective. From this we conclude that calcineurin is essential for hyphal elongation in N. crassa. Antisense expression of the catalytic subunit of calcineurin correlates with sensitivity to the immunsuppressive drugs Cyclosporin A and FK506, respectively, indicating that calcineurin playes a role in the mechanism of drug action in Neurospora crassa.

42. Gna-2: a G-protein alpha subunit from Neurospora crassa.

Rudeina A. Baasiri, Xiaohui Lu, Patricia S. Rowley and Katherine A. Borkovich. University of Texas Medical School, Houston, Texas

Heterotrimeric G proteins which consist of three subunits, alpha, beta and gamma, regulate a variety of receptor-mediated signalling pathways. In fungi, G proteins have been shown to regulate mating, sexual sporulation and are also implicated in plant pathogenicity. Genes for two N. crassa G protein a subunits (gna-1 and gna-2) have been cloned and characterized by our laboratory. Based on amino acid identity and polyphyletic clustering, gna-2 is most related to gpa1 from Schizosaccharomyces pombe. S. pombe gpa1 is essential for sexual sporulation and mating but not for asexual growth. In contrast, our studies indicate that gna-2 might be essential for asexual growth of N. crassa. Attempts at recovering gna-2 homokaryotic strains were unsuccessful. To understand the function of Gna-2, we are currently using four approaches: 1) creating mutants using Repeat Induced Point Mutation, 2) placing gna-2 cDNA under the control of the inducible quinic acid qa-1 promoter, 3) utilizing the yeast dual hybrid system to identify proteins that interact with Gna-2, and 4) using site-directed mutagenesis to create specific point mutations that have been shown to constitutively activate G proteins in other systems. This last approach will allow us to investigate the outcome of Gna-2 overactivity. N. crassa gna-2 provides a unique chance for understanding the evolution of signalling pathways in fungi; gna-2 might be a related homologue of S. pombe gpa1 that during evolution has acquired an essential function for asexual growth.

43. Biochemical, genetic, and morphological study of a Neurospora crassa G protein-ai subunit deletion mutant

F. Douglas Ivey, Petrea N. Osborne, and Katherine A. Borkovich, University of Texas Medical School, Houston, Texas.

G-protein alpha-beta-gamma heterotrimers form the molecular switches that initiate

signal transduction pathways. In response to extracellular cues such as light, nutrients, or sexual pheromones, G-proteins serve to transduce signals to downstream second-messenger-producing effectors. Our laboratory has cloned two G protein alpha-subunits from Neurospora crassa, gna-1+ and gna-2+. Gna-1 is 55% identical to members of the mammalian inhibitory class of G-alpha subunits (G-alpha-i) and is the first reported microbial G-alpha that has a mammalian homologue. Members of the mammalian G-alpha-i class are defined by possession of consensus sequences for myristoylation and ADP-ribosylation by pertussis toxin. The amino-acid transporter gene (mtr+) was used to mark a gene deletion/disruption of gna-1+ in Neurospora crassa. Southern analysis of gna-1::mtr strains showed the predicted pattern for a mtr+ insertion and loss of the wild-type gna-1+ pattern. Loss of the Gna-1 protein band on Western blots of mutant strain protein extracts and the loss of a pertussis toxin substrate in the same protein extracts verified that the strains were gna-1::mtr homokaryons. In addition, the pertussis toxin labeling experiments revealed that Gna-1 is the only G-alpha-i present in Neurospora crassa. The gna-1 mutation is pleiotropic, causing several morphological changes and slowing of apical extension. An effect of salt and temperature is also observed. Possible second messengers in the G protein signal transduction cascade are presently being investigated.

44. Identification of proteins that interact with gna-1 during signal transduction in Neurospora crassa

Qi Yang and Katherine A. Borkovich, University of Texas Medical School, Houston, Texas

Heterotrimeric GTP binding proteins, which consist of alpha, beta, and gamma subunits, are crucial intermediaries in signal transduction pathways. Neurospora crassa Gna-1 is a G protein a subunit which is a member of the Gi alpha family. The gna-1+ gene has been cloned and sequenced and gna-1 deletion mutants have been made by our laboratory. Recent results have shown that Gna-1 is involved in cell proliferation and differentiation in N. crassa. Proteins which interact with Gna-1 during signal transduction, such as receptors, beta subunits, gamma subunits and effectors, are currently being investigated. Gna-1 has been successfully immunoprecipitated from solubilized plasma membranes and coimmunoprecipitation of Gna-1-associated proteins is in progress. In addition, the Polymerase Chain Reaction is being applied as an alternative approach to identify receptor and beta subunit genes. In this case, degenerate primers have been designed according to the conserved portions of receptors and beta subunits from various organisms. Finally, the yeast two-hybrid system is being utilized in studies of protein-protein interactions. Elucidation of proteins which interact with Gna-1 will clarify the G protein signal transduction pathways in Neurospora crassa.

45. Exploring the role of protein kinase C and protein phosphorylation in hyphal development for a fungal plant pathogen

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Ustilago violacea is a basidiomycete phytopathogen that infects members of the Carnation family (Pinks). When diploid teliospores of the fungus germinate on suitable

surfaces, such as floral parts, they undergo meiosis to produce a linear tetrad of basidiospores which segregate two mating types, a1 and a2. Further development of the fungus and infectivity requires that sporidia of opposite mating type conjugate and then receive a signal from a suitable host plant to allow formation of dikaryotic hyphae. Compounds such as tocopherols and phytols stimulate hyphal growth of some conjugating strains at concentrations equivalent to hormonal action (as low as 10 uM). Moreover, such response is dominant: strains which are normally unaffected by alpha-tocopherol (VitE) when mated amongst themselves will support hyphal growth when conjugated with isolates that normally do respond. Hyphal growth is stimulated, to a lesser degree, by from 10-100 uM 1-o-hexadecyl-2-o-methyl-rac- glycerol (HEX), a specific inhibitor of protein kinase C (PKC). Phorbol ester, in the short term a specific activator of PKC, much later causes inhibition of the enzyme. After 6-12 h post exposure to concentrations of phorbol ester from 1.6-16 mM, conjugating sporidia produced abberant 3 and 4-cell mating patterns; when VitE was also present, conjugating sporidia showed a 50-75% reduction in hyphal production compared to those with VitE alone. After several days, massive hyphal production was eventually found in treatments with phorbol ester alone. Preliminary examinations of total cellular proteins suggest reduced phosphorylation in the presence of either VitE or HEX. Thus, level of phosphorylation of targets for PKC may control hyphal production in U. violacea.

46. G Protein alpha subunit of Pneumocystis carinii

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Pneumocystis carinii pneumonia results in significant morbidity and mortality among immunocompromised individuals. A lack of understanding of the cellular or sexual biology of the organism has hampered rational development of new antimicrobial therapies. G-proteins are highly conserved member of the signal transduction pathway important in many intracellular signaling pathways including the mating cascade. To characterize signal transduction pathways in P. carinii, we have cloned the G protein alpha subunit of P. carinii. Genomic DNA from a candidate species of P. carinii from rat, P. carinii carinii, was used as target DNA. Primers were designed to a highly conserved region of the G alpha subunit, and a 280 bp product encoding the GTP binding domain of the alpha subunit was amplified. The amplified product hybridized to a 450 kb chromosome of P. c. carinii. Sequence analysis revealed an open reading frame interrupted by a single 46 bp intron in a position identical to one of the introns detected in GPA1 of Cryptococcus neoformans. The predicted peptide showed 57-61% identity with known fungal G alpha proteins with greatest homology to Schizosaccharomyces pombe GPA1 and 42-57% identity with known rat G-alpha proteins. The ORF had similar codon bias to other cloned P. carinii genes with 63% AT usage in the third nucleotide position of transcribed codons. Characterization of the genes involved in signal transduction will permit a better understanding of the reproductive capacity and other cellular processes in this family of organisms that cannot be continuously cultured.

47. Calmodulin-dependent multifunctional protein kinase (ACMPK) activity is

essential for growth of Aspergillus nidulans

Kotha Subbaramaiah, Maria Gaiso and Diana C. Bartelt, St. John's University, Jamaica, NY.

ACMPK is a monomeric calmodulin-dependent protein kinase in A. nidulans which exhibits a substrate specificity similar to mammalian CaMPKII. It is encoded by a single copy gene, cmkA, which is transcribed to yield a single 1.7 kb mRNA. Three strains of A. nidulans have been constructed in which ACMPK expression can be manipulated experimentally. The first, MG1, is a strain with an additional copy of cmkA under the control the inducible alcA promoter. In Ca+- depleted medium, MG1 cells overexpressing ACMPK exhibit distinct advantages in growth and maintenance of normal morphology over cells expressing normal amounts of the enzyme. In MG2 cells, induction of the expression of a truncated form of ACMPK lacking the CaM-binding domain leads to growth arrest after several rounds of nuclear division, despite the fact that these cells are constitutively expressing full length ACMPK. KS1 cells, in which a single copy of cmkA is under the control of alcA, grow normally in medium containing ethanol as the carbon source. KSl conidia are growth arrested following several mitotic divisions when germinated on medium containing glucose, which represses ACMPK expression, indicating that ACMPK is essential for viability. (Supported by the American Cancer Society).

48. Apoptotic cell death and G1 arrest in plant and animal cells induced by fumonisin

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Fusarium monilforme is a major pathogen of corn. Most strains of this fungus produce several mycotoxins the most prominent of which is called fumonisin. Fumonisin causes a neurodegenerative disease in horses, induces hepatic cancer in rats, and pulmonary edema in swine. Studies have also demonstrated that fumonisin is correlated with increases of esophageal cancer. High levels of fumonisin can be detected in both healthy and diseased corn. Thus consumption of corn based products by both man and animals poses a serious health threat, Structurally, fumonisin resembles sphingolipids, although little is known concerning the mechanism by whlch fumonisin elicits its toxic effects. Recent findings in our laboratories indicated fumonisin alters transcription and signal transduction pathways in monkey cells. Fumonisin represses specific protein kinase C isoforms and AP-l dependent transcription. In contrast, fumonisin stimulated a simple promoter containing a single cycle AMP response element (CRE). Cells treated with fumonisin, showed inducible binding of nuclear factors with oligos containing a CRE or AP-l binding site. Furthermore, fumonisin induced programmed cell death (apoptosis) in both monkey and tomato and arrested those cells in the Gl phase of the cell cycle. Our working hypothesis is fumonisin activates or represses transcription of genes which regulate growth of animal cells. Consequently, abnormal growth occurs which contributes to the induction pathological changes. The ability of fumonisin to alter signal transduction pathways, cause Gl arrest and induce apoptosis is likely to be necessary for its carcinogenic and toxic effects.

49. The effect of neomycin on growth, inositol metabolism and protein synthesis in Neurospora crassa

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Neomycin has been used to perturb the turnover of the inositol phospholipids in animal cells and inhibit protein synthesis in bacteria. In this study, the effects of neomycin on the growth, inositol metabolism and protein synthesis in Neurospora crassa were studied. Increasing levels of neomycin (2.2-8.8 mM) prevented growth. To determine how neomycin affected growth, high concentrations of the drug (5.5 mM) were added to cultures and at various times after the addition, mycelial samples were analyzed for changes in inositol metabolism and protein synthesis. Neomycin stimulated inositol uptake and metabolism. After the drug was added, inositol increased 125% within 1 h. Within 4 h, the phosphoinositides and inositol phosphates increased, on average, 43% and 103%, respectively. In contrast, the rate of protein synthesis declined dramatically after the addition of neomycin. Within 1 h, the rate of protein synthesis declined 34%. Within 3 h, the rate declined to 54%. A continued decline in protein synthesis caused by neomycin would prevent growth in Neurospora crassa. It is, however, unclear what the effect of increased inositol metabolism would have on growth and cell signalling.

50. Isolation and functional analysis of krev-1 gene, a ras-superfamily of N. crassa

S. Ito, Y. Matsui, A. Toh-e, and H. Inoue, Tokyo Univ. and Saitama Univ., Japan
The krev-1 gene is a member of the ras-superfamily, and this gene product suppresses oncogenic ras transformation in mammals. Recently, krev-1 homologs have been isolated from several species. We used polymerase chain reaction to isolate a new member of the ras superfamily of Neurospora, and obtained a krev-1 homolog. The Neurospora krev-1 gene encoded the polypeptide that was 57% identical to those of mammalian Krev-1 at the predicted amino acid level. RFLP mapping confirmed the chromosomal position of krev-1 on LGIL near nit-2. krev-1 is expressed constitutively at low level in vegetative growth phase. To disrupt krev-1 by the RIP phenomenon, cells carrying the duplicated krev-1 were crossed. Segregants carrying the mutations in krev-1, confirmed with Southern blot analysis, showed no characteristic phenotype. To examine krev-1 function, we used site-specific mutagenesis to alter conserved residues known to be important for the function of the ras- superfamily. We are now examining the functional relationship of Krev-1 homologs between N. crassa and S. cerevisiae.

51. A deletion mutant of NC-ras-2 in Neurospora crassa

Akihito Kana-uchi, Ryoh Kobayashi, Yasuyuki Fujisawa*, and Tadako Murayama*. Tokyo Med. and Dent. Univ. Tokyo and Kanto-Gakuin Univ. Yokohama*, Japan.*
One of the Ha-ras homologues of N. crassa, NC-ras-2, has been cloned and reported (Murayama and Kana-uchi; Fungal Genetics Conference, 1993). To study the roles of this gene, we tried to disrupt the gene through RIP (Rearrangement Induced Premeiotically) using transformants containing an additional copy of the genomic DNA carrying NC-ras-2 and qa-2. Among progenies of the crosses between the transformants and the wild type, one morphological mutant ras-17 which grew poorly was obtained. Morphology of ras-17

on the solid medium was compact and colonial. Southern blot analysis revealed that the ras-17 strain has large deletion in the coding region of NC-ras-2. To know the relationships between the adenylyl cyclase and NC-ras-2 protein in N.crassa, cAMP level in the ras-17 strain was measured. The roles of NC-ras-2 gene in N.crassa were discussed.

52. The isolation of mutants in the light signal transduction pathway of Neurospora crassa

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In the filamentous fungus Neurospora crassa several photomorphogenic and biochemical events are regulated by blue light. During the asexual cycle, blue light regulates carotenogenesis in mycelium including the expression of the carotene biosynthesis genes al-1, al-2 and al-3. A new screening system has been developed in order to obtain mutants showing deficiencies in the blue light signal transduction. The gene of the neutral amino acid permease (mtr) has been cloned under the control of the al-3 promoter. This construct has been used to transform a Neurospora strain which carried mutations in the mtr and trp loci. In this transformant, thc expression of the amino acid permease was now found to be light regulated. In the presence of PFPA, a poisonous amino acid analogue, growth in the light was strongly inhibited due to the uptake of PFPA by the mtr permease. However, growth on low tryptophan concentrations was observed in the light only because of the light regulated uptake of the amino acid. Applying these two selection systems, several different mutants could be isolated after UV mutagenesis. Some of them were shown to have a "white collar" phenotype and to be "blind" for several blue light responses in Northern analysis. Using the positive selection instead, mutants were isolated which showed, in contrast to the wild type, a constitutive carotenoid biosynthesis even in the dark. The presented screening system can also be applied to recover the corresponding genes.

53. Regulation of conidial formation by cyclic AMP in Neurospora crassa

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Cyclic AMP (cAMP) has been described as one of the most important mediators in the signal transduction pathway in various organisms. The reduction of nitrogen source and carbon source induced conidial formation, but the addition of cAMP to the medium repressed it. The level of cAMP decreascd quickly after being transferred to the carbon source- and nitrogen source-free medium. A mutant (cr-1) having defective adenylyl cyclase, which catalyzes the synthesis of cAMP from ATP, formed conidia before the reduction of the nutrients. A suppressor mutant hah which suppressed the colonial growth of cr-1 and had considerably elevated level of adenylyl cyclase gene transcript did not form conidia. These results suggest that reduction of cAMP induces conidial formation and that reduction of nitrogen source and carbon source induces formation of conidia through the reduction of cAMP.

54. Purification and cloning of Ser/Thr protein phosphatases from Neurospora crassa

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Protein phosphorylation is a universal regulatory mechanism in eukaryotic cells. The phosphorylated state of a protein is affected by the conflicting activities of protein kinases and phosphatases. Nearly all Ser/Thr specific dephosphorylation reactions can be attributed to four classes of protein phosphatases (PP's): PP1, PP2A, PP2B, PP2C, which are differentiated on the basis of inhibitor sensitivity and metal ion dependence. We found that all the four main classes of Ser/Thr protein phosphatases are present in N. crassa. The catalytic subunit of PP2A (PP2AC) was purified 100-fold by using ammonium sulfate-ethanol precipitation, DEAE Sephacel, Heparin-Sepharose and MonoQ FPLC chromatography. Its apparent molecular mass proved to be 35 kD in gel filtration experiment. PP2AC was completely inhibited by 1 nM okadaic acid, 4 nM microcystin and 20 mM NaF, was insensitive to rabbit muscle inhibitor-2, and was specific for the (alpha subunit of rabbit muscle phosphorylase kinase. The catalytic subunit of PP1 was found to be less stable because its activity was gradually decreasing during purification. The pSV50 cosmid library was screened using a Drosophila PP1 cDNA probe. Four recombinant cosmids of different restriction patterns were isolated. The heterologous cDNA probe hybridized to 4-5 BamHI and HindlII fragments in a Southern hybridization experiment. On the basis of these results we assume that a gene family of at least four PPI genes is present in N. crassa.

55. Inhibition of cAMP-dependent protein kinase prevents spore germination and appressorium development in Colletotrichum trifolii.

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Colletotrichum trifolii, the causal agent of alfalfa anthracnose, is one of the most destructive fungal pathogens of alfalfa. Spore germination and the development of appressorium are requisite for this fungus to infect susceptible alfalfa cultivars, however, the signal transduction pathways responsible for triggering this developmental switch are poorly understood. We have found a relatively large amount of cAMP dependent protein kinase (PKA) activity in spores and germinated spores compared to mycelia and fully developed appressoria. Therefore, we investigated the involvement of PKA during this process. By using a PKA specific inhibitor, KT5720 at a physiologically relevant concentration, spore germination and appressorium development were virtually shut down. Further investigations using IBMX, a phosphodiesterase specific inhibitor and the cAMP analogue 8-bromo cAMP, which increase the endogenous level of cAMP, resulted in enhancement of spore germination and appressorium development. Our data strongly indicate that cAMP may act as a central mediator for differentiation by activating PKA and triggering a biochemical pathway resulting in both spore germination and appressorium development. Molecular cloning of the genes of PKA catalytic domain and regulatory domain are in progress.

56. Molecular characterization of a filamentous mutant of Ustilago horde

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Successful infection of barley by Ustilago hordei, the causal agent of covered smut, depends on the formation of a filamentous dikaryon, which is produced after mating of compatible haploid cells, which are sporidial in appearance and nonpathogenic. We report the isolation of a filamentous, haploid mutant, fil1-1, isolated following heat shock treatment. Analysis by pulsed-field gel electrophoresis (PFGE) revealed a 50 kb deletion in a 940 kb chromosome. RFLP studies using a telomere-specific repeat (TTAGGG)18 from Fusarium oxysporum as a marker, place the deletion near the end of one arm of the 940 kb chromosome. In a cross wild type (sporidial) X mutant (filamentous), the fil1 mutation and the deletion were shown to invariably cosegregate 2:2 with the wild type morphology in an ordered tetrad. In the presence of cyclic AMP, the filamentous phenotype reverts to the sporidial wild type. The same effect was observed for analogs and stimulators of cyclic AMP and inhibitors of phosphodiesterase. These data suggest that the mutation is not in the structural gene encoding the adenylate cyclase, but is possibly in a gene encoding either a G protein or the receptor protein. The putative gene(s) controlling the dimorphic switch are currently being cloned.

57. Regulation of inositol synthesis in Cryptococcus neoformans

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Cryptococcus neoformans is a basidiomycete that infects immunocompromised humans. C. neoformans grows in defined media with or without inositol supplementation. Inositol is a precursor of phosphatidylinositol (PI), an essential membrane lipid. The presence of PI in the membranes of C. neoformans grown in media without inositol supplementation indicates that this organism can synthesize inositol. The synthesis of PI in C. neoformans is different than in other yeast, however, in that it is unaffected by the presence of exogenous inositol. This study examines the regulation of inositol synthesis in C. neoformans. In eukaryotes glucose-6-phosphate is converted to inositol-1- phosphate by the enzyme inositol-1-phosphate synthase. Inositol-1-phosphate synthase was detected in crude extracts of C. neoformans. This activity was repressed by the provision of 75 uM inositol in the growth media. Regions of homology between sequences of the genes encoding inositol-1- phosphate synthase from two other yeasts and two plants were used to design synthetic oligonucleotides. PCR amplification of C. neoformans genomic DNA generated fragments that were used to probe Northern blots. Preliminary results reveal a transcript that is regulated in response to inositol in the growth media. Hence, the regulation of inositol synthesis in C. neoformans appears to be similar to other yeast, yet the regulation of PI synthesis is different. Perhaps the difference is due to this organism's unusual capacity to catabolize inositol. Clearly the regulation of inositol metabolism and the coordination of synthesis and catabolism are essential for balanced cell growth.

58. Inactivation of a single type 2A phosphoprotein phosphatase is lethal in Neurospora crassa

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A PCR approach, employing the use of degenerate oligonucleotide mixtures, was used to isolate pph- 1, a type 2A protein phosphatase gene, from Neurospora crassa. The isolated single-copy gene is 1327 nucleotides in length, contains four putative introns and encodes a 310 amino acid polypeptide. pph-1 is located between pdx-1 and col-4 on the right arm of N. crassa linkage group IV. pph-1 transcript levels are highest during the first hours of conidial germination. Failure to obtain viable progeny in which pph-1 had been inactivated via the Repeat-Induced Point mutations process and evidence that nuclei harboring a disrupted pph-1 gene could only be maintained in a heterokaryon, indicate that a functional pph-1 gene is essential for fungal growth. This is the first report providing evidence that inactivation of a single type 2A protein phosphatase results in a lethal phenotype in fungi.

59. The NIMA protein kinase is hyper-phosphorylated and activated downstream of p34(cdc2)/cyclinB: coordination of two mitotic promoting kinases

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Initiation of mitosis in Aspergillus nidulans requires activation of two protein kinases, p34(cdc2)/cyclinB and NIMA. Forced expression of NIMA, even when p34(cdc2) was inactivated, promoted chromatin condensation. NIMA may therefore directly cause mitotic chromosome condensation. However, the mitotic promoting function of NIMA is normally under control of p34(cdc2)/cyclinB as the active G2 form of NIMA is hyperphosphorylated and further activated by p34(cdc2)/cyclinB when cells initiate mitosis. To see the p34(cdc2)/cyclinB dependent activation of NIMA, okadaic acid had to be added to isolation buffers to prevent dephosphorylation of NIMA during isolation. Hyperphosphorylated NIMA contained the MPM-2 epitope and, in vitro, phosphorylation of NIMA by p34(cdc2)/cyclinB generated the MPM-2 epitope suggesting that NIMA is phosphorylated directly by p34(cdc2)/cyclinB during mitotic initiation. These two kinases, which are both essential for mitotic initiation, are therefore independently activated as protein kinases during G2. Then, to initiate mitosis, we suggest they each activate the other's mitotic promoting functions. This ensures that cells coordinately activate p34(cdc2)/cyclinB and NIMA to initiate mitosis only upon completion of all interphase events. Finally, we show that NIMA, like cyclinB, is degraded specifically during mitosis.

60. cAMP dependent protein kinase catalytic subunit (cpkA) gene is required for appressorium formation in Magnaporthe grisea

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Successful infection of rice by the fungal pathogen Magnaporthe grisea depends upon the formation of a dome shaped, highly melanized infection structure, an appressorium. The differentiation of this unique, specialized cell from the tip of an emerging germ tube is a

response to environmental stimuli. The exogenous and endogenous signalling mechanisms involved in surface recognition and the transfer of this information into the cell leading to infection related morphogenic events remain to be elucidated. We have shown that cyclic AMP, a second messenger involved in signal transduction systems, regulates appressorium formation. In other systems, the primary target for cAMP is cAMP dependent protein kinase. Activation of this kinase directly or indirectly results in specific developmental changes. To elucidate the mechanism of cAMP action in M. grisea, we have isolated, sequenced and disrupted the single gene encoding the catalytic subunit of cAMP dependent kinase (cpkA). Strains lacking the cpkA gene appear unaffected in their ability to grow and reproduce sexually and asexually. However, they are unable to form appressoria on rice or in the presence of cAMP and are non pathogenic. This is the first direct evidence that fungal pathogenesis is mediated via cAMP dependent protein kinase.

Posters I: Plant and Animal Fungal Pathogens

1. Population diversity of Colletotrichum gloeosporioides from avocado and almond using molecular techniques and pathogenicity assays

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Isolates of Colletotrichum gloeosporioides from avocado and almond fruit were compared in order to determine the genetic diversity between and among the different populations. Four almond isolates exhibited very similar nuclear banding patterns compared to avocado isolates which had differential patterns. Similar results were observed with A+T-rich DNA representative of the mitochondrial genome. PCR amplification of genomic DNA using four primers accurately grouped nine almond isolates from different geographic locations uniformly. In contrast, the avocado isolates were more diverse with 7-10 different genotypes being observed. Amplification and subsequent restriction enzyme digestion of the 4-5 ITS region of ribosomal DNA failed to distinguish between C. gloeosporioides from a diverse host range. Avocado isolates produced varying lesions on avocado and almond fruits whereas the almond isolates infected at a uniform rate. Certain avocado isolates seemed to produce perithecia in culture whereas almond isolates remained asexual. This suggests that in asexually reproducing populations such as the C. gloeosporioides almond isolates little DNA variation is expected to occur as compared with the sexually reproducing avocado isolates where multiple genotypes are found.

2. Genetics of virulence and linkage mapping in Phytophthora sojae

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Phytophthora sojae belongs to the Oomycetes which are characterised by gametangial meiosis, thus having a diploid somatic phase, which contrasts with the majority of the

true fungi which are haploid for most of their life cycle. Until recently the genetics of virulence/avirulence in P. sojae was considered intractable due to its homothallic nature. A race l and a race 7 isolate were co- cultured in vitro and, using RAPD markers, ten hybrids were identified among 354 oospores analysed. One F1 hybrid was allowed to self fertilise and produce an F2 population of 247 individuals. Fifty-three F2 individuals were selected at random for genetic analysis. A genetic linkage map has been constructed from this cross consisting of 15 major linkage groups and ten small linkage groups using 233 RAPD markers, 30 dominant RFLP markers, 10 co-dominant RFLP markers and four avirulence genes. Segregation of virulence against soybean resistance genes Rpsla, 3a and 5 revealed that the avirulence genes Avrla, 3a and 5 were dominant to virulence. Avirulence against these three resistance genes appeared to be conditioned by one locus for Avrla and two independent, complementary dominant loci for both Avr3a and Avr5. Segregation of virulence against Rps6 was in the ratio of 1:2:1 (avirulent : mixed reaction : virulent), suggesting a semi-dominant allele at a single locus.

3. Protease and cutinase in the pathogenic interaction between Pyrenopeziza brassicae and Brassica napus

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Pyrenopeziza brassicae, a haploid hemibiotroph and a member of the Ascomycotina, is the causative agent of light leaf spot disease of brassicas. Understanding the mechanism of pathogenicity by P. brassicae is of central importance in the development of alternative strategies for disease control. Extracellular enzymes are considered to facilitate penetration and growth of fungi in planta. Consequently, we are examining the roles of extracellular cutinase and protease in pathogenesis. Here we characterise an extracellular protease and describe its role as a pathogenicity determinant of P. brassicae. Purification of the protease and amino acid sequence analysis revealed N-terminal sequence homology with a cloned Aspergillus oryzae protease gene. Together with the observed inhibition of enzyme activity in vitro by ovoinhibitor, this suggests that the P. brassicae enzyme is an alkaline serine protease. Indirect evidence that expression of the extracellular protease is essential for pathogenicity by P. brassicae is as follows: (i) the protease is produced in planta; (ii) both pathogenesis (path) and protease (prot) traits co-segregate in mutants of P. brassicae; and (iii) complementation of both path and prot phenotypes was achieved using a single 40 kb genomic clone. One prot mutant is able to penetrate the host cuticle but shows restricted growth in planta and does not cause tissue necrosis. We have isolated several putative protease clones from a casein induced P. brassicae cDNA library which are currently being analysed. Initial studies have shown cutinase activity in cutin-induced culture filtrates of P. brassicae. We are characterising and purifying this cutinase prior to cloning its structural gene.

4. Isolation and characterization of phase specific clones of grass pathogen Claviceps purpurea

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The ergot fungus Claviceps purpurea infects the florets of rye and other grasses causing a tissue replacement disease. The infected ovary is fully replaced by fungal tissue. During this process a distinct border between fungus and plant is established and the fungus switches from a conidium producing form (sphacelium) to an alkaloid producing resting structure (sclerotium). We are characterizing the interaction between C. purpurea and its host Secale cereale. We have isolated several clones carrying genes which might be higher expressed or less expressed in the plant than in axenic culture or which even might be uniquely expressed in planta. Two clones were chosen for further analysis because they seem to carry regions which are expressed in the honeydew (plant colonization, conidia producing) phase, but not in axenic culture. The clones are currently being evaluated: 1) location of transcribed regions, 2) nucleotide sequence of transcribed regions, 3) specificity for in planta transcription and 4) transcription during parasitic stages other than the honeydew phase.

5. RAPD analysis and an aspergilloma legal case

Ian K. Ross, University of California, Santa Barbara
RAPD markers were used to trace the origin of a claimed work-associated case of aspergilloma caused by Aspergillus fumigatus. The counter argument was that the patient could have been infected outside the work place, and that the disability was therefore not work associated. Samples were taken from the patient's work place, from the patient's house, and from houses of neighbors and other control houses. Several decamers were used to attempt to differentiate among the various isolates obtained from the sampling. Whereas the control houses had around 3-5 strains of A. fumigatus, all different as determined by RAPD analysis, and each appearing in only one sample, the patient's house was infested with one dominant strain that appeared in nearly all the samples. This strain was indistinguishable by RAPD analysis from a strain isolated from the patient's office and the next door boiler room and other parts of his work area. The legal interpretations of these data will have to await the actual trial. The DNA was isolated by a novel non-grinding method using xanthogenates that avoids hazardous aerosols.

6. Analysis of the role of cytokinins in a hemibiotrophic plant pathogen interaction

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The fungal plant pathogen, Pyrenopeziza brassicae is a haploid member of the Ascomycotina and is the cause of light leaf spot disease of Brassicas, which is one of the most important diseases of oilseed rape in the U.K. P. brassicae is a hemibiotroph, proliferating sub- cuticularly from its initial site of infection and gaining nutrition from living cells before switching to necrotrophy at the onset of sporulation. During its biotrophic phase, a relatively balanced physiological relationship exists between P. brassicae and its host. The objective of this research is to ascertain whether cytokinins of fungal origin play a role in maintaining the biotrophic phase of P. brassicae development. Cytokinin production by P. brassicae in vitro has been established and quantified by ELISA using highly specific polyclonal antibodies to zeatin riboside and

isopentenyladenosine riboside. The role of cytokinins in hemibiotrophy is now being assessed by (i) screening for UV generated cytokinin deficient mutants and (ii), using PCR- derived cytokinin biosynthetic gene sequences to insertionally inactivate the corresponding fungal genes. Current progress in these areas will be reported.

7. Map-based cloning of pathogenicity determinants of Nectria haematococca

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Pathogenicity of Nectria haematococca (MPI) on unwounded hypocotyls of Cucurbita maxima cv. Crown (buttercup squash) is a quantitative trait, with estimates of the number of effective factors ranging from 3 to 15, dependent on cross. To locate, and ultimately clone the genes controlling pathogenicity, a set of markers linked to the quantitative trait loci (QTLs) and a yeast artificial chromosome (YAC) library from a high pathogenicity isolate are being developed. A population of 200 random ascopsore progeny was derived from a cross between a high pathogenicity and a low pathogenicity isolate. Random amplification of polymorphic DNA (RAPD) analysis revealed 40 markers that distinguished the parents. Currently, bulked DNA samples from either high or low pathogenicity progeny are being screened to assess linkage of these markers with QTLs controlling pathogenicity. High molecular weight DNA is being prepared by preparative pulsed field gel electrophoresis to enable a YAC library to be constructed. YACs containing both marker and QTL will be identified by library screening. Sub-cloning and chromosome walking will lead to isolation of QTLs, the importance of which will be established by complementation of a low pathogenicity isolate by transformation.

8. Exploring the use of Neurospora crassa mutant erg-1 for the isolation of pisatin detoxifying genes through heterologous complementation

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The fungal pea pathogen Nectria haematococca expresses a cytochrome P450 which detoxifies the pea phytoalexin pisatin by demethylation. A pisatin demethylase (pdm) encoding gene (PDA) was originally isolated from N. haematococca genomic DNA by detecting heterologous expression of the demethylase activity in Aspergillus nidulans. However, isolation of PDA genes in A. nidulans by selection for enhanced tolerance to pisatin has not been possible due to the high intrinsic pisatin tolerance of A. nidulans wildtype. Papavinasasundaram and Kasbekar (1993) have shown that ergosterol (erg) mutants of Neurospora crassa are sensitive to pisatin. In order to develop a method for the isolation of PDA genes by selection we examined the expression of a N. haematococca PDA gene in N. crassa erg-1. We found the gene to be expressed in N. crassa and pisatin demethylating erg-1 transformants showing increased tolerance to pisatin. Spores of erg-1 pdm+ transformants can be identified in mixes with erg-1 pdm spores by means of pisatin selection. These results suggest that pdm genes can be isolated by heterologous complementation of the pisatin sensitivity of erg-1. Papavinasasundaram and Kasbekar (1993). J. Gen. Microbiol. 139:3035-3041.

9. Mutants of Cercospora nicotianae altered in their response to active-oxygen-generating photosensitizers

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Fungi in the genus Cercospora produce a singlet-oxygen-generating photosensitizing toxin cercosporin which plays an important role in the ability of these fungi to parasitize plants. Cercosporin shows generalized toxicity to plants, bacteria, mice, and many fungi, but Cercospora species are resistant. This resistance has been associated with the ability of these fungi to transiently reduce and detoxify cercosporin in contact with fungal hyphae. In order to further characterize the mechanism of resistance, cercosporin-sensitive mutants of C. nicotianae were isolated. Two classes of mutants were identified. One class consisted of five mutants which were highly sensitive to cercosporin and to five other singlet-oxygen-generating photosensitizers. These mutants were unable to reduce cercosporin and could be protected against cercosporin toxicity by the reducing agents ascorbate, cysteine, and reduced glutathione. Mutants of this class were significantly less pathogenic than wild type. The second class consisted of a single mutant which was only partially inhibited by cercosporin. This mutant was unaltered in cercosporin-reducing ability and in resistance to the other photosensitizers. It was slightly decreased in pathogenicity, and could be protected against cercosporin toxicity by cysteine and reduced glutathione, but not by ascorbate. None of the mutants was altered in endogenous levels of ascorbate, cysteine, reduced glutathione, or total soluble or protein thiols. Current efforts are directed at isolating the genes involved in resistance by mutant complementation.

10. Carotenoid-minus disruption mutants of Cercospora nicotianae show no increased sensitivity to cercosporin or other photosensitizers

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Numerous phytopathogenic Cercospora species synthesize the photoactivated phytotoxin cercosporin, the presence of which is correlated with the ability of these fungi to cause plant disease. Due to its mode of action cercosporin exerts a very general and broad toxicity, not only to plants, but to bacteria, most fungi and even mice. Cercosporin absorbs light energy and interacts with molecular oxygen to generate the highly toxic active oxygen species, singlet oxygen. Even though micromolar concentrations of cercosporin are fatal to most cells, Cercospora species can synthesize up to millimolar concentrations without autotoxicity. Because carotenoids are known to be the most potent biological quenchers of singlet oxygen, we evaluated their role in Cercospora resistance to cercosporin. Targeted gene disruption of a carotenoid biosynthetic gene was used to create carotenoid minus derivatives of a wild-type and of two cercosporin-sensitive strains of C. nicotianae. These carotenoid-minus disruption strains were no more sensitive to cercosporin or to five other photosensitizers than the strains from which they were derived. Pathogenicity tests also indicated inability to produce carotenoids did not otherwise

11. Cosegregation of Avr4 and Avr6 in Phytophthora sojae

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Phytophthora sojae is an oomycete pathogen of soybean that causes a severe root and stem rot. To date, at least 13 host plant resistant genes (Rps) have been identified at 7 genetic loci, each conferring resistance to specific races of the pathogen. In this study, 55 F2 progeny resulting from a cross of race 2 (avirulent on Rps4 and Rps6) and race 7 (virulent on Rps4 and Rps6) were tested for their ability to cause disease on soybean plants carrying the Rps4 or the Rps6 resistance gene. Avirulence on Rps4 and Rps6 was dominant and linked, with 41 individuals avirulent on both of these genes and 14 virulent on Rps4 and Rps6. These results support the suggestion that the soybean-Phytophthora relationship is a gene-for-gene interaction, and that the presumptive Avr4 and Avr6 genes are either tightly linked or are identical.

12. Population genetic structure of Fusarium oxysporum f.sp. albedinis

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Bayoud, the Fusarium wilt of date palm, first appeared in the south of Morocco and then spread in most of the moroccan palm groves and reached the western and central oasis of the Algerian Sahara. Thanks to prophylactic measures, all other date palm growing areas in the world remain free of the disease. In order to assess the population genetic structure of Fusarium oxysporum f.sp. albedinis (FOA), most of the diseased palm groves were prospected and isolates were collected from wilted palms of several varieties. 120 FOA isolates were tested for Vegetative Compatibility Group (VCG), Restriction Fragment Length Polymorphism (RFLP) and Random amplified Polymorphic DNA (RAPD). No polymorphism was observed in RFLP studies on ribosomal and mitochondrial DNA and a unique VCG was found whatever the host genotype or the geographical origin of the 120 FOA isolates. RAPD analysis separated some isolates from two Algerian oasis (Adrar and Ghardaia), but a low level of genetic diversity was found among the whole FOA population. RFLP analysis with a dispersed repetitive DNA probe allowed to detect 52 fingerprints sharing at least 80 % similarity. Such a genetic homogeneity between FOA isolates provides evidence that Fusarium oxysporum f.sp. albedinis populations originate from a unique clone which spread in Morocco and Algeria.

13. Functional analysis of the ipiO genes of the late blight pathogen Phytophthora infestans

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Phytophthora infestans is the causal agent of potato late blight, one of the most devastating diseases of potato. In order to gain more insight in the molecular and cellular processes involved in pathogenicity we have isolated P. infestans genes of which the expression is specifically induced during growth of the pathogen in the host plant. From

two of these in planta induced (ipi) genes, ipiO1 and ipiO2, high levels of mRNA can be detected in infected potato leaves and tubers during the first two days of the infection cycle whereas one day later the ipiO mRNA level is strongly decreased. In in vitro grown mycelium ipiO gene expression is induced by nutrient deprivation (Pieterse et al., MGG 244:269-277). The two ipiO genes are 98% homologous. The difference between the two proteins, IPI-O1 and IPI-O2, is limited to four amino acid and there is no homology with any known protein (Pieterse et al., Gene 138:67-77). Besides a putative signal peptide both proteins contain the tripeptide Arg-Gly-Asp (RGD) which functions as a "cell attachment" sequence in several mammalian proteins. Whether the RGD tripeptide present in IPI- O has a similar function is unknown. To determine whether or not IPI-O plays a role in pathogenicity, P. infestans transformants containing the ipiO1 gene in anti-sense orientation were made. Phenotypic characterization of these anti-sense transformants were presented and the possible role of IPI-O during pathogenicity of P. infestans on potato discussed.

14. Azole resistance in Candida species

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Since its introduction in 1988, fluconazole has been used to treat >25 million patients, including >250,000 AIDS patients. Like the other azole antifungals, fluconazole acts by inhibiting fungal ergosterol biosynthesis. The fungistatic nature of the azole antifungals has resulted in AIDS patients receiving prolonged antifungal therapy and consequently azole-resistant Candida species are now isolated from patients with end-stage disease. These organisms are frequently cross- resistant to all of the commercially available azole antifungals. Previous studies on C. albicans, C. glabrata, and Saccharomyces showed that there are three possible mechansisms for resistance to azoles, viz: changes in the target enzyme, cytochrome P-450-dependent 14alpha-sterol demethylase; changes in delta-5-6 sterol desaturase, another enzyme in ergosterol biosynthesis; and permeability resistance. We have been investigating the mechanisms for fluconazole resistance in post-treatment fluconazole-resistant isolates of C. glabrata and C. albicans. Both of the isolates show cross- resistance to ketoconazole and itraconazole and both show permeability resistance to fluconazole. Studies with metabolic inhibitors showed that fluconazole resistance in the C. glabrata isolate was due to energy-dependent efflux of the drug from the cells. In contrast, fluconazole resistance in the C. albicans isolate appeared to be due to a block in uptake of the drug. We are currently attempting to clone the genes responsible for fluconazole resistance in these organisms.

15. Regulation of HMGR expression in the trichothecene-producing fungus Gibberella pulicaris (Fusarium sambucinum)

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Trichothecenes are sesquiterpenoid toxins produced by several Fusarium species. The importance of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) in the regulation of trichothecene production was investigated in G. pulicaris R-6380. A portion

of the gene encoding HMGR (Ipr1) was isolated from G. pulicaris R-6380 using anchored PCR. The isolated gene fragment specifies the C- terminal 340 amino acids of HMGR which contains the highly conserved catalytic domain of this enzyme. The G. pulicaris sequence is 76% homologous with the corresponding region of the HMGRs from S. cerevisiae. The Ipr1 gene is present in a single copy and hybridizes to an mRNA of 3.1 Kb. Transcription of Ipr1 in G. pulicaris was analyzed in both the wild-type strain R-6380 and the mutant strain BC51 (Tri5-) which is blocked in the first step of the trichothecene pathway. Under growth conditions that support trichothecene production Ipr1 mRNA levels increased 3-fold in R-6380 cultures and 6 to 7-fold in BC51 cultures over an 18 h time period. During this same time period the levels of HMGR activity also increased 2 to 3-fold in R-6380 and 5 to 6-fold in BC51. These results suggest that increases in both Ipr1 expression and HMGR activity are closely correlated with trichothecene biosynthesis in G. pulicaris, but that the observed increases in HMGR activity do not require trichothecene biosynthesis.

16. Characterization of the trichothecene pathway gene Tri7 from Fusarium sporotrichioides

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The biosynthesis of trichothecene mycotoxins occurs via a pathway involving multiple isomerization, oxygenation, and esterification reactions. The clustering of trichothecene pathway genes has permitted rapid progress in the characterization of this complex sesquiterpenoid pathway. At least four trichothecene pathway genes are closely linked in Fusarium sporotrichioides. We have identified a fifth pathway gene (Tri7) located 898 bp upstream from Tri3. The Tri7 gene encodes a protein of 399 amino acids with no significant homology to sequences present in the protein databases. Comparison of the Tri7 cDNA and genomic sequences revealed the presence of a single intron of 52 bp. Hydropathy analysis predicted the presence of several membrane spanning segments indicating that TRI7 is most likely an integral membrane protein. Disruption of Tri7 resulted in an altered trichothecene production phenotype characterized by the accumulation of HT-2 toxin but does not alter the expression of other pathway enzymes. Analysis of Tri7 transcription revealed a pattern of expression during trichothecene biosynthesis identical to that of other pathway genes. Further efforts to characterize the function of TRI7 are underway.

17. Reduced virulence of a trichothecene nonproducing mutant of Gibberella zeae on field grown wheat

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Wheat head scab caused by Gibberella zeae is a serious problem for wheat growers worldwide. Head scab causes both yield losses and contamination of grain with trichothecene mycotoxins that are injurious to human and animal health. Trichothecenes are also potent phytotoxins which has led to the hypothesis that they play a role in plant

diseases caused by trichothecene producing fungi. . It was previously shown that disruption of the Tri5 gene encoding trichodiene synthase blocks trichothecene toxin biosynthesis and reduces the virulence of G. zeae on wheat under growth chamber conditions. In this study, conducted under permit from USDA-APHIS, the virulence of a G. zeae strain carrying a disrupted Tri5 and the virulence of its Tri5+ progenitor were examined under field conditions. Hard red spring wheat cultivars Wheaton and Butte 86 were grown in central Illinois and inoculated at anthesis by injecting individual heads with fungal conidia or with water. After one month, the heads were harvested and the grain was analyzed for yield and quality, and for kernel infection with G. zeae Tri5+ strains and the Tri5- disruptant by fungal strain isolation on a selective medium and PCR. The Tri5- disruptant was able to infect wheat kernels, but was significantly less virulent ($P < .05$) than the Tri5+ progenitor on both wheat cultivars as measured by kernel number, weight and germinability, and the frequency of infected kernels. These results indicate that trichothecene mycotoxins contribute to the ability of G. zeae to cause wheat head scab under field conditions.

18. Isolation of the trichodiene synthase gene (Tri5) from the macrocyclic trichothecene producing fungus Myrothecium roridum

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Macrocyclic trichothecenes are antibiotic/phytotoxic compounds that are produced by both fungi and plants. Some macrocyclic trichothecenes produced by Myrothecium roridum are identical to trichothecenes found in two species of the plant genus Baccharis. Production of macrocyclic trichothecenes by M. roridum has been implicated as a possible virulence factor in plant diseases caused by this fungus, however, role of these compounds in the biology of Baccharis species is unclear. To investigate macrocyclic trichothecene biosynthesis we have isolated a gene encoding trichodiene synthase (Tri5) the first step in the trichothecene pathway from M. roridum. Cosmids carrying Tri5 were identified by probing an M. roridum cosmid library with the Tri5 gene from F. sporotrichiodes. Tri5 was subcloned as two 1.7 Kb Hind III fragments from a single cosmid clone (MRcos13). Sequence analysis revealed a coding region of 1211 bp which specifies a protein of 385 amino acids. A 58 bp intron sequence was found in the same position as the intron present in Tri5 from Fusarium species. The predicted amino acid sequence has approximately 70% homology with other trichodiene synthases. Preliminary results indicate that homologs of other trichothecene pathway genes are also present on MRcos13 suggesting that macrocyclic trichothecene pathway genes are clustered.

19. Evidence for chromosome transfer between biotypes of Colletotrichum gloeosporioides

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In Australia there are two biotypes (A & B) of C. gloeosporioides which cause distinct

anthracnose diseases on Stylosanthes spp. Molecular analysis has revealed that the biotypes are genetically distinct. Laboratory pairings of nitrate reductase mutants have indicated that there is no vegetative compatibility between biotypes although isolates within each biotype are compatible. These data suggest the biotypes are genetically isolated clonal lineages. The fungus has a very variable electrophoretic karyotype and this is particularly striking for small chromosomes which are easily resolved. A detailed study of the distribution of two dispensable homologous chromosomes will be presented. One of these chromosome homologues is 1.2 Mb in size and strain-specific in biotype B but a 2 Mb homologue is present in all biotype A strains studied so far. Recent field isolates have now revealed some strains carrying both 1.2 and 2 Mb chromosomes in what appears to be an exclusively biotype B background genome. Molecular analysis of this 2 Mb chromosome appears to support the notion that it is the result of a very recent transfer from biotype A. These results suggest that genetic exchange between biotypes can occur in the field. Pathogenicity tests indicate that the biotype B strain containing the 1.2 and 2 Mb chromosomes can produce chlorotic symptoms on host species only susceptible to biotype A strains. These data suggest that some pathogenicity functions are encoded on the 2 Mb chromosome. It is suggested that the presence of the 2 Mb chromosome permits survival of biotype B genotypes on hosts such as S. scabra which is the predominant species grown commercially.

20. RAPD Characterization of Alternaria alternata isolates from California tomato fields.

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The genetic diversity of 69 isolates obtained from blackmold lesions of ripe tomatoes was assessed using RAPD markers. Two major groups (Gp1 and Gp2) of isolates were identified independently by principal component analysis, and by unweighted pair group method arithmetic average (UPGMA) of Jaccard and simple matching (SM) similarity coefficients. The isolates showed a high degree of homology within each major group (Jaccard similarity (73%; SM = 81%), and 34 of 137 markers were monomorphic for all of the isolates, although the difference between Gp1 and Gp2 was large (Jaccard similarity = 50%; SM = 60%). There was no association between the genetic similarity of isolates and their geographic origin, suggesting wide dispersal of isolates across California. All isolates in the survey were tested for their ability to cause stem canker disease on susceptible tomato cultivars. None of the Gp1 isolates and only three of the 14 Gp2 isolates caused stem canker symptoms. These three isolates clustered together with 11 other isolates in Gp2, indicating a high degree of similarity between these isolates and other isolates causing blackmold disease. However, none of the 137 scored bands could be used to distinguish the three stem canker isolates from the 66 other isolates included in this study. Seven additional toxin- producing isolates were analyzed with RAPDs and found to contain Gp2-specific bands, suggesting that toxin-production may be associated with phenetic group Gp2 only. The biological basis for the large difference between Gp1 and Gp2 isolates is presently unknown.

21. Identification of corn fungal pathogens using RAPD analysis

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We have used RAPD analysis to develop a test which can be used to distinguish races of Northern leaf spot (Cochliobolus carbonum anamorph: Bipolaris zeicola) from each other and to specifically detect the presence of the genome of race one of this corn pathogen found in Iowa. Differentiation of several strains of Bipolaris was accomplished by using the RAPD banding patterns produced with a single 10 base primer (5'ccaccgcgcc). This primer amplifies a prominent band of approximately 550 basepairs from the genome of B. zeicola race one (HC-23). The 550 bp fragment specific to HC-23 was cloned using the TA cloning vector pCRII (Invitrogen) and sequenced. The sequence of this cloned fragment was used to design a pair of 30 base primers which specifically amplify an internal 440 base pair fragment of the cloned sequence from (HC- 23) in the presence of a large excess of genomic DNA from Zea mays and/or DNA from other fungi. These results show the feasabilty of developing a rapid field test for the presence of agronomically important pathogens infecting field corn.

22. Possible retrotransposon in the R-pathotype of Tapesia yallundae the causal agent of eyespot disease of cereals

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T. yallundae, the perfect stage of Pseudocercosporella herpotrichoides, is the pathogen responsible for eyespot disease of cereals. Pathogenicity tests in conjunction with in-vitro growth characteristics have been used in the past to classify eyespot isolates into the R- and W- pathotypes. On nutrient-rich agar medium, R-types exhibit slow growth with feathery colony margins, whereas W-types grow at twice the rate of the R-types with even colony margins. During growth in-vitro, R-types often exhibit cultural instability, forming variable sectors. Reversion of sectors to the parental phenotype suggests the possible activity of a transposable element. To investigate this hypothesis, primers were constructed from homologous regions of known fungal transposons (from Cladosporium fulvum and Magnaporthe grisea) to use in PCR amplification. A fragment generated by this approach has been cloned and sequenced and further investigation is underway to establish the presence and activity of a retrotransposon.

23. Expression of peroxidase, phenylalanine-ammonia lyase, ethylene and cutinase genes in Brassica napus in response to infection by virulent and weakly virulent isolates of Leptosphaeria maculans

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The relative levels of mRNAs were monitored by Northern blot hybridization of leaf tissue RNA samples, at varying intervals following infection. Variation in the level of peroxidase mRNA was observed with the use of radiolabelled Expressed Sequencing Tags (EST) clones as probes. A high level of peroxidase mRNA was induced in plants

inoculated with both pathotypes. Hybridization with radiolabelled PAL gene DNA, showed no detectable PAL message in uninfected tissue, while induction was observed after infection with both isolates. Similarly, ethylene induction--also a defense-related response--was followed in infected leaf tissue. In addition, the status of cutinase gene expression was assessed during infection. Cutinase mRNA was induced when the plant was infected with both isolates. With the avirulent strain as the inoculum, at 24 h post-infection the cutinase mRNA level was high, registering a steady decline thereafter. The opposite trend was witnessed with the use of the virulent strain as inoculum, i.e. low levels at 24 h and a steady increase thereafter.

24. A virus-resistant mutant of Cryphonectria parasitica

James J. Polashock and Bradley I. Hillman, Rutgers University, and Bao Chen and Donald L. Nuss, Roche Institute of Molecular Biology.
NB58F is a virus-resistant mutant derived from a sector of an infected isolate of Cryphonectria parasitica, the chestnut blight fungus. Morphologically, NB58F is distinguishable from both infected and non-infected isolates of C. parasitica. Mating, RFLP, and fingerprint studies have demonstrated that the NB58F morphology and virus resistance are inherited together as nuclear traits. Molecular karyotype analysis has shown that a small (~3 mB) chromosome is present in NB58F and not in the wild-type isolate. The NB58F morphology and virus resistance were inherited with the 3 mB chromosome in ascospore progeny. Although NB58F has not been stably infected with any cytoplasmic virus, it supports stable infection with the mitochondrial virus from strain NB631. Short term infection of NB58F with full-length transcripts of the cytoplasmic virus CHV1-713 was successful, demonstrating that cytoplasmic dsRNA replication can occur in NB58F. However, virus-infected transfectants were not stable and virus was lost upon frequent sectoring. Together, these results suggest that the NB58F mutation may affect movement of cytoplasmic viruses while not affecting mitochondrial movement.

25. Reversion of a disrupted toxin biosynthetic gene restores high virulence in Gibberella zeae

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Gibberella zeae is an important pathogen of cereal crops and produces trichothecene mycotoxins, including deoxynivalenol (DON). Previously, to determine whether trichothecenes contribute to the virulence of G. zeae, we generated DON nonproducing mutants of the fungus by transformation mediated disruption of the gene (Tri5) encoding the first enzyme in the trichothecene biosynthetic pathway. The virulence of Tri5- transformants, in which gene replacement had occurred, was reduced on some host plants relative to the Tri5+ strain from which they were derived. To confirm that the reduced virulence of Tri5- transformants was specifically due to the loss of Tri5 function and not some unrelated effect of transformation, we generated revertants of G. zeae by selfing a transformant that had undergone additive gene disruption. According to PCR analysis, three out of 160 single ascospore progeny had undergone reversion from Tri5- to Tri5+.

However, only one of the three revertants produced DON. The DON producing revertant exhibited wild type levels of virulence on wheat seedlings (cv. Wheaton) while all the DON nonproducing progeny examined, including the two DON- revertants, had the same reduced levels of virulence as the parental Tri5- transformant. These data indicate that the reduced virulence of the DON nonproducing transformants resulted from disruption of Tri5 and provide further evidence that trichothecene production contributes to the virulence of G. zeae.

26. RAPD-bulked segregant analysis based mapping of a Gibberella fujikuroi gene involved fumonisin biosynthesis

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The maize pathogen Gibberella fujikuroi mating population A (Fusarium moniliforme) produces fumonisins, a family of secondary metabolites that are toxic to a number of plant and animal species. A locus, designated fum1, that confers the ability to produce fumonisins was previously identified from a cross of fumonisin producing (fum1+) and nonproducing (fum1) strains of G. fujikuroi (Xu and Leslie, 1993 FG Newsletter 40A:25). We are using RAPD combined with Bulked Segregant Analysis to map and eventually clone fum1. Bulked DNA consisted of pools of DNA from 25 fumonisin producing or fumonisin nonproducing ascospore progeny. To date, 260 RAPD primers have been screened. We have identified two primers, OPA16 and OPH3, that amplify a fragment from the fum1 parent and fum1 pooled DNA, but not from the fum1+ parent or pool. RAPD analysis with DNA from individual progeny indicated that the markers amplified by OPA16 and OPH3 are located on opposite sides of fum1 at distances of 14.4 and 13.1 centimorgans, respectively.

27. The interaction between a non-pathogenic mutant of Glomerella magna and plant hosts

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Biochemical and microscopic analyses have been performed on anthracnose resistant and susceptible plants infected with the wild type (L2.5) and a non- pathogenic mutant (path-1) of G. magna. The temporal and spatial expression of phenylalanine ammonia lyase and peroxidase enzyme activities were monitored in plants inoculated with either L2.5, path-1, or colonized for 48 hours with path-1 followed by inoculation with L2.5 (cross protected). Cross protection against L2.5 by path-1 was not systemic and appeared to be tissue specific. The growth of L2.5 and path-1 in anthracnose susceptible and resistant plant tissues was observed by differential staining and microscopic assessment. The production of appressoria in plant tissues was also monitored microscopically. G. magna specific PCR primers were also used to monitor the growth of these fungi through plant tissues. The mRNA differential display system was used to identify transcript differences between L2.5 and path-1.

28. Isolation of pathogenicity and virulence genes of Cladosporium fulvum

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Cladosporium fulvum is a hemibiotroph pathogen of tomato which causes leaf mould on its host plant. A C. fulvum isolate (Cf4) was mutagenized by transformation with a plasmid carrying a hygromycin resistance gene (pAN7-1). Individual tranformants were isolated and used to inoculate tomato seedlings and transformants that failed to form the normal conidiophores on the leaves 2 weeks after inoculation were selected for further analysis. 4550 tranformants were screened in this way and 15 mutants were found. Eight of these mutants do not differ in any obvious way from wild type C. fulvum when grown on artificial media. Two classes of phenotypes can be described: (I) pathogenicity mutants, i.e. those which do not show complete the infection cycle on the tomato leaf (3 mutants) and (II) virulence mutants i.e. transformants which do eventually form conidiophores on the tomato leaves but do so with a noticeable delay and/or cause evidently reduced symptoms on the plant (5 mutants). In the poster, we report on progress made in characterising some of these mutants and in cloning DNA from regions flanking the plasmid insertion sites. Our immediate aim is to establish whether, in any case, the phenotype is determined by insertion of the plasmid in the target gene.

29. Plant flavonoids and isoflavonoid phytoalexins stimulate germination of Fusarium solani spores independent of nutritional stimulation

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Flavonoids exuded by plant roots act as important signals for most rhizobial bacteria to initiate an interaction with legumes leading to the formation of nitrogen-fixing root nodules. We show here that flavonoids, including stress-related isoflavonoids, induce spore (macroconidia) germination in Fusarium solani f.sp. pisi, (isolates of N. haematococca MPVI) a soil-borne fungal pathogen of pea. The action of specific flavonoids in stimulating germination paralleled the specificity of nod gene induction in the bacterial pea symbiont, Rhizobium leguminosarum bv. viciae. A similar parallel was observed between F. solani f.sp. phaseoli, a bean pathogen, and the bean symbiont R. leguminosarum bv. phaseoli. The flavonoid-responsive germination appears to utilize a signal pathway different than nutrient-responsive germination because the former is prevented by inhibitors of cAMP-dependent protein kinase (PKA), while the latter is not. Germination of macroconidia in root exudates was significantly inhibited by the PKA inhibitor, indicating that flavonoids may be as or more important than nutrients as a stimulatory signal in root exudates. Thus, flavonoids in root exudates may play two roles in legumes, initiating interaction with beneficial bacterial symbionts but also inducing F. solani to emerge from dormancy and initiate a pathogenic interaction with its host plant.

30. Characterisation of the pathogenicity gene MPG1 from the rice blast fungus Magnaporthe grisea

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Vries, Joseph. G.H. Wessels(3). (1)University of Exeter, Exeter, EX4 4QG, UK, (2)Purdue University, West Lafayette, IN 47907, (3)University of Groningen, Haren, The Netherlands.

Magnaporthe grisea infects its host by producing a specialized cell known as an appressorium. This cell works by adhering tightly to the leaf surface and generating high internal turgor which is translated into the mechanical force necessary to break the underlying plant cuticle. Recently, we identified a gene known as MPG1 which appears to play an important role in the elaboration of appressoria. The gene was identified as a fungal transcript produced abundantly in planta. Temporal analysis revealed that MPG1 was highly expressed as soon as 18h after inoculation of rice seedlings and was also expressed during disease symptom expression 72- 96h later. A directed gene replacement showed that MPG1 is required for efficient appressorial development and mpg1-mutants therefore showed a reduced pathogenicity phenotype. MPG1 appears to encode a hydrophobin-like protein with homology to the Sc3, Sc1 and Sc4 genes from Schizophyllum commune, the rodA gene from Aspergillus nidulans and the eas gene from N. crassa. Consistent with this, M. grisea mpg1 mutants show an 'easily wettable' phenotype showing that cell surface hydrophobicity of aerial hyphae is reduced. Hydrophobins are unusual proteins which are known to be produced during aerial growth of fungi. Hydrophobins appear to undergo self-assembly into high molecular weight amphipathic complexes when they reach interfaces between liquids and air. Such physical characteristics would predict a number of potential roles for MPG1p in appressorial development and pathogenesis. It is, for example, conceivable that MPG1p acts either as a structural component of the appressorium, or as an adhesion protein. Its secretion and potential incorporation into cell wall complexes may therefore be a rate limiting step in the transduction of the inductive signals required for appressorial morphogenesis. In order to test these hypotheses a number of strategies have been adopted to purify and characterise MPG1p and to determine its precise role in the pathogenesis of M. grisea. Progress in these areas was discussed.

31. Evidence of a horizontal transfer event between phytopathogenic fungi in the genus Leptosphaeria and its correlation to host-range expansion

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Phoma lingam (teleomorph: Leptosphaeria maculans) and Phoma wasabiae are closely related species that cause similar disease symptoms on different plants in the crucifer family. All the examined isolates of Phoma lingam that are aggressive to Brassica species possess a repetitive element, LMR1. With one exception, no isolates of the related Phoma wasabiae, that is aggressive to the crucifer Wasabi japonica, have been found to contain LMR1. The apparent horizontal transfer of the element to this isolate correlates with the expansion of its host-range to include Brassica juncea. The structure of LMR1 will be described and models will be presented for how it could become an extrachromosomal element, thus facilitating transfer. In addition, our hypotheses about how this transfer may induce host-range expansion are presented.

32. Cercosporin efflux and partial auto-resistance are encoded by the LE6 gene

Robert Upchurch, Terrence Callahan, Maura Meade. North Carolina State University.
The fungal pathogen of soybean Cercospora kikuchii produces the polyketide phytotoxin cercosporin, an essential pathogenicity factor for the development of disease. Light is required for the induction of cercosporin synthesis. We have used light induction to isolate light-enhanced cDNAs by a subtractive hybridization technique. One of these cDNAs, LE6, shows enhanced transcript accumulation 20 fold higher in light and is correlated with the accumulation of cercosporin in culture. The nucleotide sequence of this cDNA contains a putative open reading frame of 1818 base pairs that encodes a predominatly hydrophobic, cysteine rich peptide of 606 amino acids with a molecular weight of 65,424 and an isoelectric point of 5.08. Kyte-Doolittle analysis of the peptide indicates that LE6 contains six potential alpha helical regions, three of which stretch long enough to span the plasma membrane. Gene disruption of LE6 results in dramatically reduced cercosporin production in the light, loss of pathogenicity on soybean, diminished transcript accumulation of another light-enhanced cDNA, an altered pigment accumulation profile, and substantial loss of auto-resistance to cercosporin. An amino acid sequence homology search has identified two regions of homology common to efflux-mediated drug resistance determinants in the amino half of the protein, suggesting that LE6 belongs to the subgroup of the major facilitator protein superfamily that catalyzes efflux-mediated drug resistance.

33. Physical, genetic, and molecular characterization ofthe TOX2 locus, a complex locus controlling pathogenicity in Cochliobolus carbonum

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The TOX2 locus of C. carbonum race 1 encodes the biosynthetic activities required for the production of HC-toxin. HC-toxin, a cyclic tetrapeptide, is a host-selective toxin which is the key molecule involved in the pathogenicity of the fungus on specific genotypes of maize. In all wildrace 1 strains tested, there are two copies of the TOX2 locus. The gene encoding the major HC-toxin biosynthetic activity, HTS1, has been cloned and characterized. HTS1 encodes an enzyme, HTS, which is involved in non-ribosomal peptide bond formation between the amino acids in HC-toxin. Several other genes (TOXA, TOXC, TOXD) unique to race 1 strains have also been cloned and are currently being analyzed. The TOXA gene encodes a putative integral membrane HC-toxin efflux protein. The TOXC gene contains an open reading frame with amino acid sequence similarity to yeast fatty acid synthases and may be involved in the biosynthesis of the novel HC-toxin amino acid, AEO. The TOXD gene is a Tox+ unique gene of unknown function. In addition, there is evidence of Tox+ unique regulatory gene(s). The organization of the genes within the TOX2 locus was analyzed using contour-clamped homogeneous electric field (CHEF) gel electrophoresis and sexual crosses.

34. Comparison of three xylanase genes in the plant pathogenic fungus Cochliobolus carbonum

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Three endo-xylanases genes have been cloned from C. carbonum. Xyl1 is the major endo- xylanase and accounts for 85%-94% of the xylanase activity when grown in culture as well as the major protein peak when culture filtrate is fractionated by HPLC cation exchange. A degenerate oligonucleotide had been generated from direct internal amino acid sequence of Xyl1, and this oligo was used to clone XYL1 and XYL3. XYL2 was cloned from a cDNA library prepared from fungus grown on corn cell walls using XYL1 as a heterologous probe. XYL2 and XYL3 have a 62% and 42% identity respectively to XYL1 at the amino acid level. In addition, all three of these xylanases are similiar to xylanases from other organisms. The expression of the three xylanases is currently being examined. XYL3 expression has not been seen nor a cDNA clone been found in the cDNA library prepared from fungus grown on corn cell walls. Transformation-mediated gene disruption of XYL1 was already conducted and pathogenicity tests of the mutant indicate that XYL1 is not required for pathogenicity. Transformation-mediated gene disruption of XYL2 and XYL3 is currently in progress. The single endoxylanase mutants will be crossed with each other to obtain multiple disruption mutants and these will be tested for pathogenicity on maize.

35. Genetics of gibberellin biosynthesis by Gibberella fujikuroi

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The rice pathogen Gibberella fujikuroi is well known as a producer of large amounts of gibberellins which induce the superelongation ("bakanae") disease on infected rice seedlings. By GC MS it could be demonstrated that the ability to produce gibberellins is widespread among fungi, but only rice pathogenic isolates of the species Gibberella fujikuroi (mating population C) seem to be deregulated and produce these phytohormones in high amounts. The genetics of gibberellin biosynthesis and the relationship to the biosynthetic pathway in higher plants are not well understood until now. Therefore, we have started to isolate the genes of the isoprenoid pathway as well as specific gibberellin genes. So far, the genes for HMG-CoA-reductase, the geranyl-geranylpyrophosphate and farnesylpyrophosphate synthetases were isolated by screening of genomic and expression libraries of this fungus with heterologous probes or with PCR-fragments synthesized on the basis of sequence homologies to other fungal prenyltransferases. Sequence comparison with analogous fungal or plant genes showed that the genes of this pathway are very conserved.

36. Victorin-deficient REMI mutants of Cochliobolus victoriae demonstrate a requirement for victorin in pathogenesis

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C. victoriae is a pathogen of oats and produces victorin C, a cyclized pentapeptide which specifically affects oats containing the Vb allele; all other oat genotypes are resistant to

both the toxin and the fungus. We have used Restriction Enzyme Mediated Integration (REMI) to mutate genes involved in victorin C biosynthesis. Eight mutants altered in victorin C production were isolated after screening approximately 620 hygromycin B-resistant transformants by automated HPLC analysis of crude culture fluids. One is a null mutant, several are leaky (< 0.5-5% of wild type), and two are overproducers of victorin C (3-4 X that of wild type). The null mutant produces no detectable toxin and is unable to cause disease symptoms on oats in leaf uptake, root inhibition, and whole plant assays. The leaky mutants cause symptoms on whole plants but much more slowly than wild type. The null mutation is untagged, as determined by insertion point rescue and gene disruption experiments. Similar analyses are being conducted on the other mutants with altered victorin production. To identify genes encoding a particular enzyme involved in victorin biosynthesis, we have prepared degenerate primers and isolated PCR products with sequence similarity to known cyclic peptide synthetases. These products will be used to investigate enzyme functions by gene disruption methods.

37. Molecular cloning of the gene for the host-specific toxin produced by Pyrenophora tritici-repentis

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We are investigating the key events that regulate specificity in the disease, tan spot of wheat, caused by the fungus Pyrenophora tritici-repentis. The host specific toxin(s) produced by P. tritici-repentis is a protein, which provides a straight forward experimental approach to the molecular cloning of the gene. Analysis of purified toxin by mass spectroscopy indicated a molecular weight of 13.2 kD. Polyclonal and monoclonal antibodies were raised against purified toxin. Western analyses indicated that the antibodies reacted with and are specific to a 13.2 kD band associated with toxic activity. Time course studies comparing toxin production and toxic activity in culture filtrates were performed. Poly (A+) RNA was prepared from mycelia from time periods for the maximum rate of toxin production. In vitro translations of isolated total mRNA were performed and the relative abundance of the Tox message evaluated by indirect immunoprecipitations of total translation products with anti-Tox antibody. Results indicated the presence of a single, ca. 20kD translation product that reacted with anti-Tox antibody. Immunoprecipitation analyses suggest the presence of a precursor form of the toxin as expected for a secreted protein. A lambda cDNA library was prepared and screened with anti-Tox antibody. Antibody-positive recombinants were identified at a high frequency that reacted with both the monoclonal and polyclonal antibodies. We have sub-cloned a ca. 750 bp cDNA from an antibody positive recombinant and are currently conducting nucleotide sequence analysis.

38. Pathogenicity related gene expression in the entomopathogenic fungus Beauveria bassiana

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The Deuteromycete fungus, Beauveria bassiana along with other fungi, is being developed as a potential biological control agent for insect pests. The pathogenicity of an entomopathogenic fungus is largely determined by its ability to penetrate the host cuticle,

overcome the host defense responses and propagate inside the host body. We have mainly been working on the invasion processes by the fungus which include the coordinated secretion of cuticle-degrading enzymes and the differentiation of penetration structures. In our laboratory, we have cloned genes which are induced when the mycelia is grown in nutrient-deprived media containing a host (cockroach) cuticle. These genes encode an array of enzymes and a putative toxin, indicating strategies by which the fungus may penetrate host barriers and subdue the insect.

39. Sexual and asexual mechanisms influence genetic variation in Pythium ultimum

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We are interested in studying genetic, morphological, and pathogenic variation in Pythium ultimum, a homothallic soil-borne oomycete. P. ultimum isolates are capable of outcrossing under lab conditions. Genetic analysis of isolates belonging to the Pythium taxonomic group HS (self- sterile isolates with cultural characteristics similar to P. ultimum) demonstrated that these isolates can cross with homothallic P. ultimum isolates. The group HS isolates may represent heterothallic variants of P. ultimum. F2 segregation ratios and karyotypes detected by PFG electrophoresis are consistant with diploidy for several homothallic and group HS isolates. Diploid populations segregating for sporulation rate and sensitivity to inhibitory chemicals have been established for genetic analysis. Exceptions to diploidy also exist in P. ultimum. For example, three alleles were detected in isolate Pu18 with RFLP marker PG123. Variation in allele number, karyotype, growth rate, and pathogenicity was detected in subcultures generated from single hyphal tips and single sporangia of Pu18. These results suggest that polyploidy and heterokaryosis may contribute to non-Mendelian allele numbers and that asexual mechanisms can contribute to variation. We are in the process of extending our observations to field populations. Using co-dominant PCR markers and RFLP markers we have found that field isolates are often heterozygous at unlinked loci and genetically distinct individuals are frequently isolated from the same lesion. These results support the occurrence of outcrossing in nature.

40. Location of pisatin demethylase genes on diverse dispensable chromosomes in the phytopathogenic fungus Nectria haematococca

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Conventional genetic analysis has identified six genes (PDA) in the ascomycete Nectria haematococca that encode for the ability to demethylate the pea phytoalexin pisatin. It has been observed that the phenotype associated with PDA genes is occasionally lost during crosses. Miao, et. al. (Science 254:1773) showed that the loss of Pda in isolates carrying PDA6-1 was due to loss of a 1.6 Mb dispensable chromosome carrying PDA6-1. Progeny from crosses involving two independently segregating genes, PDA1 and PDA5, also exhibited loss of Pda. Southern analysis confirmed that loss of Pda was due to loss of one or both genes. CHEF analyses showed that PDA1 is on a 1.5 Mb chromosome that is

absent in PDA1 progeny. PDA5 is found on a 4.9 Mb chromosome in the original Pda+ parent from these crosses but on polymorphic chromosomes, in the range of 3.5 to 5.7 Mb, in progeny from this parent. Analyses of crosses between PDA5 carrying parents showed loss of PDA5. All these PDA5 progeny had an aberrant colony morphology which suggested that not all DNA lost along with PDA5 is dispensable. Electrophoretic karyotyping of field isolates and their progeny has shown that chromosomal bands carrying different PDA genes are absent in Pda progeny. Southern analysis of CHEF gels using DNA from a PDA1-bearing chromosome as a probe, has shown that most of the PDA chromosomes thus far tested cross-hybridize with this chromosome, one notable exception being the PDA5 chromosome. There is no hybridization of the PDA1 chromosome with the chromosomes of Pda isolates, indicating that all or part of these PDA-bearing chromosomes are dispensable.

41. Transformation of Botrytis cinerea: system and applications

Walid Hamada, Martine Boccara and Gilbert Bompeix, Laboratoire de Biochimie et Pathologie Vegetales, UPMC, 75252 Paris, France
Botrytis cinerea, the causal agent of grey mould on various fruits, is an economically important pathogen of grape. Different hydrolytic enzymes secreted by B. cinerea have been characterized, among them, pectin methylesterase (PME, Reignault et al., 1994) might be involved in its pathogenicity. The control of B. cinerea by fungicides is not satisfactory as this fungus is variable genetically and resistant strains have appeared all over the world. A transformation system is an essential tool for developing new weapons against this fungus. Using a plasmid carrying the hygromycin phosphotransferase gene (hph) of E. coli (Hamada et al., 1994), we have obtained transformants expressing different levels of resistance to hygromycin. The hph gene was integrated into B. cinerea genome, often as tandem arrays and in different locations and was expressed at variable levels. Further investigations of these transformants are needed to understand variable gene expression as well as to analyse the segregation of integrated copies. Transformation will be helpful to clone fungicide resistance genes by complementation and to generate specific mutants by gene disruption (for exemple for PME). Developing transformation as a tool of mutagenesis will help to understand the pathogenicity of B. cinerea. We are improving transformation using REMI (restriction enzyme-mediated insertion). Hamada, Reignault, Bompeix, Boccara (1994) Current Genetics 26:251-255. Reignault, Mercier, Bompeix, Boccara (1994). Microbiology in press

42. Structural analysis of melanin biosynthetic genes of Colletotrichum lagenarium

Yasuyuki Kubo(1), Yoshitaka Takano(2), Iwao Furusawa(2) and Osamu Horino(1), (1)Kyoto Prefectural University and (2)Kyoto University, Kyoto, Japan
Melanin biosynthesis of Colletotrichum lagenarium is essential for appressorial penetration. Melanin deficient albino mutants form colorless appressoria and can not penetrate host cucumber leaves and also cellulose membranes, a model substrate for host leaves. The melanin is considered to confer structural rigidity to appressorial walls needed for focusing the turgor forces in the vertical direction to facilitate penetration from pore formed at the basal area of appressorium. Three types of mutants defective in

different steps in melanin biosynthetic pathway have been isolated. Albino mutant 79215 (Pks) was defective in the polyketide synthesis, mutant 9201Y (Scd) was defective in the dehydration of scytalone to 1,3,8-trihydroxynaphthalene and mutant 9141 (Thr) was defective in the reduction of 1,3,8-trihydroxynaphthalene to vermelone. The three melanin biosynthetic genes, PKS1, SCD1 and THR1 were cloned and the structural analysis of these genes was made by resolving DNA sequence and their transcriptional structure. The predicted amino acids encoded by PKS1 and THR1 were 2187 and 241, respectively. PKS1 has significant homology with polyketide synthase gene, particularly Aspergillus nidulans wA involved in conidial pigmentation, and THR1 shares high homology with Ver1 gene involved in aflatoxin biosynthesis of Aspergillus parasiticus and the polyhydroxynaphthalene reductase gene of Magnaporthe grisea. The three genes were not clustered in a cosmid level.

43. Genetic improvement and the molecular basis of fungal pathogenesis

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Entomopathogenic fungi represent an untapped reservoir of pesticidal genes for the production of advanced engineered pestcides; an important consideration given that the lack of "useful" pesticidal genes for transfer has been a major constraint in the implementation of biotechnology in crop protection We are assembling a bank of pathogenicity related genes from Metarhizium anisopliae and Beauveria bassiana which could be used to transform other fungi, bacteria, or viruses to create novel combinations of insect specificity, or to produce transgenic plants with improved resistance to insect pests.To perform these studies, specific vectors are being constructed which facilitate strain construction to enhance virulence using constitutive and regulatory promoter regions for expression of homologous and heterologous genes. The potential for this approach has been demonstrated by transferring the gene for the Prl protease from M. anisopliae to Aschersonia aleyrodis, which consequently became a pathogen of late instar whitefly. We have developed a direct strategy for engineering enhanced virulence in M. anisopliae by constitutive expression of some of the many, normally inducible anti-insect proteins. Our initial candidates for this approach have been genes encoding cuticle-degrading enzymes and toxins, since the active agents are encoded by single genes and have been shown to be active in vitro against insects. Constitutive expression of Prl was obtained by transforming M. anisopliae with cDNA for Prl behind the Neurospora crassa cross pathway control promoter. Transgenic strains continued to produce Prl in the haemocoel of Manduca sexta caterpillars following penetration of the cuticle causing extensive melanization in the body cavity and cessation of feeding 30-40 hr earlier than controls infected with wild type. These studies provide the first conclusive demonstration of the utility of heterologous gene expression in molecular analysis of the insect- fungus interaction, and for strain construction of improved mycoinsecticides.

44. New mutations involved in T-toxin production by Cochliobolus heterostrophus are unlinked to the Tox1 locus

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Crosses between race T (which produces T-toxin) and race O (which does not produce T-toxin) of C. heterostrophus segregate 1: 1 for parental types, thus defining a gene (Tox1) controlllng toxin biosynthesis and high virulence to the host plant, corn. Twelve Tox1 mutants, generated previously, all map at Tox1, suggesting that this locus contains all the informatlon needed for T-toxin productlon. Tox1 is actually two loci, each on a different chromosome; both are linked to the breakpoint of a reciprocal translocation. Here we report identification of two additional loci unlinked to Tox1 which appear to be involved in T-toxin production. The two loci were revealed by REMI mutagenesis; mutants produce halos smaller than wild type in a microbial toxin assay. When mutants are crossed with race T, only parental progeny segregate (1 : 1); all Tox progeny contain the REMI plasmid, indicatlng each mutation is tagged. When crossed with race O, progeny segregate 25% wild type halo, 25% mutant halo and 50% no halo, indicating that the mutations do not map at Tox1. This conclusion is confirmed by crosses to any induced mutant which maps at Tox1, in which progeny segregate 25% wild type halo, 25% no halo, and 50% parental halos. When the two mutants are crossed to each other, proqeny segregate 25% wild type halo, 50% parental type and 25% double mutant (less leaky than either parental mutant), indicating the two mutations are unlinked to each other. The transforming plasmid with flanking DNA was recovered and retransformed into wild type race T; the original mutant phenotype was restored.

45. Phylogenetic relationship among PDA genes

Kevin McCluskey and Hans VanEtten, University of Arizona, Tucson, Arizona
DNA sequence for seven Nectria haematococca MP VI PDA genes which encode the enzymatic detoxification of the phytoalexin antibiotic pisatin is compared using a phylogenetic analysis technique. This analysis demonstrates that there are distinct lineages of PDA genes. PDA genes which encode a highly active pisatin demethylase phenotype (PDAH) share the closest relationship and have all been associated with virulence on pea. The gene PDA5 is the most divergent among the PDAH genes, consistent with its unique origin: this gene was found in an isolate from mulberry in Japan while all of the other highly active PDA genes are from isolates obtained from pea in the USA. Straney and VanEtten have shown that there is a region of DNA upstream from the translation start site of PDA 1 that is involved in the activation of PDA genes following exposure to pisatin (MPMI 7:256-266). While this sequence, called the pisatin responsive element, is found in all of the PDAH genes, this region is interrupted by a small insertion in all of the genes conferring the low activity pisatin demethylation phenotype. DNA sequence is also presented for homologs of the Nectria PDA gene in Fusarium oxysporum f. sp. pisi. Comparing all of these PDA genes with other fungal P450 gene sequences demonstrates that this is a unique family of genes in phytopathogens of pea.

46. The TOXA gene of Cochliobolus carbonum encodes a putative HC-toxin pump

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The TOXA gene of C. carbonum is tightly linked to the HTS1 gene which encodes the

multifunctional HC-toxin synthetase, HTS. Both genes are found only in toxin-producing strains of C. carbonum and TOXA message is expressed at very low levels in the cell. The TOXA gene has an open reading frame which encodes a putative protein of 58kD. Hydropathy plots of the TOXA amino acid sequence suggest that the protein is an integral membrane protein with 10-13 membrane spanning domains. The amino acid sequence is similar to bacterial and fungal proteins known to be small molecule efflux pumps, and we propose that the TOXA gene product is an HC-toxin pump. There are two linked copies of the TOXA gene in C. carbonum; deletion of either copy has no measurable effect on the cell's ability to produce HC-toxin or infect maize. However, despite many attempts, it has not been possible to construct strains with both copies of the TOXA gene disrupted. This suggests that TOXA mutants are lethal in an HC-toxin producing background due to the intracellular accumulation of HC-toxin.

47. Neutral versus selective evolution of proteins: data from the human pathogen Coccidioides immitis (Ascomycota, Onygenales)

Vassiliki Koufopanou, Austin Burt and John W. Taylor. Department of Plant Biology, University of California, Berkeley.
The question of whether evolution is mainly due to selection or random drift can be addressed using DNA sequence data from protein coding loci and comparing the ratio of replacement to synonymous substitutions within and between species. A higher ratio of replacement to synonymous substitutions between species than within indicates that species divergence represents adaptation to different environments (positive selection changing amino acids), while equal ratios within and between species suggest speciation by a random accumulation of mutations (neutral model). We are studying evolution at a variety of protein-coding genes in the ascomycete fungus Coccidioides immitis, a human pathogen causing valley fever, and its closest known relatives, the nonpathogenic Uncinocarpus reesii and Auxarthron zuffianum. We have obtained data for CHSl, coding for chitin synthase, an enzyme located on the cell membrane and participating in cell wall synthesis, and for a gene coding for 4 hydroxy-phenylpyruvate dioxygenase (4HPPD), a protein identified as a human T-cell antigen of C. immitis, found on the cell membrane and cell walls of conidia and spherules, the infective and proliferative stages of the disease in humans.

48. Molecular markers and sex in the human pathogen Coccidioides immitis

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Microorganisms are not easily studied in nature and even determining whether a species has sex can be difficult or impossible by direct observation. Coccidioides immitis, a dimorphic fungal pathogen and cause of a recent epidemic of "Valley fever" in California, is typical of many eukaryotic microbes in that mating and meiosis have yet to be reported, but it is not clear if this is because sex is absent, or just cryptic. To find out, we have undertaken a molecular population genetic study to test for the clonal structure expected if sex is truly absent. Molecular markers with nucleotide level resolution were found by a

novel approach combining PCR amplification with arbitrary primer pairs, screening for single strand conformation polymorphisms (SSCPs), and direct DNA sequencing. Both population genetic and phylogenetic analyses indicate that C. immitis is almost completely recombining, with little evidence of clonal structure. These results suggest that sex is a regular part of the C. immitis life cycle and illustrate the utility of SSCP and sequencing with arbitrary primer pairs (SWAPP) in molecular population genetics.

49. Possible regulation of pathogenicity genes by nitrogen catabolite expression in Magnaporthe grisea

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Carbon and nitrogen catabolite repression have recently been implicated in regulation of genes required for pathogenesis in both plant pathogenic bacteria and fungi. We recently demonstrated that Mpg1, a Magnaporthe grisea gene that expressed in planta and involved in pathogenicity is induced by nitrogen and carbon starvation. Thus we examined the role of nitrogen catabolite repression in the regulation of genes involved in pathogenesis. We investigated whether mutations at the major transcriptional activator, Nut-1 (for Nitrogen utilization) would render M. grisea non-pathogenic. Three hundred and eighty chlorate resistant mutants were isolated and classified according to their defects in nitrogen metabolism. Five putative nut-1 mutants were identified, and two of these mutants, Cl60 and Cl105 have reduced pathogenicity and toxin production. In addition to the commonly observed ammonium-dependent growth, they have also lost the ability to regulate Mpg1 during nitrogen and carbon starvation. Genetic analysis of Cl60 and Cl105 demonstrates that these mutations define two unlinked genes. Surprisingly, neither gene is allelic to Nut-1. We named Cl60 and Cl105 as Npr1-1 and Npr1-2 (for Nitrogen and pathogenicity regulation) respectively. We hypothesize that Npr1-1 and Npr1-2 may act as regulators that integrate nutritional starvation and pathogenesis related pathways. To begin to test this hypothesis, cloning of these genes by complementation is underway.

50. SAR gene expression in maize

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Systemic acquired resistance (SAR), induced by biotic or chemical activation offers broad host resistance to a variety of pathogens (Ross, 1961; Kuc, 1982; Metraux et al., 1991). In response to some pathogens many plants form necrotic lesions on their leaves. Lesion mimic mutants form lesions in the absence of pathogens, stress, or wounding (Walbot et al., 1983). Arabidopsis lesion mimic mutants simulate the disease response by expression of histochemical and molecular markers (Dietrich et al. 1994), formation of secondary metabolites, deposition of callose in and around necrotic lesions, and SAR gene expression. To examine if maize lesion mimics possess these characteristics, plants were grown to maturity and tissue harvested before and after lesion formation. RNA was extracted and analyzed for SAR gene expression by northern analysis. Chemical inducers of resitance also induces SAR genes in maize similar to what has been reported in other systems (Ward et al. 1992, Uknes et al 1992, 1993). References: Alexander et al., PNAS

90: 7327-7331 (1993); Dietrich et al., Cell, in press (1995); Kuc, BioScience 32:854-860 (1982); Metraux et al., in Advances in Molecular Genetics of Plant-Microbe Interactions, Vol. 1, H. Hennecke and DPS Verma, eds. pp. 432-439 (1991); Ross, Virology 14: 329-339 (1961a); Ross, Virology 14:340-358 (1961b); Uknes et al., The Plant Cell 4:645-656 (1992); Uknes et al., The Plant Cell 5:159-169 (1992); Walbot et al., in Genetic Engineering of Plants, T. Kosuge and C. Meredeth, eds., pp. 431-442 (1983); and Ward et al., The Plant Cell 3:1085-1094 (1991).

Posters II: Gene Expression/Genome Structure

1. A fungal transposon with homology to the maize Ac element

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Transposable elements are well analysed genetic traits in higher plants and animals. However, in filamentous fungi transposons were isolated only recently. Most of them appeared to be retroelements, i.e. employ a reverse transcriptase for propagation(1). Non-retroelement transposons are far less frequently described(2). This particular type of transposons has proven to be extremely useful for the development of transposon tagging systems, in both higher plant and animal systems(3). We have successfully attempted the isolation of a non-retroelement transposable element from the filamentous fungus Tolypocladium inflatum. As a result a 4100 bp repeated DNA element with 20 bp terminal inverted repeats and 8 bp target site duplications was identified. Hybridization to chromosomal restriction fragments indicates the element being present at about 20 different genomic sites. The transposon carries a large open reading frame. Its deduced amino acid sequence exhibits strong similarities to members of the eukaryotic Ac-like transposon family. The data presented prove that the repeated DNA which we named restless has properties of a transposable element. Currently the biological activity of restless is under investigation. (1) e.g.: Cambareri EB, Helber J, Kinsey JA (1994) Mol Gen Genet 242:658-665; (2) Daboussi M-J, Langin T, Brygoo Y (1992) Mol Gen Genet 232:12-16; (3) review: McDonald JF (1993) Curr Opin Genet Develop 3:855-864

2. Expression and targeted secretion of a mammalian thrombolytic protein using the gla-1 gene of Neurospora crassa

Elie Kato, Doreen Morris, Faye Nagano, Cindy Rehkemper, Cal Long, Dennis Gross, Robert Phillips and W. Dorsey Stuart, University of Hawaii, Office of Technology Transfer and Economic Development/Neugenesis Corp. joint project.

Expression vectors using the highly secreted glucoamylase-1 (gla-1) gene of Neurospora crassa were created for the targeted secretion of a mammalian thrombolytic protein (mTh) in N. crassa. The gla-1 ORF was fused in- frame to the mTh cDNA at various distances from the gla-1 translation initiation site. The expression vectors were characterized by the location of the fusion site in the gla-1 ORF. Construct 1) The fusion was created 37 amino acids into the gla-1 ORF to include the propeptide of the glucoamylase gene. Construct 2) Contained 100 amino acids of the gla-1 ORF. This construct included the gla-1 propeptide and the first/only intron of the glucoamylase gene.

Construct 3) The mTh cDNA was fused at the final codon of the gla-1 gene, 625 amino acids from the translation initiation site. A kex-2 endopeptidase recognition site was inserted between the gla-1 ORF and the mammalian Thrombolytic cDNA to cleave the fusion protein prior to secretion. Here we compare the secreted product of the three different expression vectors.

3. Analysis of a polyketide gene cluster in Aspergillus nidulans

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Sterigmatocystin, a carcinogenic polyketide, is the product of a lengthy biochemical pathway found in the Aspergillus spp., A. nidulans, A. flavus and A. parasiticus. Whereas the latter two species convert sterigmatocystin to aflatoxin in a two step process, A. nidulans makes sterigmatocystin as an end metabolite. We have identified a ~60 kb cluster of genes in A. nidulans whose products are involved in sterigmatocystin biosynthesis. This cluster contains at least 20 genes proposed to encode both enzymatic activities and a regulatory protein, AflR. At least 15 structural genes including a polyketide synthase, a and b subunits of a fatty acid synthase, several monooxygenases, dehyrogenases and reductases have been identified through sequence homologies and gene disruption studies. Transcripts of these genes are co-regulated with verA, a previously described ketoreductase necessary for sterigmatocystin biosynthesis. The regulatory gene, aflR, is located in the middle of the cluster and functionally conserved between A. nidulans, A. flavus and A. parasiticus.

4. Aflatoxin biosynthesis in Aspergillus parasiticus and A. sojae is regulated at transcription

Maren Klich, Beverly Montalbano and Kenneth Ehrlich, USDA Agricultural Research Service, New Orleans

Aflatoxin, the most potent naturally formed carcinogen, is a secondary metabolite of Aspergillus parasiticus and A. flavus. Two very closely related species, A. sojae and A. oryzae, are used in food fermentations and never produce aflatoxins. We previously established that many A. oryzae isolates lack at least one of the genes necessary for aflatoxin biosynthesis but that all A. sojae, A. parasiticus and A. flavus isolates tested had homology to these genes in Southern blot analysis. To determine if aflatoxin biosynthesis genes were being transcribed, we screened mRNAs from six A. sojae and A. parasiticus isolates by northern blot analysis using one regulatory gene (aflR) and six structural genes (pks, fas, nor, aad1, ver1 and omt1) involved in aflatoxin biosynthesis.

The A. parasiticus isolates that produced aflatoxin or were known to be blocked at the end of the pathway had homology to all of the genes tested. One A. parasiticus isolate that had lost its ability to produce aflatoxin in culture and one A. sojae isolate did not have homology with any of the genes tested. Two of the A. sojae isolates had regions of homology with the regulatory gene, and fas but did not have homology with any of the other structural genes. These results indicate that aflatoxin production is transcriptionally regulated in these two species.

5. Population genetic structure in Rhizobium leguminosarum biovar trifolii: a hierarchical analysis

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To determine the relative importance of gene flow, founder effects, selection, and mode of reproduction in Rhizobia populations, fifteen allozyme loci were used to estimate diversity and analyze the population structure in a collection of 912 Rhizobium leguminosarum biovar trifolii isolates. Data sets, comparing different sampling levels (total sample, individual population samples, and plants within a population), geographic separation (northern populations vs. southern populations), and temporal separation (southern populations collected in 1992 vs. those collected in 1993) were analyzed. Total genetic diversity among all isolates is H=0.426, with 57.6% of the variation found within individual plants. Gene flow among plants within a population is low relative to gene flow among populations within a geographic or temporal grouping. Significant differences were observed in (i) allele frequencies among populations and among plants within populations, and (ii) the frequency distribution of the most widespread and the most abundant strains. Multilocus linkage disequilibrium was calculated and significant levels were observed in the total sample and in three of the eight populations.

6. Cloning and characterization of a gene encoding a putative RNA binding protein from Neurospora crassa

Gretchen A. Kuldau and N. Louise Glass, University of British Columbia.
A series of cosmids located around pyr-4 on LGIIL were isolated in a chromosome walk from pyr-4. Northern analysis of a 19.0 kb region of one of the centromere distal cosmids identified a 3.0 kb mRNA which is relatively abundant in vegetatively grown mycelia. Four overlapping cDNAs corresponding to this message were isolated; the largest of these incomplete cDNAs was 1.8 kb. A database search using BLAST revealed significant homology between the cDNA and a broad class of RNA binding proteins. RNA binding proteins are a diverse set of important proteins including poly(A) binding protein and regulators of developmentally specific splicing. Results of one conventional RIP (repeat induced point mutation) mutagenesis experiment suggest this gene may be essential. Sequencing of a genomic clone is in progress.

7. Molecular analysis of the negative regulatory gene scon-2, a beta-transducin homolog in Neurospora crassa

Anuj Kumar and John V. Paietta, Wright State University.
Sulfur uptake and assimilation in Neurospora crassa is accomplished via a complex regulatory circuit encompassing a set of sulfur-related structural genes and a set of trans-acting regulatory genes. These sulfur regulatory genes include cys-3+, which encodes a bZIP (basic region-leucine zipper) transcriptional activator, and the negative regulatory sulfur controller gene scon-2+. The scon-2+ gene encodes a polypeptide of 650 amino acids (molecular weight 72.2 kD) belonging to the expanding b-transducin family of eukaryotic regulatory proteins. Specifically, SCON2 contains six repeated Gb-homologous domains spanning the C-terminal half of the protein. Additionally, SCON2 exhibits an amino-terminal domain that potentially defines a new subfamily of b-transducin homologs. Expression of the scon-2+ gene has been examined using Northern hybridization and gel mobility shift analysis. Northern blots of scon-2+ mRNA indicated reduced expression of scon-2+ in a cys-3 deletion strain. Gel mobility shift assays

uncovered four CYS3 binding sites within the scon-2 promoter. Taken collectively, this data suggests the presence of a control loop within the N. crassa sulfur regulatory circuit involving CYS3 activation of scon-2+ expression.

8. Quantitation of Phanerochaete chrysosporium mRNAs in soil by reverse transcription polymerase chain reaction

Rich Lamar, Barry Schoenike, Amber Vanden Wymelenberg, Philip Stewart, Diane Dietrich and Dan Cullen. USDA Forest Service, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53705.

Analysis of fungi in complex substrates has been hampered by inadequate experimental tools for assessing physiological activity and estimating biomass. We report a method for quantitative assessment of specific fungal mRNAs in soil. The method was applied to complex gene families of Phanerochaete chrysosporium, a white-rot fungus widely used in studies of organopollutant degradation. Among the genes implicated in pollutant degradation, two closely-related lignin peroxidase (LiP) transcripts were detected in soil. The pattern of LiP gene expression was unexpected; certain transcripts, abundant in defined cultures, were absent in soil cultures. Relative transcript levels of several closely related cellobiohydrolases genes and the b-tubulin gene were also measured. The approach is applicable other systems including the laccase transcripts of Trametes hirsutas. The method will aid in defining the role of specific genes in complex biological processes such as organopollutant degradation, in developing strategies for strain improvement, and in identifying specific fungi in environmental samples.

9. Comparative phylogenetic and functional analysis of frequency (frq) homologs: support for a transcriptional regulatory role

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The putative amino acid sequence of the Neurospora crassa frq protein contains sequences suggesting it is a nuclear transcription factor. Using PCR, we have cloned frq homologs from other filamentous fungi including Chromocrea spinulosa, Leptosphaeria australiensis, Podospora anserina and four additional Neurospora species. Alignment of the Chromocrea and Leptosphaeria sequences with those previously published for Neurospora and Sordaria shows that the former are about 50% identical to the latter and to each other. There are both regions of near total divergence and highly conserved regions including a predicted helix-turn-helix structure that may act as a DNA-binding domain. Amino acids at positions altered in the frq mutants are conserved among all species. Sequences consistent with frq being a transcription factor are generally conserved; most predicted post-translational modification sites are not. Transformation of the Neurospora frq9 mutant with the Chromocrea homolog rescued the pigmentation defect of the mutant but not the circadian defect; transformation with the Leptosphaeria homolog failed to rescue either phenotype.

10. The ylo-1 gene of Neurospora crassa encodes a putative polypeptide with strong similarity to mammalian class 3 aldehyde dehydrogenases

Changgong Li, Hongjian Liu, and Thomas J. Schmidhauser, Medical Biochemistry, Southern Illinois University, Carbondale.

We have completed DNA sequence analysis of a Neurospora 2.3 kilobase (kb) genomic

fragment containing ylo- 1+ as well as of a 1.7 kb ylo-1 cDNA. Northen analysis indicates a single ylo-1 gene specific transcript of approximately 2.0 kb. ylo-1 transcript accumulation is not photoregulated in mycelia or during conidiation; transcript levels remain constant during macroconidiation suggesting constitutive expression. Comparison of the genomic and cDNA sequences identified a 1602 base pair open reading frame. The putative ylo-1 open reading frame encodes a putative polypeptide of 60 kilodaltons. A database search revealed similarities between Ylo-1 and members of the aldehyde dehydrogenase family. The ylo-1 gene product is thought to catalyze the conversion of torulene or g- carotene to neurosporaxanthin a C35 apo-carotenoid that is unique to the fungi. The highest degree of similarity was observed between Ylo-1 and class 3 mammalian aldehyde dehydrogenases. Two amino acid residues placed in the active site of class 1 aldehyde dehydrogenase from human liver, Cys-302 and Glu-268 are highly conserved in the ALDH family of enzymes. This Cys residue is the only Cys residue conserved in all known ALDH structures. Both residues are conserved in Ylo-1.

11. Regulation of expression of the albino-1 (al-1) and al-2 genes of Neurospora crassa during macroconidiation

Changgong Li and Thomas J. Schmidhauser, Medical Biochemistry, Southern Illinois University, Carbondale.

The albino genes of Neurospora encode enzymes essential for carotenoid biosynthesis. The al-1 gene encodes a phytoene desaturase while the al-2 gene encodes a phytoene synthase. The levels of al-1 and al-2 transcripts change dramatically in response to light and development during the formation of the major Neurospora crassa asexual spore (macroconidia). al-1 and al-2 mRNAs accumulate in response to a developmental cue, a specific stage of conidiation, irrespective of lighting conditions. During conidiation light induces accumulation of al-1 and al-2 gene specific transcripts at each stage tested; the photoinduced increase in albino gene transcript levels was not observed in two Neurospora mutants, wc-1 and wc-2, that are defective in all physiological photoresponses. Mutations in the genes fluffyoid, fld, and fluffy, fl, block conidiation at distinct early stages, both mutations reduce photoindependent albino gene transcript accumulation under conditions that promote conidiation.

12. Development of species-specific probes complementary to the 18S rRNA sequence of the yeast-like fungus Aureobasidium pullulans

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Aureobasidium pullulans, a cosmopolitan yeast-like fungus, colonizes leaf surface and has potential as a biocontrol agent of pathogens. In an effort to develop specific probes for A. pullulans, we isolated genomic DNA from 12 strains of A. pullulans and from 16 different fungi. A 578-bp region within the 18S rRNA gene was PCR- amplified and sequenced. We also PCR-amplified the full length 18S rRNA gene from all the fungi tested. Southern blot hybridizations were performed to test the specificity of different probes. Multiple alignments of the 578-bp region of the 18S rRNA gene among all the A. pullulans strains showed few nucleotide differences. Rodotorula rubra, a phylloplane yeast, had the most closely related sequence to A. pullulans. On the basis of a single nucleotide mismatch, a 17-mer was identified which differentiated the 12 A. pullulans

strains from R. rubra. Another 17-mer probe hybridized to A. pullulans strains but not to the 16 other fungi tested. We also identified a 17-mer highly specific for Cladosporium herbarum. An additional 100 phylloplane fungal isolates are being tested by Southern blot hybridization. These probes have potential in monitoring and quantification of fungi in leaf surface and other microbial communities.

13. Cloning of genes palB and palF of Aspergillus nidulans

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Mutations in genes palB and palF increase acid phosphatase staining and decrease alkaline staining when mutant strains are grown at pH6.5. On this basis, it is possible that these genes may be involved in external pH- regulated gene expression, thus becoming an important target to be characterized at the molecular level. Alleles palB7 and palF15 are recessive and strains palB7 and palF15 do not grow at alkaline pH nor in medium with b-glycerolphosphate as the sole source of phosphate. Therefore, we have cloned the palB and palF genes by complementation using a chromosome-specific cosmid library of Aspergillus nidulans. The palB gene was subcloned as a 3.5 kb fragment with full complementing activity and which recognized a 3.0 kb mRNA. Furthermore, this fragment complemented the palE11 mutation, supporting the genetic analysis which suggested that palB and palE are the same gene. The palF gene was subcloned as a 5.0 kb fragment that fully complemented the palF15 mutation. An ~4.0 kb mRNA was identified using a internal fragment from this subclone. Two step gene replacement experiments ruled out the possibility that we had cloned supressors for the mutations palB7 and palF15. Financial support: CNPq, FAPESP, CAPES, FINEP and BID-USP

14. Glucose repression of xylanase formation by Trichoderma reesei regulates xyn1 but not xyn2 gene expression and is mediated by the Cre1 gene product

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The filamentous fungus Trichoderma reesei secretes two xylanases (XYNI and XYNII) into the medium; when grown on xylan as carbon source. No xylanase activity occurs in the supernatants of glucose cultures, whereas lactose cultures exhibit xylanase activity (lactose promotes carbon catabolite derepression). Highest levels are obtained on xylan and xylose. Northern blot analysis of xyn1-and xyn2-mRNA formation as well as the use of the E. coli hygromycin B phosphotransferase-encoding (hph) gene as a reporter system for the xyn1 and xyn2 promoters document that transcription of one of the two xylanase-encoding genes (xyn1) is regulated by carbon catabolite repression. The low constitutive transcription of xyn2 on glucose contrasts with the absence of XYNII in the culture filtrates, and suggests a further regulation of XYNII formation following transcription. The 5' upstream sequence of both genes show several nt-motifs homologous to the postulated 5'-SYGGRG-3' consensus for binding of the carbon catabolite repressor Cre1 (CreA). Using a protein fragment containing the zinc-finger domain of T. reesei Cre1, prepared by expression as a GST fusion protein in E. coli (Strauss et. al., manuscript in

preparation), these sequences were demonstrated as being functional in vitro in gel retardation assay and methylation protection foot printing techniques. Cre1 was shown to bind to several motifs present in xyn1 and xyn2. However only the xyn1 promoter contains a consensus form of an inverted repeat. In vivo deletion of 4 central nt of this motif resulted in the expression of the xyn1-hph fusion on glucose at a level comparable to that observed under carbon catabolite derepressing conditions. The functionality of this fragment was strengthened further by inserting it into the xyn2 promoter which lacks this motif. This resulted in a strong repression of the constitutive expression of the xyn2-hph fusion. Neither the loss of carbon catabolite repression of xyn1 nor the gain of carbon catabolite repression of xyn2 affected the induction of transcription of both genes by xylose and xylan. Based on these results we postulate that the transcription of xyn1 is repressed by glucose via Cre1 but that induction by xylose bypasses this repression. We also postulate that in T. reesei the single consensus motif for Cre1 binding is not sufficient to mediate carbon catabolite repression in vivo.

15. Cloning and transformant analysis of the period-2 (prd-2) clock gene of Neurospora crassa

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prd-2 is a recessive clock mutation that lengthens the period of the circadian conidiation rhythm from the wildtype value of 21.5 hours to about 25.5 hours at 25 C. Genetic mapping of prd-2 localized the gene to the right arm of linkage group V between lys-2 and am. A chromosome walk in the Volmer-Yanofsky cosmid library in the genetically determined region of prd-2 yielded a set of cosmids spanning about 150kb. These cosmids were tested in a transformation assay for the ability to complement prd-2 and restore wildtype rhythmicity. Some of the cosmids showed partial rescue of the mutant phenotype shortening the period by up to 2 hours in approximately 20% of the primary transformants. Analysis of homokaryotic microconidial isolates showed that the suppressed phenotype is stable and not an effect of heterokaryosis. Analysis of transformants from overlapping cosmids localized the suppressing DNA to a 4kb region.

16. Karyotype mapping of Aspergillus ficuum SRRC 265 acid phosphatase genes

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Four Aspergillus ficuum (niger) SRRC 265 phosphate-repressible phosphatase genes have been cloned (aphA, phoA, phyA and phyB). Several of these enzymes are economically important because they can degrade phytic acid. All four enzymes are secreted at high levels when A. ficuum SRRC 265 is grown under conditions of limited phosphate. This suggests their genes may share a common regulatory system. The chromosomal location is unknown for any of these extracellular acid phosphatase genes. To determine if any of these acid phosphatase genes are clustered we have started to map their chromosomal location by CHEF gel electrophoresis and Southern analysis. Taxonomically A. ficuum and A. niger are very similar and A. ficuum is widely considered a synonym of A. niger. We have found that the electrophoretic karyotype of A. ficuum has the same eight linkage groups as A. niger. A series of A. niger tester strains with introduced chromosomal size variations was obtained to verify chromosome

assignment of the acid phosphatase genes.

17. Manganese superoxide dismutase activity in Neurospora crassa

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As is observed in almost all aerobic eucaryotes, the filamentous fungus Neurospora crassa possesses two different forms of superoxide dismutase, one that is mitochondrial and has manganese as a co-factor (MnSOD), and another that is cytosolic and has copper and zinc as co-factors (CuZnSOD). Superoxide dismutase scavenges the superoxide anion, O2-, which is both toxic and ubiquitously generated during aerobic metabolism. Our laboratory previously isolated and sequenced sod-1, the gene that encodes CuZnSOD, and constructed a strain bearing a null allele of sod-1. We recently isolated a gene that appears to encode mitochondrial MnSOD and designated this gene sod-2. Sequence analysis of sod-2 suggests the possibility that an alternate pathway of expression of this gene may result in a cytosolic MnSOD activity, in addition to mitochondrial activity. We are examining sod-2 transcript levels and MnSOD activity in sod-1 mutant strains, and in copper-starved wildtype strains, in an attempt to establish if MnSOD activity levels or subcellular locations are altered in response to the reduction or elimination of CuZnSOD activity. We are also attempting to construct a mutant strain of N. crassa that lacks MnSOD activity. This strain will be useful in evaluating the contribution of MnSOD activity to the ability of an obligate aerobe to tolerate oxidative stress, and in confirming the mitochondrial location of the gene product.

18. Phenotypic analysis of the Neurospora crassa mei-3 mutant

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The N. crassa mei-3 gene appears to encode a homolog of the E. coli RecA protein, one function of which is recombinational DNA repair. The mei-3 mutant is sensitive to ultraviolet irradiation, other mutagens and histidine at 39oC. There is evidence that the mei-3 gene product is induced under conditions of superoxide-mediated stress. A portion of the present study derives from our efforts to construct N. crassa strains carrying mutant alleles for both mei-3 and sod-1. The sod-1 gene encodes CuZn superoxide dismutase, a scavenger of toxic superoxide radicals. The sod-1 mutant exhibits a high spontaneous mutation rate, presumably as a result of increased superoxide-mediated stress. The mei-3 sod-1 double mutant was constructed to determine whether mei-3 has a role in either the repair of oxidation damaged DNA or the exacerbation of superoxide-mediated mutagenesis. During the construction of the mei-3 sod-1 strain, which requires a crossover on linkage group IL, we isolated progeny that exhibited extreme temperature sensitivity, even in the absence of mutagens. We are currently investigating the molecular basis of some of the known mei-3 mutations and the nature of temperature sensitivity. Analysis of crosses between mei-3 mutants and strains containing conventional markers on linkage group I will determine if the temperature sensitive phenotype is tightly linked to mei-3 or other linkage-group I genes. Transformation experiments are being conducted to ascertain if the wild type mei-3 gene can rescue the temperature sensitive phenotype. We also intend to sequence several mutant mei-3 alleles to elucidate the molecular basis of the mei-3 phenotype. Finally, we are attempting to disrupt the mei-3 gene using the RIP (repeat-induced point mutation) process in order to determine if (a) the known mei-3 phenotype can be reproduced and (b) if mei-3 null mutants are viable.

19. Altering fatty acid composition in Neurospora crassa

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The fungal and plant kingdoms synthesize a large diversity of fatty acids. The fatty acid composition of membrane and storage lipids largely determines their properties. Neurospora crassa has many advantages for studying the biosynthesis of fatty acids, including the availability both of an extensive collection of mutants in fatty acid and membrane lipid synthesis, and of vectors for the expression of Neurospora and foreign genes. We are utilizing Neurospora for studying effects of mutations (ufa, pfa) in the fatty acid desaturation pathway, effects of transformation with fatty acid desaturases, and the synthesis of lipids with unusual fatty acids, such as ricinoleate.

20. Cloning and characterization of the carboxypeptidase Y homologue from Aspergillus niger

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Aspergillus niger is a filamentous fungus used industrially for the production of secreted proteins, and has the capacity to secrete proteins in the gram per liter range. As part of our efforts to begin understanding the high secretory capacity of A. niger, we have decided to clone genes coding for proteins that are known to be localized to specific organelles in the secretory pathway. To obtain a marker for the vacuole (lysosome), we have cloned the gene coding for carboxypeptidase Y (CPY). In Saccharomyces cerevisiae, it has been demonstrated that CPY is localized to the vacuole where it is processed to the mature form. Based on regions of homology found between the S. cerevisiae CPY and other carboxypeptidases, degenerate oligonucleotides were used to PCR amplify a genomic fragment from A. niger. The nucleotide sequence of a 600 bp amplification product was determined and shown to contain an open reading frame of 200 amino acids having ~69% identity to the S. cerevisiae CPY protein. The PCR product was used as probe to screen both genomic and cDNA libraries of A. niger. Both genomic and full length cDNA clones of the A. niger CPY homologue have been isolated and characterized at the molecular level.

21. Development of Fusarium graminearum as a novel host for heterologous protein expression

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Fusarium graminearum has been utilized as a commercial human food source in the UK for nearly ten years (A.P.J.Trinci. Mycol. Res. 96:1-13). The apparent absense of both plant pathogenicity and toxin production, and the well studied fermentation characteristics of this strain made it a good candidate as a host for heterologous protein secretion. In preliminary studies, we observed that F. graminearum secretes a low background of extracellular proteins. This feature is desirable in an expression system since it facilitates product recovery. As a first step, a transformation system was developed using the amdS gene of A. nidulans. We next examined the capacity of the fungus to express a trypsin-like protease gene from a closely related Fusarium species.

This enzyme is transcribed as a pre-pro enzyme which requires processing by a maturing enzyme for protease activity. Production of this protease in Aspergillus has been problematic due to inefficient processing and proteolytic degradation. A vector containing both the amdS gene and the protease gene was constructed and transformed into the fungus. The enzyme was expressed and processed correctly. The regulation of expression of the protease gene was discussed.

22. Characterization of albino mutants of Neurospora crassa

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The albino genes of Neurospora encode enzymes essential for carotenoid biosynthesis. The al-1 gene encodes a phytoene desaturase while the al-2 gene encodes a phytoene synthase. The al-1 and al-2 genes are closely linked on the right arm of linkage group I of Neurospora. Hunts for mutants defective in carotenogenesis have identified over twenty albino mutants that map to the right arm of linkage group I. However, these mutants have not been identified as alleles of al-1 or al-2. We have used DNA mediated transformation complementation analysis to assign these alleles to locus. Phytoene desaturases (pds) are common to all carotenogenic organisms including all photosynthetic organisms. Primary sequence analysis of carotenoid desaturases has established at least two classes of enzymes. Bacterial/fungal pds catalyze the conversion of phytoene to neurosproene, or more commonly, lycopene. Plant pds desaturates phytoene through a single intermediate, phytofluene, to z-caroteneprotein. A second plant carotenoid desaturase, z-carotene desaturase (zds) acts on z-carotene yielding first neurosporene then lycopene. Protein homology plots suggest that the bacterial/fungal pds are more closely related to plant zds then the plant pds. We present sequence analysis of alleles of Al-1 the Neurospora pds.

23. Characterization of genes affecting dimorphism in Candida albicans

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Candida albicans is an opportunistic human pathogen responsible for deep seated and systemic mycoses in susceptible individuals. Fungal infections have been on the rise in recent years and antifungal research has taken on renewed interest. Pathogenesis by C. albicans has been linked to the ability to switch between a budding and filamentous growth form. We are using several molecular techniques including differential display to identify genes involved in regulation of dimorphism in C. albicans. Differential display detects a significant number of mRNAs uniquely expressed in cultures within the first few hours of germ tube formation when compared to budding cells. These transcripts are being further characterized by sequence analysis and northern blots. Analysis of the genes for these mRNAs may reveal promotor motifs that co-regulate hyphae specific genes.

24. In vivo analysis of transcriptional regulation of the Aspergillus nidulans creA gene mediating glucose repression

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The creA gene of A. nidulans encodes a wide domain regulatory protein mediating glucose repression of a multitude of enzymes responsible for utilisation of less favoured carbon sources. CreA is a DNA binding protein of the C2H2 Zn finger class which binds specifically to consensus target sites composed of 5'-SYGGRG 3'. Cis acting mutations in the consensus binding sites of target genes (e.g. prnd 22) or mutations in the CreA binding domain (e.g. creAd1) lead to glucose derepression. Complete deletion of creA, however, results in lethal phenotype, which suggests an additional function of the protein beyond carbon catabolite control. Little is known about the signals mediating carbon catabolite repression by CreA. To this end we have started an investigation on the regulation of creA gene transcription. Northern blot analysis of creA mRNA revealed a complex expression profile: addition of glucose to a carbon derepressed mycelium of A. nidulans results in a transient stimulation of creA transcript formation ("early stimulation"), followed by partial repression. This effect is not observed in a creAd1 mutant strain. This data are consistent with the presence of a perfect tandem repeat of the SYGGRG consensus sequence, which has recently been shown to be functional in vivo in the genes of the proline cluster and the ethanol regulon of A. nidulans. In vicinity of this CreA target, also a putative binding site for the positive acting wide domain regulatory protein AreA, a member of the "GATA factor" proteins, which mediates nitrogen metabolite repression, is found. That this motif might be functional in vivo is supported by the finding that "early" stimulation of glucose mediated transcription is prevented in the presence of ammonia. Additional evidence is given by the observation that the steady state level of creA transcript on proline is constant at all sample times (corresponding to the derepressed level on low fructose) in mycelia pregrown on nitrate but needs an adaption time for derepression of around 10 minutes when pregrown on ammonia. We have moreover identified a putative "cAMP Responsive Element" (CRE) within the 5' regulatory region of creA. The in vivo function of those motifs are currently under investigation. To prove the involvement of the proposed target sites for activator/repressor binding in vivo, we have applied the in vivo genomic footprinting method using ligation mediated PCR. Data from these experiments will be presented and discussed with respect to the interaction of signals regulating creA expression.

25. Cloning and characterization of the signal recognition particle 54 kDa protein homologue from Aspergillus

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Aspergillus oryzae and Aspergillus niger secrete large amounts of protein in submerged cultures. Because of this characteristic, they have been used industrially to produce heterologous proteins of commercial interest. In order to begin understanding the high secretory capacity of these organisms, we are cloning homologues of known components in the yeast and eukaryotic secretory machinery. The gene coding for the signal recognition particle (SRP) 54 kDa subunit has been cloned from several organisms including the yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe. Alignment of the cloned SRP54 homologues identifies areas of strong homology between the proteins. These areas were used to design oligonucleotide primer sets for use in PCR experiments in attempts to amplify the SRP54 homologues from A. oryzae and A. niger.

The resulting amplification product from A. oryzae contained an open reading frame of 498 bp. The predicted amino acid sequence of the PCR product was found to be 71% homologous to the SRP54p from S. cerevisiae. This PCR product was subsequently used to retrieve the full length genomic and cDNA clones from A. niger libraries.

26. Regulation of the extracellular acid protease(s) of Aspergillus nidulans

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The proteases of Aspergillus species have become an area of great interest, mainly due to the economic importance of the hydrolytic enzymes produced by filamentous fungi. Although early studies of A. nidulans confirmed the existence of enzymes from the neutral and alkaline classes of endo-proteases (1), the methods used were unsuitable for the detection of acid proteases, therefore it was unknown if proteases of this class were produced by A. nidulans. Using a heterologous probe generated from the A. niger pepA gene encoding an acid protease (2), we have isolated a structural acid protease gene (prtB) from a lambda library of genomic A. nidulans DNA. Preliminary sequence data shows that prtB is also similar to an A. oryzae acid protease of the same class (3). Enzyme assays, polyacrylamide gel electrophoresis, and RNA blots have been employed to study the regulation of the structural protease genes of A. nidulans, which are expressed in response to carbon, nitrogen, or sulphur nutrient- limiting conditions. Although the alkaline protease gene (prtA) has been shown to be expressed under acid pH conditions (pH 3.5), preliminary studies suggest that the acid protease(s) may not be expressed under neutral and alkaline conditions. (1) Cohen BL (1973) J. Gen. Microbiol. 77:521-528. (2) Berka RM et al (1990) Gene 86:153-162. (3) Gomi et al (1993) Biosci. Biotech. Biochem. 57(7):1095-1100.

27. Electroporation-based transformation of freshly harvested conidia of Neurospora crassa

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This transformation method was optimized for yield and flexibility rather than efficiency. Conidia were harvested from 7- to 28-day old cultures. All cell manipulations were done in 1M sorbitol solution. After three washes by centrifugation, a suspension of 2.5X10(9) cells/ml was prepared. One to 5 ug of linearized plasmid DNA was added to 100 ul of conidial suspension. A 40 æl aliquot of the cell suspension was placed in the bottom of a 0.2 cm gap electroporation cuvette. An InvitroGen Electroporator II with a voltage gradient of 7.25 kV/cm and settings of 71 uF and 200 ohms gave good results. Transformation to histidine prototrophy (his-3) or hygromycin resistance was demonstrated in a variety of strains of N. crassa. Prototrophic transformants were selected by direct plating on minimal plates containing 1M sorbitol. A pour plate method was used to select for hygromycin resistance in which the bottom agar contained hygromycin and no sorbitol and the top agar contained no hygromycin and 1M sorbitol. An input of 10(8) cells routinely yielded 200 to 1000 transformed cells. Co-transformation with DNA constructed for expression of heterologous proteins occurred in up to 30% of selected transformants. Transformants have been found to be stable and capable of production of significant amounts of heterologous proteins in N. crassa.

28. New tools for gene cloning and chromosome assignment studies of cloned genes in Aspergillus niger

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The development of an improved gene cloning strategy by complementation of mutant alleles in A. niger is described. The strategy is based on the use of a fungal autonomously replicating pyrG vector, pAB4-ARp1. With this vector, a 10-100 fold increase in transformation frequency was obtained compared to integrative vectors. With pAB4-arp1, also the transformation frequency of a cotransformed plasmid is increased. A. niger transformants containing pAB4-ARp1 are mitotically unstable, but cotransformed plasmids strictly cosegregated with the autonomously replicating vector, as a result of recombination between both vectors. The use of pAB4-ARp1 in gene cloning was demonstrated by the cloning of two linkage group (LG) VII specific A. niger genes (nicB, lysF) with an A. niger gene library and pAB4-arp1. Furthermore, a method is described for assignment of cloned genes to Aspergillus niger chromosomes/linkage groups. With the cloning of a LG VII specific A. niger gene, all of the 8 LGs could be assigned to a chromosomal band in the electrophoretic karyotype of A. niger. The electrophoretic karyotype reveals 5 distinct bands, of which 3 consist of equally sized chromosomes. Using a set of A. niger strains with introduced chromosomal size variation, unambiguous assignment of cloned genes using CHEF-Southern analysis was demonstrated. The availability of these tester strains obviates the need of isolating or constructing mutant strains for the purpose of chromosome assignment of cloned genes.

29. Carbon catabolite-mediated regulation of genes encoding NAD-specific glutamate dehydrogenase and Hsp80 of Neurospora

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The genes encoding the NAD-specific glutamate dehydrogenase (gdh-1) and heat shock protein 80 (hspe-1) are controlled through a common carbon catabolite repression mechanism. In addition, they are individually subject to regulation by specific stimuli, i.e. heat shock (hspe-1) and the substrate inducer, glutamate (gdh-1). Expression of these two genes was monitored during growth of cells under (a) carbon source starvation, (b) optimal conditions for gdh-1 induction and (c) heat shock. Northern blot analysis using DNA fragments of cloned genes, and protein blot (Western) analysis with HSP80- and GDH-specific polyclonal IgG and GDH enzymatic activity measurements were conducted to assess gene expression. While hsp80 gene is expressed at high levels during growth under starvation conditions, it appeared to be down-regulated by the presence of glutamate. GDH is also expressed at a high level upon carbon source depletion but its expression is enhanced further by the presence of glutamate. Exposure to heat shock, subsequent to the derepression treatments, resulted in the cessation of gdh mRNA synthesis as well as degradation of pre-existing messages. The elevated expression of hsp80 may be attributable to an increased demand for molecular chaperoning activity under starvation conditions.

30. Evidence for trans-inactivation of aflatoxin biosynthesis gene expression in diploids of Aspergillus flavus

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Aspergillus flavus produces the decaketide aflatoxin that is a potent carcinogen. Strain 649 does not produce aflatoxin, and diploids formed by parasexual crosses between strain 649 and aflatoxigenic strains do not produce aflatoxin, indicating the dominate nature of the mutated afl-1 locus in strain 649. In metabolite feeding experiments, the diploids did not convert three intermediates of the aflatoxin pathway. Northern analysis indicated the aflatoxin biosynthesis genes nor, ver1, and omt1 were not expressed in diploids grown in medium conducive for aflatoxin production; however, there was low level expression of the regulatory gene aflR. A large (>150 kb) region that includes a cluster of genes involved in aflatoxin biosynthesis was found deleted from the genome of strain 649. Pulsed-field gel electrophoresis of chromosomes from strain 649 and the aflatoxigenic strain 86 indicated a larger (6 Mb) chromosome in strain 649 than the apparent homologous (4.9 Mb) chromosome in strain 86. The larger sized chromosome in strain 649 suggests that a rearrangement may have occurred in addition to the deletion These data suggest that a trans-sensing mechanism in diploids is responsible for the dominant phenotype associated with the afl- 1 locus in strain 649.

31. The expression of porcine relaxin in Neurospora crassa

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This study was to determine if cDNA encoding porcine relaxin inserted into the genome of Neurospora crassa will be transcribed, the mRNA translated, and the protein post-transcriptionally modified by host cells. Relaxin, a member of the insulin family, is involved in parturition, uterine accommodation and sperm motility. Constructs for expression of relaxin were made using combinations of promoter sequences (tubulin and mtr), signal sequences (relaxin and chymosin), and the authentic porcine relaxin sequence. Neurospora host cells were transformed with these constructs. Media from transformed cell lines was screened for the production of relaxin by immunoassays. Transformants producing anti-relaxin antibody positive material were tested by Southern analysis for multiple integration sites. The relaxin protein was verified by reverse-phase HPLC followed by immunoassays on pooled HPLC fractions. This protein appears to be produced in a mature form and secreted into the media at levels of up to 45 ug/liter.

32. The family of AMA elements in Aspergillus nidulans

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The AMA1 element was first characterized as a genomic sequence from A. nidulans which promotes extrachromosomal maintenance of plasmids in this fungus. The sequence is a perfect quasipalindrome (foldback element) of at least 7 kb, and it is represented by a single copy per genome, as demonstrated by hybridization with the unique central spacer. This complete copy is located on chromosome IV but several genomic copies of single arms are located on different chromosomes, the pattern of chromosomal location being highly variable between wild isolates. Several clones bearing sequences homologous to AMA1 (AMA-elements) were isolated from a genomic library. They share up to 95% sequence homology and differ mostly by AT-GC transitions, which suggests that they

may have evolved from AMA1 by methlyation-induced mutations. None of the clones so far characterized other than AMA1 is arranged as a foldback element. All members of the family enhance transformation efficiency and produce phenotypically unstable transformants. However, in all the transformants (except those generated by the complete AMA1 element) transforming DNAs are probably rearranged and multimerized. We discuss possible models for extrachomosomal maintenance of AMA-bearing vectors.

33. MILD R.I.P.: an ideal tool to obtain leaky mutants from essential genes in N. crassa

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The al-3 gene codes for Geranylgeranyl Pyro phosphate Synthase (GGPPS), an enzyme involved in the biosynthesis of carotenoids and of diverse prenylated compounds. The only two al-3 mutants isolated so far have a leaky albino phenotype, i.e. their carotenoid content is reduced. We used the R.I.P. (repeat-induced point mutation) mechanism to obtain new mutants of the al-3 gene upon introduction of a 1765bp fragment of the gene. Using phenotypic selection based on the colour of the mycelium to screen R.I.P. products, we obtained a much lower frequency of al-3 mutants than expected (only 0.3%), all with a leaky phenotype ranging from light orange to light yellow. We analysed the nucleotide sequence of the endogenous a1-3 gene from five mutants obtained by R.I.P. following cloning of a PCR-amplified product. In all the mutants, R.I.P. had introduced very few mutations (from two to four). Studies carried out suggest that al-3 is an essential gene and therefore heavily mutagenized ascospores do not survive. The data presented here show that varying degrees of R.I.P. exist and therefore the R.I.P. mechanism may have an intrinsic ability to introduce a highly variable number of mutations. We suggest that the mild mutagenetic effect of R.I.P. on duplicated sequences may represent a useful in vivo method of mutagenesis, to obtain functional mutant alleles of essential genes in Neurospora crassa.

34. The impact of an antisense transcript (aflRas) on the expression of aflatoxin biosynthesis pathway genes and the pathway-specific regulatory gene aflR

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Aflatoxins are toxic and carcinogenic polyketides produced by Aspergllus flavus and A. parasiticus, which contaminate many important food crops. Aflatoxin biosynthesis is regulated by the gene aflR, however, the mechanisms by which this gene regulates pathway genes and is itself regulated are currently unknown. During the cloning of aflR, cDNA clones were isolated which represented the complementary strand of the regulatory gene. To determine if an antisense transcript is produced in vivo and the impact of such transcripts on aflatoxin regulation, we will determine the temporal expression of these two genes. Total extractable RNA will be isolated from a time-course study of A. flavus cultures grown on aflatoxin inducing and non-inducing media. Transcripts representing aflR and (aflRas) will be identified by Northern analysis and reverse transcription-polymerase chain reactions. RT- PCR is a highly sensitive, strand-specific RNA detection assay. We will also use RT PCR to determine the expression of pathway genes. If aflR is regulated in part by aflRas, we would expect to see differential expression of these two genes and an impact on the expression of the aflatoxin

biosynthesis pathway genes.

35. Cloning and characterization of TB3, a kinase-encoding gene from Colletotrichum trifolii expressed during hyphal elongation

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Colletotrichum trifolii is a fungal pathogen which incites anthracnose of alfalfa. To initiate research on molecular communication in this fungus a kinase-encoding gene (TB3) and the corresponding cDNA were cloned and sequenced. The deduced amino acid sequence of TB3 showed high homology to a Neurospora crassa, serine/threonine protein kinase, cot-1, required for hyphal elongation. Importantly, the carboxyl terminal catalytic domains of TB3 and cot-l are highly conserved but the amino terminal regions are divergent. Southern hybridizations showed that TB3 is part of a gene family in Colletotrichum trifolii. Furthermore a TB3 homolog is present in a related fungus Colletotrichum gloeosprioides f. sp. aeschynomene. We have also developed a procedure for isolating large numbers of germinating conidia and appressoria of Colletotrichum trifolii. Northern analysis indicated that TB3 is expressed in several fungal cell types. TB3 expression was highest in germinating conidia and vegetative mycelia and lowest in conidiating mycelia. These data show that TB3 is functionally similar to cot-1 and is important for hyphal elongation.

Posters II: Gene Expression/Genome Structure

36. Quelling: gene inactivation during mitosis in Neurospora crassa by transformation with homologous sequences

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When a wild-type strain of Neurospora crassa is transformed with different portions of the carotenogenic albino-1 or albino-3 genes, up to 30-35% of the transformants show an albino phenotype. The albino transformants presented a variety of phenotypes ranging from white or yellow to dark yellow colour. The ectopically integrated sequences provoke a severe impairment of the expression of the endogenous al-1 or a1-3 genes. This phenomenon, termed quelling, is found to be spontaneously and progressively reversible. In fact, all of the albino transformants have an unstable phenotype and revert progressively to wild-type or intermediate phenotypes over a prolonged culturing time. The phenotypic reversion is characterised by a progressive release of the transcriptional inhibition and seems to correlate with a reduction in the number of the ectopically integrated sequences. However, there is no strict correlation between the copy number of the ectopic sequences and the intensity of quelling. Heterokaryons with nuclei from quelled and wild type strains show a quelled phenotype indicating that the inhibition of expression by quelling acts in trans. The nature of the molecular events determining the onset of quelling is unclear. They are likely to involve some kind of interaction between the resident genes and ectopically integrated exogenous sequences. Recent evidence on a possible mechanism was presented.

37. Molecular cloning and expression of laccases from the white-rot basidiomycete

Polyporus pinsitus

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Two laccases have been purified from the extracellular medium of an 2,5-xylidine culture of the white-rot basidiomycete Polyporus pinsitus. These proteins are dimeric, comprised of two subunits of 63 kDa as determined by SDS-PAGE, and glycosylation accounts for 5-10 kDa of the total mass. Under non-denaturing conditions, the two purified laccases I and III have pIs of 6-6.5 and 3.5, respectively. The laccases have optimal activity at pH 5-5.5 and pH <4 with syringaldazine and ABTS as substrates, respectively. The genes LCC1 and LCC2 coding for the two purified laccases (I and III) have been cloned and their nucleotide sequences determined. LCC1 and LCC2 have 8 and 10 introns, respectively. The predicted proteins are 79% identical at the amino acid level. LCC1 expression is induced by 2,5-xylidine, while LCC2 expression appears to be constitutive. LCC1 has been expressed in Aspergillus oryzae, and the purified recombinant protein has the same pI, spectral properties, stability and pH profiles as the purified native protein. Three additional laccase genes have been cloned from P. pinsitus. The genes LCC3, LCC4 and LCC5 contain 12, 11 and 11 introns, respectively. The position of several of the introns is conserved among all 5 genes. The karyotype of P. pinsitus was determined by CHEF, and 8 bands ranging in size from approximately 5.7 to 2 Mb were resolved of which 2 appear to be doublets. The 5 laccase genes have been mapped to specific chromosomes. LCC1 and LCC2 are on a chromosome of 5.7 Mb. LCC4 and LCC5 are on a chromosome of 3.7 MB, and LCC3 is on a chromosome of 2.5 Mb.

38. Structure and function analysis of N. crassa NAD(P)H-nitrite reductase

James D. Colandene and Reginald H. Garrett, University of Virginia.

Nitrate assimilation, widespread among plants, fungi and bacteria, is the predominant means by which inorganic nitrogen is converted into a reduced, biologically useful form. Via this two-step process, nitrate is converted into nitrite by the enzyme nitrate reductase in a two-electron transfer reaction, and nitrite is converted into ammonium by nitrite reductase in a six-electron transfer reaction. The focus of these studies is nit-6, the N. crassa gene encoding nitrite reductase. N. crassa nitrite reductase is a soluble protein composed of two identical subunits. This enzyme utilizes either NADH or NADPH as electron donor, and possesses two iron sulfur (4Fe/4S) clusters, two siroheme groups, and two FAD molecules. Besides its native activity, nitrite reductase has a number of partial activities which are presumed to be functions of discrete structural domains of the protein. These include the FAD-dependent NAD(P)H-cytochrome c reductase and dithionite-nitrite reductase activities. We have constructed a full-length nit-6 cDNA and partial nit-6 constructs encoding, the putative domains of the protein and have expressed them in E. coli and yeast in order to perform structure-function studies. Results indicate that the N-terminal end of the protein which putatively encodes NAD(P)H and FAD binding domain possesses the FAD-dependent NAD(P)H-cytochrome c partial activity. The C-terminal portion of the protein putatively responsible for the binding and coordination of the (4Fe/4S) clusters and siroheme appears to possess the dithionite-nitrite reductase activity.

39. The location, rate of expression, and activation domains of the regulatory

protein CYS-3

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The sulfur circuit of Neurospora crassa consists of a set of unlinked structural genes. These structural genes include three major regulatory genes (cys-3, scon-1 and scon-2) and six sulfur-catabolic enzyme genes (aryl sulfatase, choline sulfatase, sulfate permeases I and II, methionine permease and an extracellular protease). cys-3 acts in a positive manner to turn on the sulfur catabolic enzyme genes and is regulated by scon-l and 2; sulfur availability and, possibly, by itself. scon-l and 2 are negative regulatory genes that inhibit the expression of the cys-3 gene when sulfur levels within the cell are high. Mobility shifts and western blots were used to detect CYS-3 protein in nuclear extract samples (both nuclear and cytoplasmic fractions) of wild type and mutant strains of Neurospora crassa grown under repressed or derepressed conditions. The mobility shifts indicate the following: 1) CYS-3 protein is localized to the nucleus; 2) CYS-3 protein is present in wild type derepressed samples and scon- samples (repressed and derepressed conditions); 3) no CYS-3 protein is detected in cys-3 strains. The western blots appear to support these findings. The same methods are being used to determine the length of time needed to induce CYS-3 protein production. The results so far indicate that it takes approximately three hours for CYS-3 protein to be made. The potential activation surfaces of cys-3 are being determined using the yeast hybrid system. Results so far have been inconclusive.

40. Laccase gene-specific sequences from selected white-rot and brown-rot fungi

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Laccase gene-specific sequences from 12 strains representing 10 genera of white-rot and brown-rot fungi were isolated, using a PCR amplification procedure employing degenerate primers corresponding to the consensus sequences of the copper-binding regions in the N-terminal domain of known basidiomycete laccases. All the fungi included in this study, except Pleurotus ostreatus and Fomes fomentarius, gave a PCR amplified product of about 200 bp. A few of the fungi gave additional PCR products suggesting laccase gene polymorphisms in these organisms. Several of the PCR products including those from the white-rot fungi Trametes versicolor, Phlebia brevispora, Ganoderma lucidum and Lentinula edodes, and the brown-rot fungus Gloeophyllum trabeum, were sequenced. These nucleotide (nt) and predicted amino acid (aa) sequences were compared to the corresponding, previously published, laccase gene sequences of white-rot fungi. The results indicated the presence of laccase gene-specific sequences in a number of wood-rot fungi included in this study. The results further showed a high degree of nt (54%-74%) and aa homology (72%-97%) between the laccase gene-specific sequences (and deduced aa sequences) of white-rot fungi, but not those of Ascomycetes. Demonstration of laccase gene-specific sequences as well as laccase activity in the brown-rot fungus G. trabeum was of particular interest because brown-rot fungi were not previously shown to contain laccases.

41. Transcription initiation and termination sites of ribosomal RNA gene clusters of Neurospora crassa

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The nontranscribed external spacer region containing the transcription regulatory sequences of rDNA of Neurospora crassa has been characterized which shows many interesting sequences like the presence of Sal I box, putative rRNA processing site, pyrimidine rich region and secondary structure of the rDNA typically present in almost all eukaryotes. The entire region (nt) has been cloned and sequenced. The transcription initiation and termination sites were localized by S1 mapping and primer extension analysis. Primer extension analysis suggested that the transcription starts from a G at location 2537 whereas the transcription termination site is located in the region 471-475. The nontranscribed spacer region also contains sequences which are binding sites for known nuclear factors. (Supported in partly by the EPA (#R813126) and from an Institutional Grant (#2S06GM 08016) from the NIGMS-NIH and by a Collaborative Core Unit, Graduate School of Arts and Science, Howard University to SKD).

42. An aflatoxin biosynthesis regulatory protein (AFLR) is a sequence-specific DNA binding protein

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In previous work, a gene (aflR) was isolated from an Aspergillus parasiticus cosmid clone whose expression was correlated with expression of several aflatoxin pathway genes. The open reading frame of aflR encodes a protein (AFLR) with a GAL4-type zinc binuclear cluster, which suggested that it may be a DNA-binding protein. Using synthetic oligonucleotides in electrophoretic mobility shift assays, we found that recombinant AFLR bound with sequence specificity to a palindromic site, TTAGGCCTAA, 121 bp upstream of its own translation start site. AFLR also bound to portions of the DNA from the promoter regions of several aflatoxin pathway genes. The genes tested were part of a 50 kb gene cluster and included genes for norsolorinic acid reductase, a polyketide synthase similar to that of A. nidulans, an aflatoxin pathway-specific fatty acid synthase, two reductases involved in versicolorin A synthesis, and sterigmatocystin O-methyltransferase. These results suggest that in addition to regulating its own expression, AFLR may coordinately regulate the expression of multiple genes in the aflatoxin biosynthetic pathway.

43. Analysis of gene clustering: transformational study of the ethanol-gene-cluster in Aspergillus nidulans

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Ethanol-utilization in Aspergillus nidulans is mediated by alcohol-dehydrogenase I and aldehyde- dehydrogenase encoded by alcA and aldA respectively. Both genes are under the transcriptional control of the specific activator AlcR and the general carbon catabolite repressor CreA. The alcR and alcA genes are closely linked on chromosome VII; aldA is located on chromosome VIII. We have identified seven other genes at the same locus as alcA and alcR on chromosome VII. They are transcriptionally controlled by AlcR and CreA and were therefore called alcQ, alcT, alcN, alcP, alcO, alcM and alcS. We analysed in two alc-deletion strains the function of gene-clustering; we were able to show that alcR

and alcA integrated at different loci (trans) in an alc500 deletion strain did not restore growth on ethanol. Transformation of the same strain with a phagemid containg six alc genes in cis (alcR, alcO, alcA, alcM and alcS) leads to wild-type phenotype on ethanol. Several gene combinations in cis and trans were tested for growth on ethanol and other related carbon sources as well as transcription regulation. We will further discuss gene and cluster-function.

44. Multiple control signals in the proline utilisation gene cluster of Aspergillus nidulans

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Aspergillus nidulans can utilise proline as the sole source of carbon and nitrogen. The genes involved form a cluster which comprise three structural genes, prnB, C and D, a positively-acting regulatory gene, prnA, and a fifth gene, prnX, of unknown function. The 1.7 kb intergenic region between genes prnB and D has been shown to play a crucial role in the regulation of thc expression of all prn genes with exception of prnA. Proline catabolism is subject to three levels of control: Specific induction, mediated by the PrnA protein, carbon catabolite repression, mediated by the negatively-acting protein CreA, and nitrogen metabolite repression, mediated by the positively-acting protein AreA. PrnA, CreA and AreA belong to the zinc-containing class of DNA binding proteins. A complete molecular characterization of their binding to the prn intergenic region has been carried out by band shift and footprint analysis. Three PrnA binding sites have been found in the prn cluster, two in the prnB-D intergenic region, and one in the prnB-C intergenic region. At least two of them are functional in vivo. A PrnA binding site situated 3' of gene prnB is sufficient to its inducibility. The complete molecular characterization of 17 AreA binding sites in the prnB-D intergenic region has allowed to define a consensus binding sequence 5'HGATAD3'. The functional importance of the pyrimidines contained in the binding site has been determined for the first time for a GATA factor. The most proximal sites to the start points of genes prnB and D are functional in vivo, as determined by transcriptional analysis and growth tests of deletion mutants in the prnB-D intergenic region. The positive action of AreA is not due to repressor displacement. The expression of the prn genes is conditionally dependent on AreA. Strains carrying null areA alleles can utilise proline as carbon and nitrogen sources in the absence of CreA. A 700 bp deletion in the prn intergenic region leads to unconditional dependence on AreA for proline utilization. We postulate the existence of a third positive regulatory element whose target lies in this region. We have carried out band shift assays and deletion analysis and have found a 80 bp DNA fragment which binds specifically to hitherto unidentified protein

45. Identification of NIT4 binding sites within the nit-3 promoter

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Expression of nit-3 and nit-6, structural genes in Neurospora crassa which encode nitrate and nitrite reductase, respectively, requires limitation of primary nitrogen sources and induction by means of nitrate. Transcriptional activation of these genes also requires the action of NIT2 and NIT4, positive regulatory proteins. NIT2 is a global acting regulatory protein, also needed for expression of a variety of other nitrogen metabolizing enzymes,

while NIT4 is a pathway specific regulatory protein. NIT4 contains 1090 amino acids with a putative Cys6 zinc c!uster DNA binding domain, similar to that of GAL4, found near the amino terminus. A NIT4/bGAL fusion protein has been expressed and purified, and its DNA binding specificity determined. Two NIT4 binding sites of varying strengths in the nit-3 promoter are identified through mobility shift and DNA footprinting experiments. The stronger site contains the palindromic sequence TCCGCGGA, while the weaker site contains the related sequence TCCGTGGC. Similar sequences are found in the nit-6 promoter. Further examination of this octomeric binding site and its flanking sequences is currently being done.

46. Evidence for a GATA-1-like DNA binding protein in N. crassa whose expression responds to nitrogen metabolism

Peter T. Gates and Reginald H. Garrett, University of Virginia

Employing gel-shift assays and DNA-affinity chromatography, we have isolated DNA-binding proteins from N crassa crude cell extracts that bind specifically to the consensus sequence NGATAC. Such GATA sequences are specifically recognized by the fungal transcription factors AREA and NIT2, which regulate nitrogen metabolism in A nidulans and N. crassa, respectively. Protein-DNA complexes are observed with oligonucleotides containing either one NGATAC consensus site or multiple consensus sites in various orientations. A lower molecular weight protein DNA complex is unaffected by the presence of physiological levels of ammonium, glutamine, or histidine, metabo lites which repress nitrate assimilation and other aspects of nitrogen metabolism in filamentous fungi. In contrast, formation of a higher molecular weight protein DNA complex does appear to be inhibited by physiological levels of these metabolites. Interestingly, the affinity purified proteins bind with high affinity and sequence specificity to single-stranded DNA containing one or more NGATAC consensus sequences. Protein sequence analysis of the affinity-purified fractions has not reveale any AREA or NIT2. Multiple GATA-binding activities, independent of NIT2 or its homolog AREA, and differing in sensitivity to the nitrogen status of the cell, may exist in N. crassa, as shown recently in Aspergillus nidulans.

47. Pleiotropic deficiencies of the laccase-derepressed mutant lah-1 are caused by constitutively increased expression of the cross-pathway control gene cpc-1

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We previously isolated two Neurospora crassa mutants that showed abnormal expression of the laccase gene (lacc). One is the lah-1 mutant which derepresses lacc expression. The other is a cpc-1 mutant, which is not able to induce laccase activity. To investigate relationship between lah-1 and cpc-1 on lacc expression, we further characterized these mutants. The lah-1 mutant showed pleiotropic deficiencies such as slow growth in vegetative cultures, poor protoperithecia formation and hypersensitivity to cycloheximide. The cpc-1 mutant showed abnormality in early stage of conidial germination and also high sensitivity to cycloheximide. Genetic analyses of lah- 1cpc-1 double mutant showed that the cpc-1 mutation is epistatic to lah-1. Moreover we observed that expression of trp-3 gene is constitutively elevated in the lah-1 mutant. The derepressed level of trp-3 gene in the lah-1 mutant reduced to basal level in the lah-1cpc-1 double mutant. These results

suggest that in the lah-1 mutant, the expression of cpc-1 gene is constitutively derepressed

48. Analyses of N. crassa mei-3 gene products and expression

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The mei-3 (meiosis) gene of Neurospora crassa plays important roles in DNA repair and meiotic recombination. In this study we produced anti-MEI3 antibody rised against recombinant MEI3 protein, and using the antibody we detected a 38kDa polypeptide in perithecia, fruit body of N. crassa, by Western analysis. From sequencing of both cDNA and genomic clones, mei-3 gene had one 1195 bps open reading frame and two introns, and deduced molecular weight (39kDa) was in good agreement with the data obtained from Western analysis. The 39kDa MEI3 product is homologous to proteins of Rad51 family, and contains the conserved "domain I" domain II" and carboxy-terminal domains found in this family. The mei-3 expressions in wild type and five different mei-3 mutants increased in response to MMS and UV as determined by Northern blotting. Two genomic sequences of mei-3(SA10) and mei-3 (N289) were determined. These mei-3 genes contained several differences from mei-3 gene of wild-type, resulting in truncated proteins.

49. Abnormal mitochondrial DNA in uvs-4 and uvs-5 mutants of Neurospora crassa

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The mutagen sensitive uvs-4 and uvs-5 nuclear mutants progressively degenerate upon prolonged asexual propagation (Schroeder 1970 Mol. Gen. Genet. 107:291-304). Since this phenotype is characteristic of some respiratory mutants we have examined uvs-4 and uvs-S for defects in mitochondrial function. After recovery from sheltering heterokaryons, both mutants develop high levels of CN-resistant respiration and become cytochrome a and b deficient upon repeated subculturing. The growth rate of both mutants progressively declines, but only uvs-5 dies after being subcultured 4 or more times. During the degeneration, abnormal forms of mtDNA appear in both mutants. High amounts of a plasmid-like derivative of mtDNA accumulate in uvs-4, whereas mtDNA deletions accumulate in uvs-5. The plasmid-like mtDNA derivative of uvs-4 is a 167-bp tandemly repeated sequence that consists of a PstI palindrome, the promoter and 104 base pairs of the 23 S mt rRNA gene. Heterokaryon tests have shown that the uvs-4 mutation is essential for the generation and maintenance of the plasmid-like element. The mechanisms which give rise to the plasmid-like element in uvs-4 and mtDNA deletions in uvs-5 are still unknown. Supported by the Muscular Dystrophy and American Heart Association, and a NSERC post-doctoral fellowship to G.H.

50. The elongation factor 1alpha (EF-1alpha) of Neurospora crassa

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The translation elongation factor 1a (EF-lalpha) plays an essential role in protein synthesis in eukaryotic cells. This protein transfers aminoacyl-tRNAs into the acceptor site of the ribosome in a step that requires GTP. The tef-1 gene encoding the EF-1alpha protein was cloned from Neurospora crassa. The sequences of genomic DNA and cDNA

clones showed that the tef-1 gene contained one ORF of 1380 bp length which is interrupted by three short introns. The deduced polypeptide contained 460 amino acids. The level of tef-1 mRNA was low in conidia but high in growing cells. When mycelia were transferred to poor nutrient media the level of tef-1 gene mRNA decreased remarkably. The pattern of tef-1 expression was similar to that of genes for ribosomal proteins. Southern blot analysis showed that Neurospora genomic DNA contained only one copy of the tef-1 gene. The tef-1 mutant was produced by RIP. This strain showed very slow growth. The tef-1 gene was mapped between arg-3 and leu-4 loci on linkage group I by RFLP mapping and between T(OY321) and ser-3 loci by genetic mapping.

51. Excision of UV products during liquid holding in Neurospora

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Excision of pyrimidine dimer and that of (6-4) photoproduct were measured ln Neurospora crassa wild type and the mus-18 mutant during liquid holding. Pyrimidine dimers were lost within 60 min in wildtype irradiated at 150 J/m2, while (6-4) photoproducts were processed faster than dimers. In mus-18, most dimers were left unprocessed over 6 hr. Unexpectedly, excision of photoproducts were also defective in this mutant. Combination of liquid holding and photoreactivation in UV-induced reversion assay revealed that pyrimidine dimer was a main cause of high mutagenicity of UV in mus-18, although this damage seemed cytotoxic rather than mutagenic to wildtype Neurospora.

52. Molecular cloning and characterization of a rhamnogalacturonan acetylesterase from Aspergillus aculeatus. Synergism between rhamnogalacturonan degrading enzymes

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The main backbone in plant cell wall pectins can be devided into linear homogalacturonan polymers of (1-4) linked alpha-D-galacturonic acid (GalUA) and highly branched rhamnogalacturonan (hairy) regions, composed of alternating alpha-(1,2) linked L-rhamnosyl and alpha-(1,4) linked galacturonic acid residues, with arabinan, galactan or arabinogalactan side chains attached to the rhamnose residues. Most pectic substances are furthermore substituted with acetyl or methyl groups at the GalUA residues in the backbone. We have recently cloned and characterized two structurally and functionally different rhamnogalacturonan degrading enzymes, RGase A and RGase B, from the filamentous fungus Aspergillus aculeatus. A prerequisite for the action of rhamnogalacturonases is that the acetyl esters have been removed from the backbone. Thus, the presence of a rhamnogalacturonan acetylesterase (RGAE) in A. aculeatus, highly specific for the deacetylation of rhamnogalacturonan regions of pectin suggests that this enzyme is essential for the action of RGases in vivo. This communication describes the isolation and characterization of the rha1 gene encoding RGAE I from A. aculeatus. With the goal of elucidating the role of RGAE I in the enzymatic degradation of plant cell wall rhamnogalacturonan we have undertaken high level expression of the rha1 gene in Aspergillus oryzae and characterization of the purified, recombinant enzyme. In addittion, we show that the recombinant rhamnogalacturonan acetylesterase (rRGAE)

acts in synergy with RGase A as well as RGase B in the degradation of apple pectin rhamnogalacturonan.

53. Cloning and characterization of two structurally and functionally divergent rhamnogalacturonases from Aspergillus aculeatus

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The pectic substances of plant cell walls are composed of homogalacturonan (polymer of alpha-1,4-galacturonic acid) and rhamnogalacturonan (polymer of alternating alpha-1,4-rhamnose and alpha-1,2-galacturonic acid). Whereas pectinases, which cleave in the homogalacturonan regions, have been extensively studied, rhamnogalacturonases have only recently been described. In the present study two rhamnogalacturonases from the filamentous fungus Aspergillus aculeatus have been cloned and characterized. A cDNA library from A. aculeatus was constructed and a novel Rhamnogalacturonase B was isolated by expression cloning in yeast. For this purpose a new plate screening assay was developed, specific for the detection of rhamnogalacturonase activity. The Rhamno galacturonase A, known from previous reports, was shown not to be expressed in yeast in an active form. Therefore, Rhamnogalacturonase A was purified, peptide sequences were obtained and full-length cDNAs encoding the enzyme were isolated using a polymerase chain reaction generated product as a probe. Comparison of the deduced primary structures indicates that the two rhamnogalacturonases are structurally different. The cloned genes were transformed into Aspergillus oryzae for high level expression. The recombinant enzymes were purified and characterized, revealing significant differences in substrate specificity, as well as in pH and temperalure optima and stability. Data from the hydrolysis of apple rhamnogalacturonan with the recombinant rhamnogalacturonases suggest that the two enzymes exert their action at different sites in the backbone.

54. Characterization of a general amino acid control transactivator in the chestnut blight fungus Cryphonectria parasitica and its regulation in virulent and hypovirus infected strains

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The transcription factor CPC1 governs general amino acid control in Neurospora crassa. We have cloned a homologous gene from the plant pathogenic fungus Cryphonectria parasitica and examined its regulation in isogenic virulent and hypovirus-infected strains. Northern analysis revealed that amino acid starvation elicits an increase in C. parasitica cpc-1 transcript levels. Although amino acid starvation produced similar increases in cpc-1 transcript levels in isogenic virulent and hypovirus-infected strains, the diminished accumulation of transcripts for several amino acid biosynthetic genes in the hypovirulent strain is consistent with a virus-mediated reduction in cpCPC1 activity. Suppression of cpc-1 transcript accumulation was observed in the hypovirus-infected strain under growth conditions that derepress laccase (lac-1) transcription in C. parasitica. Our results define a general amino acid control transactivator in a plant pathogenic fungus and provide further evidence that a virulence-attenuating hypovirus alters the transcriptional response of the host fungus to changes in nutrient availability.

55. Specific recognition of the DNA-targets of AlcR, the transactivator of the ethanol regulon in Aspergillus nidulans, involves an arginine residue in its NH2-terminus

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The transcriptional activator of the ethanol regulon, AlcR, is a binuclear zinc cluster able to bind onto specific targets on the alcR and alcA promoters. These targets are organized as invert and direct repeats with the consensus core 5'CCGCA-3'. We tested the importance of the AlcR NH2-terminal part on the specific in vitro binding of the GST-AlcR fusion protein. Two constructions were assayed in gel band shift experiments. The fusion product with a truncated AlcR NH2-end (the 6th first residues are missing) was able to bind specifically onto the two types of DNA targets with a low affinity for the invert repeats (10(-3) M) and a higher affinity for the direct repeats (10(-7) M) targets. With the fusion protein containing the complete NH2-terminus of AlcR, the affinity was only increased for the invert repeats by 10000 fold (10-7 M). This enhanced binding activity was shown to be directed by only one arginine residue within the NH2-terminus. Footprint interferences experiments showed a decrease of the number of contacted Gs in the invert repeat target. This demonstrates that the AlcR amino terminus is essential for the correct recognition of invert repeats targets. In order to test these results in vivo, a reporter strain of Aspergillus nidulans was built. It contains the uidA gene, encoding the beta-glucuronidase (GUS), under the control of the alcA regulating sequences. Transformants lacking the NH2-terminus of AlcR were tested for growth on ethanol and GUS activity was determined in comparison with a transformant strain containing the AlcR NH2-terminus.

56. Studies of Neurospora crassa CYS3 protein basic region and identification of its consensus and optimal DNA binding site

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CYS3 is the positive acting regulatory protein involved in the sulfur utilization control circuit in Neurospora crassa. CYS3 activates the expression of a set of genes which encode sulfur-related catabolic enzymes under sulfur limitation conditions. CYS3 belongs to the bZip protein family and shares extensive sequence homology with other family members in the leucine zipper and basic region. Here we report the characterization of native DNA binding sites recognized by CYS3. From systematic mutational analysis, we hare defined the consensus of CYS3 binding sequence, 5'-ATGPuPyPuPyCAT-3', a ten nucleotide palindrome, and have found 5'-ATGACGTCAT-3' acts as an optimal binding site. We also have studied two uncharged residues, SER113 and PHE116, in the basic region of the CYS3 protein, and found that S113 is directly involved in a nucleotide specific interaction and that F116 also contributes significantly to DNA binding affinity.

57. DNA binding of FacB, a transcriptional activator of acetate utilization genes of Aspergillus nidulans

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The facB gene of Aspergillus nidulans encodes a transcriptional activator which mediates

acetate induction of the amdS gene (encoding acetamidase) and genes required for acetate metabolism via the glyoxylate bypass. Cloning and sequence analysis of facB revealed a Zn(II)2Cys6 DNA binding cluster, a putative leucine zipper-like dimerization motif and potential acidic activation domains (H.M. Martin, S. Sapats, J.A. Sharp, M.E. Katz, M.A. Davis and M.J. Hynes, unpublished). DNA binding studies are being used to investigate the regulatory function of facB. The FacB protein has been expressed in Escherichia coli as a Maltose Binding Protein fusion. This fusion has been used in gel mobility shift assays to demonstrate that FacB is a DNA binding protein that binds to specific sequences from FacB- regulated promoters. Footprinting assays have been used to define two dissimilar sequences to which FacB binds in the amdS promoter. An analysis of binding to mutant FacB binding sites has been performed. In vitro mutagenesis has been used to alter specific cysteine residues in the DNA binding domain of FacB. A facB allele containing a mutated Zn(II)2Cys6 cluster fails to complement a facB null mutant for growth on acetate. Thus, the DNA binding cluster is essential for FacB function. A fusion protein containing a mutated zinc cluster has been expressed in E. coli to confirm that DNA binding activity is abolished.

58. Methylation and gene silencing in Neurospora

Jeff Irelan and Eric Selker. University of Oregon.

Regional inhibition of gene expression by epigenetic means, or gene silencing, is responsible for phenomena as diverse as silencing of mating type information in yeast to imprinting in mammals. DNA methylation is thought to be involved in many gene silencing phenomena. Due to the existence of mutants deficient in methylation, Neurospora crassa offers an opportunity to determine the role of methylation in various aspects of gene silencing. We found that silencing by al-1 trangenes, or quelling, is not affected in a mutant, dim-2, that lacks cytosine methylation. In contrast, we found that methylation resulting from RIP can result in epigenetic silencing of a single- copy gene, hph, located between the elements of a duplication of the am gene. The effect of methylation on gene expression varies widely from case to case, and probably occurs by inhibition of transcription. Spontaneous revertants of hph are re-silenced, although this re-silencing can require many cell divisions, indicating that epigenetically induced gene expression states are somewhat stable. The spectrum of gene expression states, the role of methylation in the stability of the epigenetic mark, and the use of epigenetically silenced genes to isolate and characterize methylation mutants was discussed.

59. Detection of a protein which binds specifically to the upstream region of the pcbAB gene in Penicillium chrysogenum

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An electromobility shift assay (EMSA) was previously used to screen for specific proteins binding to the upstream region of the pcbAB gene, one of the three genes encoding enzymes for penicillin biosynthesis in Penicillium chrysogenum. A specific DNA-protein interaction was detected within a fragment covering the region -387 to -242 relative to the pcbAB translational start codon. The involvement of a 7 basepair motif TGCCAAG in the binding was demonstrated in this work by cross competition EMSAs.

The detection of this protein and pcbAB-mRNA in culture extracts occurred at the same time point fermentations suggesting that the protein might be a transcription activator.

60. Cosmid and YAC clone anchored genetic map of Magnaporthe grisea

Jinrong Xu and John E. Hamer, Purdue University, West Lafayette, IN 47907-1392
The rice blast fungus M. grisea contains repetitive sequences called MGR sequences. One of these repetitive elements, MGR586, is dispersed over the genome and has been used in the construction of a genetic map. A cosmid library in pcosAX and a YAC library in pYAC4 were constructed from the M. grisea mapping strain 4375-R-26. Cosmid and YAC clones containing the MGR586 sequence were identified by colony in situ hybridization. These clones were then mapped onto the MGR586-based genetic map. This genetic map, with anchored MGR586 cosmid and YAC clones, will be useful to construct a regional physical map and for other genomic analyses.

61. Origin of a new plasmid by heterologous recombination between a circular and a linear plasmid in Neurospora mitochondria.

Tony Griffiths and Xiao Yang, Botany Department, UBC, Vancouver, Canada V6T 1Z4.
A strain of *N. intermedia* from China contains three prominent linear plasmids plus two different circular plasmids. In one subculture of this strain, a new linear plasmid arose spontaneously. The plasmid was named Harbin-L Simultaneously, one circular and three linear plasmids disappeared. Restriction analysis and sequencing revealed that the new linear plasmid was formed by a double recombination event which slotted part of a circular plasmid into part of one of the linear plasmids. The sequences at the junctions show that two 'crossovers' occurred at sites where there was seven base pairs of homology between the parental plasmids. One of the open reading frames of the new linear plasmid spans a junction site, so is composed of sequences from both parental elements.

Posters II: Sexual and Asexual Reproduction

1. Molecular characterization of the veA gene from Aspergillus nidulans

Patricia A. Kennedy and Lawrence N. Yager, Temple University, Department of Biology, Philadelphia, PA.
Light is an important environmental stimulus that induces conidiation in Aspergillus nidulans. The ability to respond to light is dependent on the allelic state of the veA (velvet) gene. Wild-type strains require light for asexual development, whereas strains bearing the veA1 mutation conidiate regardless of the presence or absence of light. Previous genetic analysis suggested that veA is a negative regulator of early development. We have cloned the veA gene by mutant complementation, and have performed genetic tests and CHEF gel electrophoresis to show that we have correctly isolated this gene. The veA gene encodes a 2.5 kb mRNA that is present throughout development and in uninduced cultures. This mRNA is also present in the veA1 mutant. We have used the genomic veA gene fragment as a probe to isolate a cDNA clone from a lambda Zap Express library. The DNA sequence of this cDNA was presented and the possible role

that veA performs during early conidiation discussed.

2. Genetic and molecular analysis of a suppresssor of fluG mutations in Aspergillus nidulans

Hyung-Ok Lee and Lawrence N. Yager, Temple University, Department of Biology, Philadelphia, PA.

Mutations in the fluG gene disrupt the ordered sequence of events that result in the formation of conidia (asexual spores) in Aspergillus nidulans. Lee and Adams (1994, Genes Dev. 8:641-651) have proposed that FluG functions as an enzyme for the production of an extracellular signal that controls the initiation of sporulation. Although the phenotype of all fluG mutants is the proliferation of undifferentiated masses of vegetative mycelia, several alleles show additional phenotypes. One mutant disrupts the ability of colonies to conidiate in red light, whereas three others suppress the veA1 mutation. (Red light is required for conidiation for wildtype colonies and the mutation allows conidiation to occur in the absence of light.) We have isolated a mutant, designated suA1fluG, that suppresses all fluG mutations. The suA1fluG mutant is recessive to its wild-type allele and maps on chromosome II. We have cloned this gene by mutant complementation and have isolated a cDNA clone, which is presently being sequenced. The gene encodes a 2.5 kb mRNA that is present both in uninduced and induced cultures. Since the suppressor appears to bypass FluG functions, its possible role in controlling those events involved in the intiation of sporulation was discussed.

3. The acoC gene is necessary for developmental competence and vegetative growth in Aspergillus nidulans

Kathryn W. Sutton(1), Erh-Hsin Ling(2) Sewell P. Champe(2) and Lawrence N. Yager(1), . (1)Temple University, Department of Biology, Philadelphia, PA; (2)Waksman Institute, Rutgers University, Piscataway, NJ.

Conidiation in Aspergillus nidulans is normally suppressed in submerged culture, but occurs readily if colonies are exposed to an air interface. The transfer of colonies from liquid to solid medium, termed induction, initiates and synchronizes conidiation. However, induction can only initiate conidiation if colonies have attained a certain change in state, called developmental competence. Butnick et al. (1984, J. Bact. 160:533-540) describe the isolation and characterization of three temperature sensitive mutants that are blocked in the acquisition of competence. One of these mutants, acoC193, is not only defective in attaining competence, but displays a lethal phenotype if colonies are exposed to light at the restrictive temperature. Combined temperature/ light-dark shift experiments indicate that the light lethal phenotype persists after the acquisition of competence, suggesting that acoC is involved in a growth maintenance function. Although conidiation in wild-type colonies is induced by exposure to red light, preliminary spectral analysis shows that red wavelengths are not responsible for the lethal phenotype. We have cloned acoC and show that it encodes a 1.9 kb mRNA that is present in uninduced and induced colonies. DNA sequence analysis is currently being performed on genomic and cDNA isolates. Our results suggest that acoC is involved in early functions associated with the acquisition of competence and in other activities required for vegetative growth.

4. Isolation and characterisation of the B mating type genes of Coprinus cinereus

Suzanne O'Shea, Crawford Kingsnorth, John Halsall and Lorna Casselton. University of Oxford, Oxford, UK

A successful, mating in Coprinus cinereus leads to the development of a fertile dikaryon with binucleate cells and characteristic clamp connections. The B mating type genes regulate specific steps in the developmental programme that establishes the dikaryon (nuclear migration) and also in its maintenance (clamp cell fusion). We describe a genomic subtraction technique that enabled us to isolate the B6 mating type locus. The genes that confer B6 mating type specificity reside within a 10 kb DNA sequence that fails to cross-hybridise to genomic DNAs from several monokaryons that have different versions of the B locus (e.g. B1, B3, B5, B42). This suggests that different alleles of the B genes have little sequence similarity. By taking common flanking sequences we have isolated a second B locus, B42. We have identified a number of genes within the B6 and B42 specific DNAs and have followed the regulation of their transcripts through different stages of development. We will report on the DNA sequences which allow us to predict the likely functions of some of thc genes and relate this to their regulation.

5. A system of balanced lethals controls the segregation of mating type loci in the oomycete, Phytophthora infestans

Howard S. Judelson, University of California, Riverside.

The genetic basis of mating type is being studied in the oomycete, Phytophthora infestans. Previous studies have failed to establish the number of loci that determine the two mating types, A1 and A2, and how alleles of these loci interact; this latter point is important since oomycetes are diploid and therefore potentially heterozygous at the mating type loci. To characterize these loci in P. infestans, thirteen markers linked to the A1 and A2 phenotypes were identified using RAPD (random amplified polymorphic DNA) markers. Fifty alleles of the loci were subsequently scored in four crosses (360 meioses) as RAPDs or as other PCR or RFLP markers. Genetic and physical mapping indicated that all loci linked to the A1 and A2 determinants resided in a single region, consistent with the existence of a single mating type locus. A curious 300 kb region of structural hybridity between the chromosomes, tightly linked to the mating type locus, was detected; its function is unclear but it may account for the distorted segregation of A1 and A2 type described in many earlier studies. Strikingly, a non-Mendelian pattern of segregation was observed for markers in the mating type region as only two of the four expected genotypes were detected in viable progeny. This pattern is equivalent to that caused by balanced lethal loci. By restricting progeny to only two genotypes, this apparently enables the unambiguous determination of the two mating types in the heterothallic oomycetes.

6. Control of sexual morphogenesis in Pyrenopeziza brassicae: cause of light leaf spot in brassicas

Matthew Robb, Padma Venkatasubramanian and Alison M. Ashby. Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA. UK.

Pyrenopeziza brassicae is a heterothallic ascomycete and is the cause of light leaf spot disease of brassicas. We are studying sexual morphogenesis using both biochemical and molecular approaches. Biochemical analysis has lead to the partial purification of a

lipoidal sex factor, produced by mated cultures but not by single isolates of the fungus. The sex factor (SF) inhibits asexual sporulation and stimulates ascocarp development and therefore functions as a developmental signal. Molecular analysis has focused on cloning of the mating type loci and the analysis of protein profile changes in single mating type isolates in response to sex factor. Heterologous probing using the N. crassa A mating type probe has successfully identified the MAT 1-2 locus from P. brassicae. Protein profile analysis revealed a major protein present in SF induced single isolate cultures and fertile ascocarps which was not in untreated single isolates. The protein (sex factor induced; SFI) has been purified by hydrophobic interaction FPLC. Primers were generated by reverse translation of internal amino acid sequence and used in PCR reactions against genomic DNA. A single unique PCR product was obtained and used to probe a genomic library identifying three putative positive clones. Recent progress in analysis of sexual morphogenesis in P. brassicae was reported.

7. Isolates of Glomerella cingulata mate on a non-host plant

Cindy R. Cisar, Alyson B. Thornton, and David O. TeBeest, Department of Plant Pathology, University of Arkansas, Fayetteville

Glomerella cingulata (teleomorph of Colletotrichum gloeosporioides) is an agriculturally important fungus pathogenic on many plant species. Self- sterile, sexually compatible isolates of G. cingulata (from pecan) were crossed on northern jointvetch (NJV) plants under simulated natural conditions. Stems of 1-2 month old northern jointvetch (Fabaceae) plants were inoculated with one of two G. cingulata isolates from pecan (Juglandaceae), placed in growth chambers, and after one week the same sites were inoculated with the other sexually compatible isolate. After incubating the plants for one more week in growth chambers the co-inoculated NJV stems were excised and placed in Petri dishes containing either a minimal agar or moistened sterile filter paper and incubated at room temperature with a 12 hour photoperiod for 3 weeks. As controls, NJV plants were also inoculated with only one of the G. cingulata isolates. G. cingulata perithecia with ascospores were observed on 75% of the stems inoculated with both isolates. No perithecia were observed on NJV stems inoculated with only one of the G. cingulata isolates from pecan. Twenty-eight single ascospore progeny were randomly isolated from these crosses and compared to 26 progeny isolated from crosses between the same two isolates of G. cingulata on agar medium. Progeny were examined for evidence of sexual recombination using mating type and several RFLPs as markers. All of the progeny were hybrids of the two parental isolates. The implications of completion of the sexual cycle by a fungal plant pathogen on a non-host was discussed.

8. Expression of three genes of the Neurospora crassa A idiomorph in the vegetative and sexual phases

Ferreira, A. V.-B. and Glass, N. L. Department of Botany and Biotechnology Lab, University of British Columbia, Canada.

mt A-1 is a 1.2 kb gene contained in the A idiomorph of N. crassa which confers mating identity and heterokaryon incompatibility. Mutational analyses have shown that the remaining 4 kb of the A idiomorph is involved in the formation of perithecia and ascospore production. We focused our research on the characterization of any genes present within the 4 kb region and on the expression of the mating-type genes during the

life cycle of N. crassa. Using RT-PCR two cDNAs were amplified and sequenced. mt A-2 is a 1147 bp long gene and contains three introns. It codes for a putative protein of 326 amino acids which has a very acidic C-terminus tail, a common characteristic of transcription activators. mt A-3 is a 1103 bp long gene and also contains three introns. The mt A-3 ORF encodes a protein of 324 amino acids which contains an HMG domain, acidic N and C tails and a proline rich segment in the C-terminus end. These features suggest that mtA-3 may also be a transcriptional activator. The mt A-2 and mt A-3 gene products have similarity to various mating-type gene products including the P. anserina SMR1 and SMR2, respectively. To investigate if expression of the mating-type genes are restricted to one phase of the N. crassa life cycle, transcriptional analyses of mt A-1, mt A-2 and mt A-3 were done using RT-PCR and Northern hybridization. All three genes are expressed during both the vegetative and sexual phases of the N. crassa life cycle. These results suggest that the mating-type products of mt A-1, mt A-2 and mt A-3 are regulated at the post- transcriptional level. Studies are in progress in order to determine the roles of mt A-2 and mt A-3 during the vegetative and sexual cycle of N. crassa.

9. Loss of meiosis in Aspergillus

David M. Geiser, Michael L. Arnold, and William E. Timberlake, University of Georgia, Athens, GA

The genus Aspergillus has a remarkable diversity of propagative strategies, including strictly mitotic, meiotic, homothallic, and heterothallic species. Many species not known to produce ascospores retain characters that are often correlated with ascospore production, including the production of Hulle cells, and the production of sclerotia and sclerotium-like structures. We inferred patterns of evolution in the genus Aspergillus using DNA sequences from the internal transcribed spacers (ITSs) of the nuclear ribosomal repeat, the mitochondrial small ribosomal subunit (mtssu), and the 5 non-translated (5 NT) region of the trpC gene. The trpC 5 NTS was highly variable in comparison to both the ITSs and the mtssu. The overall phylogenetic patterns suggest as least four independent losses of ascospore production in the genus, leading to taxa that do not produce ascospores in culture, but retain apparent vestiges of ascospore production. These findings are consistent with a model where strictly mitotic taxa arise frequently in Aspergillus, but do not form stable long-term evolutionary lineages.

10. Control of DNA replication and mitotic checkpoint function by the Aspergillus nidulans nimO gene

Steven W. James, Gettysburg College.

We are investigating the role of the Aspergillus nimO gene in DNA replication and mitotic checkpoint function. Flow cytometric and cytological analyses showed that when conidia of a temperature sensitive lethal nimO18 mutant were germinated at the restrictive temperature (44 C), they were unable to replicate their DNA, but 35-40% of nuclei nevertheless progressed into mitosis and arrested with condensed chromatin. These results identify nimO as a candidate regulator of G1/S or S phase, and further suggest that this gene influences the checkpoint that prevents mitosis (M) from occurring until DNA synthesis has been completed. Sequencing of a nimO cDNA revealed a predicted protein of 72.8 kd that shares 29% identity with DBF4, a budding yeast G1/S regulator which associates with origins of replication and with the CDC7 kinase in order to initiate DNA

synthesis. nimO and DBF4 are especially strongly conserved in a C-terminal domain containing a single, novel motif which bears strong resemblance to Cys2-His2 zinc fingers. A deletion of this 60 bp motif from a nimO gene fused with the inducible alcA gene promoter rescued the ts-lethality of nimO18 for growth, but not conidiation, when high-level expression was induced by ethanol. On glycerol, which permits low basal expression, the strains grew poorly and were likewise unable to conidiate. Thus, the putative zinc finger appears non-essential for mycelial growth when the variant gene is highly expressed, but may be necessary for the accelerated cell cycling that must occur during conidiation. We are continuing to examine the function of this motif and to test nimO function through gene disruption.

11. Construction and characterization of brlA(p):alcA gene fusions in Aspergillus nidulans

James E. Jurgenson, Jason Abbas, and Jeff S. Pallas, University of Northern Iowa, Cedar Falls, Iowa 50614.

In order to study the temporal and developmental regulation of the bristle A gene in Aspergillus nidulans we have constructed several gene fusions to the bristle A promotor. We have decided to use the expression of alcA as a negative selection marker allowing aconidial mutants containing this construct to survive on media containing allyl alcohol. This approach can be used to identify genes which code for bristle promotor specific trans acting transcriptional factors. In addition we have constructed promotor fusion to the argB gene and to lacZ. BrlA(p)::argB gene fusions in argB null strains will allow positive selection of bristle regulators. We are using the brlA(p)::lacz fusion as a reporter of normal developmental expression of the brlA gene. All of these constructs contain the 4 kb of the A. nidulans genome containing the brlA promotor sequences. The fusions have been constructed using PCR amplification of gene and promotor segments and overlapping PCR to construct the gene fusions so that the initiation codon of the reporter/selectable marker exactly replaces the coding sequence of the brlA structural gene. Results of these experiments and expression of the gene fusions in transgenic strains of A. nidulans were presented.

12. Multiallelic recognition: nonself-dependent dimerization of the bE and bW homeodomain proteins in Ustilago maydis

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In the plant pathogenic fungus Ustilago maydis sexual and pathogenic development are controlled by the multiallelic b mating type locus. The b locus encodes a pair of unrelated homeodomain proteins termed bE and bW. Only combinations of bE and bW of different allelic origin are active. Allelic differences are clustered in the N- terminal domains of bE and bW. To investigate the underlying molecular mechanism for this intracellular self-nonself recognition phenomenon, the Saccharomyces cerevisiae two hybrid system was used. The results demonstrate that the bE and bW polypeptides dimerize only if they are derived from different alleles. Dimerization occurs just through the N-terminal variable domains and does not involve the homeodomain motifs. The non- self dependent dimerization of bE and bW was confirmed with a biochemical interaction assay. Different point mutants of bE2 were isolated that lead to function in combination with

bW2 in Ustilago maydis. Such combinations of polypeptides were also able to form heterodimers in the two hybrid system. We suggest a model for self-nonself recognition in which variable cohesive contacts direct dimerization.

13. Molecular characterization of the A1 mating type factor of Coprinus bilanatus

U. Kües, Institut für Mikrobiologie, ETH Zürich, M.P. Challen, Horticulture Research International, Wellesbourne

The A factor of Coprinus cinereus is one of two multiallelic mating type loci which determine compatibility between monokaryons and regulate the development of a dikaryon. The A factor consists of two subloci, a and b, each of which contain two classes of genes encoding homeodomain transcription factors, HD1 and HD2. The products of compatible A factors are brought together by mating. The active interactions are formed between compatible HD1 and HD2 protein pairs; combinations from the same locus are nonfunctional (Kües et al., MGG 245:45, 1994). Heterologous expression experiments gave evidence for a related A factor system in Coprinus bilanatus. HD2 products of C. cinereus were shown to be functional in C. bilanatus and seem to interact with native HD1 proteins. However, HD1 genes of C. cinereus appeared to be inactive in C. bilanatus hosts (Challen et al., MGG 241:474, 1993). Molecular cloning of the C. bilanatus A factor revealed two closely linked subloci, a metalloendopeptidase (mep) gene and a pab1 within 40 kb. This arrangement is similar to that found in C. cinereus. Activity of the cloned C. bilanatus A1 factor was demonstrated through transformation of C. bilanatus A2 and A3 hosts and several different C. cinereus strains. Heterologous transformations using individual C. bilanatus A genes are thus far consistent with our earlier observations. C. bilanatus HD2 genes appear to be inactive in C. cinereus whereas HD1 genes seem to interact with native HD2 products to elicit clamp cell development. It is hoped that further studies will lead to a greater understanding of these protein-protein interactions. Work was supported by the Violette and Samuel Glasstone Foundation, University of Oxford.

14. Evolution of A factor specificity genes in Coprinus cinereus

U. Kües, Institut für Mikrobiologie, ETH Zürich

The A mating type factor of C. cinereus is a multiallelic locus which allows mating monokaryons to recognise self from nonself. To be compatible, cells must have different A factor specificities. These specificities are determined by two types of genes encoding proteins with distinct homeodomain DNA binding motifs, HD1 and HD2. These genes are transcribed in pairs and a single A factor may have up to four functionally redundant gene pairs (a, b, c and d) (1). Genes within the same A factor, as well as gene alleles in different A factors, have a low overall homology. Different A factors can share alleles. A6 and A42 both have a copy of the HD1 gene d1-1 and gain different specifities through their b pair alleles (2). Gene and allele specificities result from sequence divergences in their 5' ends (3) and their evolution from a common origin is a fascinating problem. Although functionally identical, the d1-1 copies in A6 and A42 are distinguished by restriction polymorphism. Sequence comparison of the 5' sequences of d1-1 of A6 and A42 (3) revealed base pair insertions and deletions and a number of base differences, several leading to amino acid changes in the gene products. These changes are nonrandom. The DNA binding motif is conserved and in the N-terminal specificity

region there are three amino acid exchanges which do not interfere with protein specificity. The positions of these exchanges are especially interesting if the N-terminal region form coiled coil dimerization motifs similar to those found in the yeast mating type protein a2 and if specific amino acid positions in the coils influence dimerization affinities with compatible and incompatible HD2 proteins. Supported by the BSERC to work with Dr. L.A. Casselton and by the Violette and Samuel Glasstone Foundation, University of Oxford. (1) Kües and Casselton, J. Cell Sci. 104:227, 1993; (2) Kües et al., EMBO J. 13:4054, 1994; (3) Kües et al., Plant Cell 6:1467, 1994.

15. Molecular analysis of a sexual development gene of Neurospora crassa

Sandra T. Merino and Mary Anne Nelson, University of New Mexico

The sexual development process in Neurospora crassa is quite complex and requires the concerted effort of many genes. Previously, subtractive hybridization was used to isolate genes expressed during sexual development, called sdv for sexual development genes. A reverse-genetic approach is being used to determine the functions of the sdv genes. In this study, the RIP (repeat-induced point mutation) process has been used to create mutations in the sdv-15 gene. RIP-mediated disruption of the sdv-15 gene resulted in a mutation causing a recessive sexual defect; the gene was renamed asd-3, for ascus development, to denote the function of the gene product. In asd-3 x asd-3 crosses, early sexual development is normal and many asci are formed, but ascospores are never delineated. Confocal microscopic analysis has been used to demonstrate that the morphology of asd-3 asci is grossly aberrant. The lengths of the asci are comparable to those of wild type asci, but the width and shape vary widely. Spore formation in homozygous asd-3 crosses is defective, with asci containing few or typically no spores. Ascospores appear immature in comparison to wild type, and no spores are shot from the fruiting body. Preliminary sequence analysis indicates that the asd-3 gene product is a sugar-transporter. Our studies indicate that the asd-3 gene product is essential for normal sexual development in the fungus Neurospora crassa.

16. STUA-directed gene expression during Aspergillus nidulans development

James R. Dutton and Bruce L. Miller, University of Idaho.

Conidiophore development in Aspergillus nidulans requires a tightly regulated sequence of balanced, interacting gene expressions that direct correct spatiotemporal cell differentiation. The stunted gene (stuA) is a key morphological modifier in this process, mutations in which result in shortened conidiophores lacking intermediate cell types. stuA encodes a putative transcription factor with a DNA binding site that shows strong homology to those of Swi4, Mbp1 and Phd1 from S. cerevisiae and Cdc10, Sct1 and Pst1 from S. pombe. Swi4 and Mbp1 are transcription factors responsible, in association with Swi6, for periodic activation of transcription during the S. cerevisiae cell-cycle. Phd1 enhances pseudohyphal growth of diploid S. cerevisiae. Cdc10, in association with Sct1, is required for the initiation of DNA synthesis in S. pombe. An initial search for genes under StuA control employing a yeast one-hybrid system indicated that StuA is competent to direct gene expression through upstream activating regions containing either MCB or SCB cell-cycle boxes. These hexamer motifs are recognised in S. cerevisiae by Mbp1/Swi6 and Swi4/Swi6 respectively and are important in gene regulation throughout the S. cerevisiae cell cycle. We investigate the role of similar

sequences in StuA-controlled gene expression in Aspergillus nidulans and discuss their implication in both directing differentiation and cell-cycle control during conidiophore development.

17. The Aspergillus nidulans modifier gene medusa encodes a protein that can form dimers with the transcriptional factor bristle

Karen Y. Miller and Bruce L. Miller, University of Idaho.

One of the key steps during development in Aspergillus nidulans is the transition from multinucleate filamentous growth to uninucleate budding. Null mutations in medusa (medA) and abacus (abaA) genes and hypomorphic bristle (brlA) mutations cause branching chains of reiterated uninucleate cells (metulae) and delay or prevent conidiation. Earlier work suggested that MEDAp is required for normal patterns of brlA expression and may directly or indirectly co-activate abaA expression. An extra copy of brlA suppresses medA mutations restoring both normal conidiophore morphology and abaA expression. The degree of the suppression is allele and temperature dependent. These results suggested a possible protein-protein interaction between BRLAp and MEDAp. We have constructed a GST-BRLA protein fusion that is expressed in E. coli. When this fusion protein is bound to a glutathione column, it specifically binds in-vitro made MEDAp. We have made a series of carboxy terminal deletions of MEDAp. Preliminary results have delineated a ninety amino acid region necessary for the BRLAp-MEDAp interaction.

18. Characterization of genes controlling sexual development in Neurospora crassa, including gene identities and the control of their expression

Mary Anne Nelson, Sandra T. Merino, David Parks and Sheila Luna, University of New Mexico

Twenty genes expressed specifically or preferentially during sexual development in N. crassa are being characterized. (1) The control of expression of these sexual development (sdv) genes is being examined. All sdv genes are expressed in response to nitrogen starvation, and some require the A and a mating type products for their expression. Other sdv genes are expressed only if the white collar (wc) products, in addition to the mating type products, are also present. (2) In previous work, we showed that the N. crassa asd-1 product is required for sexual development; in its absence, aberrant asci lacking ascospores are formed. Sequence analysis has shown that this gene encodes a product closely related to a rhamnogalacturonase of Aspergillus aculeatus. This protein is abundant in young but not more mature asci. (3) The car1 gene of Podospora anserina encodes a product required for karyogamy; in the absence of this product, many croziers but no asci are formed. The Podospora car1 gene, isolated by Veronique Berteaux-Lecellier and Marguerite Picard (University of Paris) was used to isolate the homologous N. crassa gene. These car1 genes, which share extensive homology, encode a protein similar to the human PAF1, or peroxisome assembly factor (an integral membrane protein required for the import of many peroxisomal proteins).

19. Phenotypic analysis and cloning strategy for rad12, a gene required for meiosis and DNA repair in Coprinus cinereus

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Our lab studies meiosis and DNA repair in Coprinus cinereus, an organism in which meiosis is synchronous. rad12 is required for both processes. We have undertaken a series of experiments to characterize the phenotype of three different rad12 mutant alleles, rad12-1, rad12-4, and rad12-15. Quantitative measurements of oidial survival indicate that all three rad12 alleles are sensitive to gamma irradiation but insensitive to UV irradiation relative to the wildtype. Recombination within the tract of the ribosomal RNA gene repeats was not significantly different in the mutants when compared to the wildtype. The progression of rad12 mutants through meiosis was monitored by examining surface spreads at one, six, and ten hours after karyogamy. All three rad mutants showed defects in chromosome synapsis. While attempts to clone the rad12 gene from a wildtype cosmid library using DNA-mediated transformation have not proven to be successful, this strategy did provide us with a tag which allowed us to jump to the region of the rad12 locus. We have been walking from this tag towards the gene, transforming clones from each step of the walk as well as monitoring the walk genetically, using RFLPs. We have defined a 15 kb region which complements the repair phenotype in the mutant. Currently, we are examining the expression patterns of transcripts produced in this region, in order to determine whether one of them could be a likely candidate for the rad12 gene.

20. Meiotic defects in the rad9-1 mutant of Coprinus cinereus

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We have isolated 38 radiation-sensitive mutants of the basidiomycete Coprinus cinereus, which define at least 10 complementation groups for gamma survival. Four complementation groups, rad3, rad9, rad11 and rad12, represent genes required both for survival of gamma radiation and for meiosis. Mutants in each of these four groups arrest during meiosis and produce mushrooms with greatly reduced numbers of viable spores. Genetic pathway analysis has shown that these four genes are in the same pathway for the repair of gamma ray damage. Using a chromosome-specific cosmid library, we have cloned one of these four genes, rad9. This 8 kb gene encodes a transcript of about 6.8 kb, which is induced during meiosis and after gamma irradiation. A closely linked, coordinately expressed gene encodes an RNA helicase of the DEAH box family. Silver staining of surface spreads of rad9-1 strains indicates that its chromatin partially condenses and pairs. Time course studies of chromosome behavior during meiosis in wild-type and rad9-1 strains have shown that the rad9-1 mutant is defective from early stages of meiosis. Although the rad9-1 strain undergoes karyogamy at the normal time, differences between mutant and wild-type cells are apparent immediately after karyogamy. Comparisons between rad9-1 and wild-type strains indicate that telomere condensation and pairing may occur in rad9-1, but that further condensation, pairing, and synapsis of the chromatin is inhibited. At 12 hours after karyogamy, when wild-type strains have completed both meiotic divisions, rad9-1 chromatin shows evidence of degradation.

21. Dimerisation is the key to self non-self recognition between A mating type proteins of Coprinus cinereus

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The A mating type genes of the basidiomycete Coprinus regulate part of the developmental programme that coverts the asexual monokaryon into a fertile dikaryon. The A locus is complex and contains several paralogous pairs of genes that encode two classes of homeodomain containing proteins that we term HD1 and HD2. A compatible combination that triggers A- regulated development is an HD1 gene from one mate and an HD2 from the other. We have asked how the cell distinguishes between several HD1 and HD2 proteins that are present in unmated cells and the compatible combinations that are brought together by mating. We will describe our in vivo and in vitro experiments which identify an N-terminal domain in both classes of proteins that only permits heterodimerization between compatible proteins. We will also describe experiments that identify other functional domains of the A proteins.

22. The first non-mammalian homologue of the PAFl gene (Zellweger syndrome) discovered as a gene involved in caryogamy in the fungus Podospora anserina

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The fusion of haploid nuclei (caryogamy) is a vital part of the sexual cycle. To date, in fungi, this process has been well studied only in the yeast S. cerevisiae (1). However, caryogamy in filamentous ascomycetes is more complex than in unicellular yeasts. In particular, it does not occur immnediately after fertilization but it is a deferred stage occuring in specialized cells. The car1 mutants of P. anserina, completely defective for caryogamy, have been isolated during a systematic search for sporulation deficient mutants (2). The car1 gene was cloned by complementation. Surprisingly, this gene encodes a protein that shows similarity to the mammalian PAF1 protein (Zellweger syndrome, 3). Altogether, the molecular, physiological (inability for the car1 mutants to grow on oleic acid), genetical (revertants analysis) and ultrastuctural approaches (immunofluorescence and electron microscopical analyses) gave evidence that the P. anserina car1 protein is actually a peroxisomal protein. Therefore, this study shows that peroxisomes are required at a specific stage of sexual development, at least in P. anserina, and that a functional homologue of the PAF1 gene is present in a lower eucaryote. The data will be discussed in terms of the possible functional role of peroxisomes in the caryogamy process or, more likely, in the cell determination or differentiation programs.
(1) M. D. Rose (1991) Annu. Rev. Microbiol. 45:539-567. (2) J. M. Simonet & D. Zickler (1978) Mol. Gen. Genet. 162:237- 242. (3) N. Shimozawa, T. Tsukamoto, Y. Suzuky, T. Orii, Y. Shirayoshi, T. Mori and Y. Fujiki (1992). Science 255:1132-1134.

23. Mating type genes in Podospora anserina are required for sorting mat+ and mat- nuclei from syncytial cells into the ascogenous hyphae

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In the heterothallic ascomycete Podospora anserina, fertilization is followed by a series of mitotic divisions of the parental nuclei leading to a syncytium, from which one mat+ and one mat nucleus migrate into the ascogenous hyphae. The four mating type genes, FPR1 in the mat+ allele, FMR1, SMR1, and SMR2 in the mat allele, are required to match and

sort mat+ and mat nuclei from the syncytium, whereas the N-terminal parts of FPR1 and FMR1 are sufficient for fertilization (1,2). We have examined how the four mat genes control the sorting of mat+ and mat nuclei by: i) analyzing the sexual behavior of strains mutated in FPR1, FMR1, SMR1 or SMR2 (3). ii) testing mat interactions through the analysis of the sexual behavior of a mat+ strain containing the mat genes. SMR1 information is expressed in a mat+ context whereas FMR1 and SMR2-controled information are either inactivated or nucleus-specific. iii) testing mat protein interactions with the yeast two hybrid system. An interaction between FMR1 and SMR2, and a dimerization of FPR1, have been shown, but no interaction between FPR1 and mat proteins can be detected with this method. These data have been used to propose that FPR1 and FMR1-SMR2 inhibit the development of uniparental mat+ and mat ascogenous hyphae respectively. SMR1 would be required for sorting mat+ and mat nuclei. (1) R. Debuchy and E. Coppin 1992 Mol Gen Genet. 233:113-121. (2) R. Debuchy, S. Arnaise and G. Lecellier 1993 Mol Gen Genet. 241:667-673. (3) D. Zickler, S. Arnaise, E. Coppin, R. Debuchy and M. Picard 1995 Genetics, in press.

24. Genetic control of sexual compatibility in Tapesia yallundae

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Different pathotypes of Tapesia yallundae (anamorph Pseudocercosporella herpotrichoides) may be distinguished on the basis of host range on cereal crops. These include the W-, C-, S- and R-types. Sexual compatibility in the W-type is controlled by a two-allele heterothallic mating system and in vitro mating tests have shown a similar mechanism in the C- and S-types. Furthermore, the C- and S-types are sexually compatible with the W-type. Recombination between isolates was demonstrated using randomly amplified polymorphic DNA (RAPD) markers. However, attempts to induce crossing between the R-type and other pathotypes were unsuccessful, indicating that the R-type may be considered as a separate species. Work is in progress to clone the mating-type genes (MAT1-1, MAT1-2) from T. yallundae to study possible variations between pathotypes and how this may effect sexual compatibility. Approaches being used include: (1) use of degenerate PCR primers designed to amplify a partially conserved region of the MAT1-2 locus from other Ascomycete species (N. crassa, P. anserina, C. heterostrophus, S. cerevisiae); (2) oligonucleotide probing using a family of probes from conserved regions of MAT loci from other Ascomycete species; (3) heterologous probing using MAT genes cloned from other Ascomycete species.

25. The mushroom-inducing gene Frt1 of Schizophyllum commune encodes a putative ATP-binding protein

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Fruiting bodies (mushrooms) can be induced to form in normally non fruiting strains of the Basidiomycete fungus Schizophyllum commune by the ectopic genomic integration of a cloned gene called Frt1. The Frt1 gene encodes a predicted polypeptide of 192 amino acids that does not have significant similarity to any entries in the protein sequence databases. A conserved sequence found in nucleotide-binding proteins, called the P-loop motif, has been detected in the N-terrninal half of the predicted FRT1 protein. A potential site for Mg2+-binding was predicted to reside next to the P-loop at Thr24. No motifs

specific to GTP-binding proteins were detected in the FRT1 protein, but a potential site for ATP-binding is predicted to reside in the C-terminal half of the polypeptide. The possible functional significance of these and other regions within the FRT1 protein were examined using site- directed mutagenesis, followed by transformation of these mutant alleles of Frt1 back into S. commune. Mutation of the middle glycine of the P-loop completely abolished the fruit-inducing activity of cloned Frt1. Mutant alleles containing an alanine residue substituted for Thr24 had no fruit-inducing activity. Experiments are in progress to test the in vivo effect of mutations within the putative ATP-binding region. Taken together, the results of our mutagenesis experiments suggest the possibility that activity of the FRT1 protein could be altered by nucleotide- binding and coordination of Mg2+.

26. Distribution of Gibberella fujikuroi by biological species, mating type, and female fertility in a Malaysian maize field

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Samples of Gibberella fujikuroi (Fusarium, section Liseola) were collected along a transect through a corn field in northern Malaysia. These strains were characterized for mating population, mating type within population (where possible), and female fertility. The preponderance of the sample was found to be in mating population A. Of these, the majority (approximately 70%) were of a single mating type (A+). Distribution of both mating type and species was significantly non-random, implying a highly patchy and perhaps clonal pattern of dispersal in the field. The relative effects of distribution, mating type disequilibrium, and female sterility on the effective size of these populations were discussed.

27. Motherhood and the price of sex

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Natural populations of heterothallic biological species (= mating populations) in the Fusarium moniliforme species complex (sexual stage Gibberella fujikuroi) consist of males and hermaphrodites. The ratio of males to hermaphrodites varies with the biological species. In the "A" mating population the male:hermaphrodite ratio is 50:50, in the "D" population it is 70:30, and in the "F" population it is 90:10. If P(h) is the proportion of hermaphrodites in the population, then a stable equilibrium can be found for any P(h) that is dependent on the relative fitness of females and males (q), where q=(l-P(h))/(l+P(h)). Hermaphrodites always make a larger contribution to the next generation because they contribute all of the female gametes and some of the male gametes. At equilibrium, hermaphroditism costs l-q, otherwise the equilibrium quickly shifts to a population that is all hermaphrodites. In the "A" mating population q=0.33, in the "D" mating population q=0.54, and in the "F" mating population q=0.81. This model also suggests that mating type should be independent of genes for female sterility, and the available data from these species are consistent with this conclusion. The effective population number appears to be much more limited by the availability of hermaphrodites

than it is by imbalances in mating type frequencies.

28. Reciprocal heterologous expression of MAT genes from Cochliobolus heterostrophus and its asexual relative Bipolaris sacchari

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To investigate the cause of asexuality in fungi, we have chosen a pair of closely related species, one sexual (C. heterostrophus) and one asexual (B. sacchari). A key experimental strategy available to address the question of asexuality in B. sacchari is reciprocal heterologous expression. B. sacchari genes can be expressed in C. heterostrophus to ask if they are functional in a strain with a complete mating pathway. The counterpart is to express C. heterostrophus genes in B. sacchari to ask if B. sacchari can mate if it carries 'good' mating type genes. We have already determined that B. sacchari has a cruclal piece of the mating pathway ie., the MAT locus itself. The B. sacchari MAT-2 homolog is 98% similar to C. heterostrophus MAT-2 and is functional when transformed into C. heterostrophus. Yet transfer of a C. heterostrophus gene of opposite mating type into B. sacchari does not make the latter sexual. No B. sacchari MAT transcript can be detected in B. sacchari. When B. sacchari expresses a C. heterostrophus MAT gene, transcription is no longer tightly regulated, as it is in C. heterostrophus. The B. sacchari transgenic strains do not mate with B. sacchari; with C. heterostrophus they sometimes initiate, but don't complete, sexual development. Both improper MAT regulation and missing or defective mating-specific genes could explain asexuality in B. sacchari.

29. HSP90 and HSP70 are present in a heteromeric complex in the steroid hormone responsive oomycete Achlya ambisexualis

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In vertebrates HSP90 is reported to exist in heteromeric complexes with a number of cellular proteins, including kinases, transcription factors and steroid hormone receptors, as well as other heat shock proteins (HSP70, HSP56). In the oomycete Achlya, steroid hormones regulate sexual development, The response to hormone is thought to be mediated by a steroid hormone receptor. Immunoprecipitation of either cellular or in vitro translated proteins with monoclonal antibodies to HSP90 from Achlya (AC88), mouse (8D3) or rat (2D12), showed that HSP90 (85kD) in Achlya is found in a heteromeric complex with proteins of 110kD, 74kD, 64kD, 61kD, 56kD, 47kD and 23kD. Proteins of similar molecular weight are observed in vertebrate steroid receptor heterocomplexes. Western analyses, using antibody AC88 (antibody to HSP90) and BRM-22, an antibody to HSP70, showed that the 85kD and 74kD proteins found in the heteromeric complex, were HSP90 and HSP70 respectively. Our results suggest that the Achlya steroid receptor is likely found in a heterogeric complex with HSP90, HSP70, and with other proteins with sizes similar to the proteins reported to be complexed with animal steroid receptors. (Supported by NSERC Canada)

30. Genes involved in vegetative incompatibility reaction in Podospora anserina

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The three genes, het-c, het-e and het-d, involved in the two nonallelic. incompatibility het-c/het-e and het-c/het-d systems have been cloned. HET-C protein is similar to a glycolipid transfer protein. A strain containing a disrupted het-c locus is affected in ascospores production. A het-e gene encodes a 1356-amino acid protein which exhibits two domains. In the N-part of the protein, there is a P-loop motif characteristic of GTP binding protein. In the C-part of the protein, there are ten direct repeats of a motif of 42 amino aids which are characteristic of beta- transducin like protein. Mutation in the P-loop motif results in a loss of the incompatibility phenotype. It has been shown in P. anserina that mutation in some genes named mod genes results in the suppression of a barrage reaction. Moreover, resulting strains have defects in secondary ramification and reproductive female organ production. On the basis of the wild type phenotype restoration of a modD mutant strain, a gene has been cloned. It encodes a polypeplide which has homology with the N. crassa adenylate cyclase. But, by RFLP, the modD locus and the adenylate cyclase locus are distinct. Transcriptional and/or translational regulation of genetic expression and enhancement of the proteolytic activity had been shown in cells undergoing cell death associated to incompatibility. Two genes under transcriptional regulation have been cloned. Study of their expression is in progress. The cloned gene papA, encoding an aspartylprotease, has been disrupted.

31. Comparison of pheromone receptor genes of Schizophyllum commune

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The mating type locus B(alpha)1 of S. commune contains a pheromone receptor and putative pheromone genes. The pheromone receptor Bar1 shows homology to other pheromone receptors such as Ste2 and Ste3 of Saccharomyces cerevisiae and pral and pra2 of Ustilago maydis. It also contains sites conserved among other G protein-linked receptors of the seven transmembrane domain family. The fact, that the mating system in S. commune provides nine different allelic specificities at the mating type locus Ba makes it a model system to investigate ligand interaction between different allelic specificities of pheromones and receptors. For S. commune multiple isolates for each mating specificity exist. In order to obtain evidence for functional domains, different alleles for the BAR1 gene will be compared. Southern hybridization experiments could show that strains sharing the specificity B(alpha)1 exhibit strong hybridization signals. This indicates higher homology of the alleles of BAR1, all sharing the mating specificity B(alpha)1, as compared to strains with other mating specificities, which also hybridize, but to a lesser extent.

32. Complementation of Cochliobolus heterostrophus mating type deletions with homologous and heterologous MAT genes

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In C. heterostrophus, mating is controlled by a single locus (MAT) with dissimilar, alternate forms. Partial diploids carrying both a resident MAT gene and a MAT transgene can self and are dual maters. Selfs form perithecia but very few asci and ascospores. Crosses are fertile when transgenic strains are mated with a strain of opposite mating type to the resident MAT gene of the transgenic strain, but fertility is low when mated with a

strain of opposite mating type to the transgene, suggesting that the resident MAT gene interferes with transgene function. Interference was eliminated by deleting the resident MAT gene by gene replacement. Deletion strains are completely sterile when crossed to tester strains. In a gain-of-function analysis, we determined the minimal requirement for full fertility by re-transforming deletion strains with various fragments of homologous MAT DNA. To investigate the functional conservation of MAT genes, we transformed deletion strains with MAT DNA from 4 sexual species (C. carbonum, C. victoriae, N. crassa and P. anserina) and one asexual species, Bipolaris sacchari. Genes originating from closely related species were able to completely restore the wild type phenotype whereas those from the more distantly related N. crassa and P. anserina resulted in only partial complementation. Strains carrying the latter produced low numbers of perithecia that were barren.

33. Viral repression of fungal sex pheremone expression. Implications for virus-host interactions

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We have found that a virus of the chestnut blight fungus, Cryphonectria parasitica, perturbs its host's sexual cycle by repressing transcription of mating type-specific genes. We have cloned mating-type specific genes from both mating types of the fungus, and have found that the virus represses their transcription. Two of the repressed genes encode a Mat1-1-specific pheromone required for completion of the sexual cycle. Vegetative compatibility (vc) genes function to prevent cytoplasmic exchange of viruses in this fungus, and represent the only known class of virus- resistance genes of the host. Viral suppression of mating prevents recombination of vc genes, which should result in fungal populations with fewer vc groups, and thus with fewer barriers to viral transmission. Studies of viral- infected and non infected populations of the fungus support the hypothesis that this mechanism of host mating suppression has evolved to enhance viral spread in host populations. These results are as predicted by the Red Queen hypothesis of the evolution of sex.

34. Sexual reproduction in ascomycetes: roles of the N. crassa mating type polypeptides

Melissa L. Philley, Adlane Ferreira, N. Louise Glass*, and Chuck Staben (University of Kentucky and University of British Columbia*)*

The N. crassa mating type idiomorphs encode polypeptides that play crucial roles in vegetative incompatibility, mating, and formation of ascogenous hyphae. RIP experiments also suggest a role for the mating type genes within the ascogenous hyphae. The sole product of the mt a idiomorph, MT a-1, is a sequence-specific DNA binding protein of the HMG box family. The presumed regulatory targets of MT a-1 include genes necessary for mating pheromone biosynthesis and response. MT a-l DNA binding activity is not necessary for vegetative incompatibility function, but it is necessary for mating functions. The mt A idiomorph encodes at least three products. One of these products, MT A-3, is also a member of the HMG box family. MT A-3 produced in E. coli binds DNA fragments that are also bound by MT a- 1. The interactions of the mating type polypeptides with DNA and with each other as well as the biochemical and biological

consequences of such interactions are under investigation

35. Cloning conidiation genes from Colletotrichum graninicola

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Colletotrichum graninicoa can produce two types of conidia; one type is falcate-shaped and the other is oval- shaped. The conidia are not only morphologically distinct but also differ in ontogeny. To better understand the differentiation and regulation of the development of the conidia, we constructed a cDNA library representing genes expressed during the development of oval conidia. Three cDNA clones, oval-5, oval-7 and oval-11 were recovered from the library by differential screening. The oval-5 clone hybridizes to a 2.2 kb transcript, oval-7 to a 1.8 kb transcript, and oval-11 to a 6.0 kb transcript. Oval-7 shares homology with the sequences of superoxide dismutase (SOD). The transcript of oval-7 starts early in development, increases at the stages when most oval conidia are produced and then decreased at later stages of development when levels of oval-5 and oval-11 are more abundant. The genes corresponding to oval 7 and oval-11 are developmentally regulated and their expression is associated with the production of both type of conidia. The transcript of oval-5 is also developmentally regulated but its expression seems to be exclusively associated with the development of oval conidia. Gene disruptions are in process to address the function(s) of the genes corresponding to these clones

36. Evolutionary analysis of the b1 gene within the A mating-type of Coprinus cinereus

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Self-incompatibility mechanisms prevent inbreeding in many organisms, including fungi. Although of great importance to the organism's evolution, little is known about the forces acting upon the genes controlling self- incompatibility. I am studying self-incompatibility in the basidiomycete Coprinus cinereus to better understand how self-incompatibility genes evolve. In this fungus, recognition of self versus non self occurs at the sub-cellular level to determine mating compatibility for sexual reproduction. This sub-cellular mechanism is governed by the mating type genes. I am studying the evolution of the b1 mating-type gene to better understand how this operates in C. cinereus. Because inbreeding (self mating) does not occur, a region of b1 must discriminate between self and non- self. As natural selection favors outcrossing (non-self mating), a large number of different b1 alleles exist in natural populations. My work examines how b1 has evolved at the molecular level and how these changes may provide for self-non self discrimination for self-incompatibility. This work provides a better understanding of how selection can produce extreme variation in b1, yet still maintain identical function for self incompatibility.

37. Genetic characterization of conidiation mutants of Colletotrichum graminicola

Juan Wang and Robert M. Hanau, Purdue University, West Lafayette.
Colletotrichun graminicola (Teleomorph: Glomerella graminicola) causes anthracnose leaf-blight and stalk rot of maize. Asexual reproduction by means of conidia serves as a major mechanism of dispersal. Therefore, the process of conidiation can be an important target for disease control. Three distinct conidiation phenotypes have been identified

among 10 field isolates under investigation. These phenotypes are: (i) light-dependent conidiation (wild- type), (ii) light independent conidiation (Con1-), and (iii) aconidial phenotype (Con2-). In the wild-type, growth in the dark results in the absence of conidia and a fluffy colony morphology whereas growth m the light results in abundant conidia and little aerial hyphae (i.e., lack of the fluffy morphology). The Con2 mutant produces very few conidia and has a fluffy colony morphology whether grown in the light or dark. The Con1 mutant does not have a fluffy colony morphology and produces conidia at comparable levels in the light and dark. Thus, reduced conidiation is always associated with abundant aerial hyphae and a fluffy colony morphology. This correlation was also observed in progeny from crosses between the wild-type and either of the mutants, indicating a genetic basis for linkage between the absence of conidia and fluffiness. Crosses of the wild-type with either mutant showed a 1:1 segregation of wild-type and the respective mutant phenotype. This indicates that the Con1 and Con2 mutants each differ from the wild-type at a single locus. Genetic analysis has shown that Con2 is linked to PYR1 which has previously been physically mapped to chromosome 1 (Rollins and Hanau, unpublished data). In order to clone the Con2 gene by chromosomal walking, tightly linked RAPD markers were identified by bulked-segregant analysis. Other strategies involving transformation mediated complementation are also being employed.

38. Complex control of the brlA developmental regulatory locus in Aspergillus nidulans

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brlA is a primary regulator of asexual development in the filamentous fungus, Aspergillus nidulans. Activation of brlA is necessary and sufficient for conidiophore development. It is known that brlA has two overlapping transcripts, designated brlA(alpha) and brlA(beta). From previous studies, we found that expression of brlA is complex in that activation of the two brlA transcripts is regulated at different levels. brlA(beta) is regulated both transcriptionally and translationally while brlA(alpha) is regulated transcriptionally. To better understand transcriptional controls for brlA(beta) regulation, we have made 5' deletions of the essential ~2 kb upstream control sequences of brlA(beta) transcript fused to E. coli lacZ reporter gene. The results from deletion experiments indicate that there are probably several cis-acting control sequences involved in the regulation of brlA(beta). We have also made various fragments of the 2 kb brlA(beta) control sequences fused to otherwise inactive amdS::lacZ fusion as a complementary approach to our deletion analysis. In this way we identified two ~600 bp fragments extending from -2034~ -1426, and -929~ -380 that confer activity on the inactive amdS promoter. We are in the process of more precisely defining these ~600 bp fragments to identify a minimal sequence that is required for developmental regulation in asexual development. In addition, we are investigating putative trans-acting factors which are involved in the activation of brlA(beta) using Saccharomyces cerevisiae as a heterologous expression system. Thus far we found that BrlA(alpha), AbaA and FlbD are likely involved in the activation of brlA(beta) transcription.

39. Cloning of the vegetative incompatibility locus het-C of Neurospora crassa

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Somatic incompatibility, manifest as regulation of heterokaryon formation, appears to be

a nearly universal feature among filamentous fungi. In Neurospora crassa at least eleven loci mediate heterokaryon formation. Stable heterokaryon formation between two strains is prevented when different alleles are found at one or more of the het loci. To better understand the molecular basis of this self/non-self recognition we have cloned one of these loci, het- C, by means of a chromosome walk from the nearby marker pyr-4. Six overlapping cosmids were isolated and their location on LGII and orientation centromere distal or proximal to pyr-4 was determined using RFLP analysis. Additional RFLP analysis indicated several of the cosmids extended beyond het-C. Partial duplication strains heterozygous at a het locus are phenotypically similar to incompatible heterokaryons. Based on this, we have used a transformation assay in which cosmids are introduced into strains carrying different alleles at het-C by DNA mediated transformation. Results of this analysis indicate that two of the six cosmids contain het-C. Further transformation with fragments of one of these cosmids shows het-C is located within a 10 kbp SphI-EcoRI fragment. Transformation of Tn-5 mutagenized versions of this cosmid indicate het-C lies at least partially within a 3.5 kbp BglII fragment.

40. Characterization of two incompatibility genes at het-6 in Neurospora crassa

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Het-6 is one of eleven heterokaryon incompatibility loci in Neurospora crassa. Het-6 was originally identified in strains having inhibited hyphal growth due to heterozygous partial duplications in the left arm of linkage group II. With near isogenic strains, we have confirmed that het-6 is, in fact, a heterokaryon incompatibility locus: different alleles at het-6 are sufficient to prevent heterokaryon formation under forcing conditions. Cosmids in the vicinity of het-6 were isolated by differential hybridization signals when CHEF separated chromosomes, with or without translocations containing het-6, were used to probe the Orbach/Sachs library. RFLP patterns in progeny with crossovers between het-6 flanking markers were used to locate cosmids with respect to het-6, and to determine the direction and progress of a chromosome walk past het-6. Crossover points were identified on either side of het-6, within a region spanned by two contiguous cosmids. Based on a transformation assay, one of these cosmids contains two, non-homologous regions of DNA, each of which confers incompatibility-like activity. One of these regions is located at, or near to, the RIP-mutant marker, un-24. The other is located about 14 kbp centromere proximal to un- 24. DNA fragments which confer het function have been isolated from both regions and DNA sequence analysis is in progress.

41. Identification of genes whose overexpression leads to growth inhibition in Aspergillus nidulans.

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Overexpression of Aspergillus nidulans developmental regulators like brlA, abaA, and flbA causes vegetative cells to stop growing and to differentiate into reduced conidiophores. We reasoned that additional developmental regulators could also be identified by a similar ability to inhibit vegetative growth and/or induce development when overexpressed. To test this possibility, we constructed an alcA(p)-inducible expression library by placing A. nidulans genomic DNA fragments under the control of

the alcA(p). We expect to identify mutants that fall into two phenotypic categories: FAB (Forced Activator of brlA) and FIG (Forced Inhibitor of Growth) which may include mutants overexpressing structural genes leading to growth cessation (Liu et al., 1992 Genetics 132:665). To test the utility of the library for identifying FAB and FIG genes, we screened 20,000 A. nidulans transformants for inhibited growth on alcA(p)-inducing media. To date we have identified 52 unique clones with a FIG phenotype and 16 with a FAB phenotype. We have recovered the transforming plasmids from two of the growth inhibited strains and eight of the brlA-inducing strains and have shown that when transformed back into a wild type A. nidulans strain they result in either growth inhibition or brlA-induction on alcA(p)-inducing media. We have designated the two growth inhibition genes recovered as figA and figB and the brlA inducing genes as fabA - fabP. Sequence analysis of figA shows it is predicted to encode a >950 codon open reading frame whose predicted amino acid sequence has similarities to several structural proteins including trichohyalin, collagen, and myosin. Sequence analysis of fabM shows it is predicted to encode a ~650 codon opening reading frame whose predicted amino acid sequence shares high homology to several poly-A binding proteins of yeasts and animals. We are creating a fabM deletion strain to deterrnine its involvement in development.

42. flbD encodes a Myb-like DNA binding protein that coordinates initiation of Aspergillus nidulans conidiophore development.

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The timing of asexual fruiting body formation during Aspergillus nidulans colony development is precisely regulated so that conidiophores are typically produced 1-2 mm behind the growing edge of the colony. Mutations in any of four A. nidulans genes flbB, flbC, flbD, or flbE result in colonies that are delayed at least 24 h in their ability to initiate conidiophore development resulting in fluffy colonies with conidiophores forming in the center at least 12-15 mm behind the growing edge. The requirement for each of these four genes in determining the timing of developmental initiation precedes transcriptional activation of the primary developmental regulatory gene brlA, indicating a possible role for each in developmentally regulated activation of brlA expression. The wild-type flbD gene was isolated and shown to encode an ~1.6 kb mRNA that is present throughout the A. nidulans life-cycle. The deduced FlbD protein sequence predicts a 308 amino acid polypeptide with significant identity at its N-terminus to the DNA binding domain of the Myb family of transcription factors indicating that FlbD likely functions as a sequence-specific transcriptional activator. Although conidiophore development does not normally occur in submerged culture, forced overexpression of flbD in submerged hyphae caused inappropriate activation of brlA expression and resulted in production of complex conidiophores that produced all of the distinct cell types observed in wild-type conidiophores including viable spores. This ability of flbD overexpression to activate conidiation requires brlA, flbB, and flbA (another early developmental regulator), but does not require flbC, flbE or fluG. We propose that FlbD functions during normal development by activating other genes (such as brlA) and that FlbD activity is normally controlled post-transcriptionally by an unknown mechanism.

43. Characterization of the control elements of brlA in Aspergillus nidulans.

Sangtae Han and Thomas H. Adams. Department of Biology. Texas A & M University.*

brlA is a primary regulator of asexual development in the filamentous fungus, Aspergillus nidulans. Activation of brlA is necessary and sufficient for conidiophore development. It is known that brlA has two overlapping transcripts, designated brlAa and brlAb. From previous studies, we found that expression of brlA is complex in that activation of the two brlA transcripts is regulated at different levels. brlAb is regulated both transcriptionally and translationally while brlAa is regulated transcriptionally. To better understand transcriptional controls for brlAb regulation, we have made 5' deletions of the essential ~2 kb upstream control sequences of brlAb transcript fused to E. coli lacZ reporter gene. The results from deletion experiments indicate that there are probably several cis-acting control sequences involved in the regulation of brlAb. We have also made various fragments of the 2 kb brlAb control sequences fused to otherwise inactive amdS::lacZ fusion as a complementary approach to our deletion analysis. In this way we identified two ~600 bp fragments extending from -2034~ -1426, and -929~ -380 that confer activity on the inactive amdS promoter. We are in the process of more precisely defining these ~600 bp fragments to identify a minimal sequence that is required for developmental regulation in asexual development. In addition, we are investigating putative trans-acting factors which are involved in the activation of brlAb using Saccharomyces cerevisiae as a heterologous expression system. Thus far we found that BrlAa, AbaA and FlbD are likely involved in the activation of brlAb transcription.

44. fluG and flbA induced conidiophore development in Aspergillus nidulans requires brlA

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We have been characterizing two early developmental regulatory genes called flbA and fluG. Mutations in these two genes result in a "fluffy-invasive" phenotype with colonies producing an abundance of aerial hyphae instead of undergoing conidiophore development. flbA mutant colonies have a distinct fluffy phenotype having an abundance of interwoven aerial hyphae that undergo autolysis during maturation. The predicted FlbA sequence has 30% identity to the Saccharomyces cerevisiae Sst2 protein which is required for regulating yeast mating pheromone adaptation response. fluG mutant colonies are aconidial under normal conditions, however, the mutant phenotype can be extracellularly complemented by growing next to wild type or other developmental mutants (e.g. brlA, flbA but not fluG). The predicted fluG product is a 97 Kd cytoplasmic protein and the C-terminal half of the sequence has significant similarity to prokaryotic glutamine synthetase (GSI). These results have lead us to suggest that FluG plays an enzymatic role in the production of an extracellular factor and that flbA regulates the response to this signal in controlling the initiation of A. nidulans conidiophore development. Overexpression of flbA or fluG in vegetative hyphal cells using an inducible promoter resulted in conidiophore development in the submerged culture indicating that flbA and fluG can function directly in activating development. In addition, overexpression of flbA or fluG also activates the expression of an essential developmental regulator called brlA. The brlA locus has two major transcripts called brlAalpha and brlAbeta, Han et. al. have proposed that brlAbeta plays the major role in the initiation of conidiphore development and brlAalpha is required for positive feedback in maintaining brlA activity during conidiophore development. Our results support this

model that sporulation induced by overexpression of fluG or flbA requires brlAbeta but not brlAalpha. The genetic interactions between fluG and flbA and other genes required for development are discussed.

Posters III: Gene Expression/Genome Structure

1. Molecular cloning of cDNA encoding Neurospora crassa wall-associated proteins.

Dorothy McColl and P.J. Vierula, Biology Department, Carleton University, Ottawa, Ontario, Canada. K1S 5B6.

A significant fraction of the cell walls of the filamentous fungus, Neurospora crassa is thought to be proteinaceous. To study this cell component, we have been analysing proteins which are found to co-purify with the cell walls. Using antisera raised against a crude cell wall preparation as a probe, several clones have been isolated from a cDNA expression library. A partial sequence of one of these clones has revealed homology to a thiamine- regulated protein in yeast. Results of the characterization of this gene were presented.

2. The Aspergillus nidulans catA locus encodes a developmentally regulated catalase.

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The Aspergillus nidulans CAN5 cDNA clone was isolated as a developmentally regulated transcript. Here we present the complete sequence of the corresponding gene. Comparison of this gene with sequence data bases shows significant identity to know catalases and has been denominated catA (developmental catalase). CHEF gel electrophoresis and hybridization of this gene with a chromosome-specific cosmid library showed that catA is in chromosome III. Northern Blot analysis indicates that catA is regulated during sporulation and is accumulated in spores. Catalase activity determination using native gel electrophoresis demonstrated that there are at least two catalases in A. nidulans; one catalase has low molecular weight and is induced during the first hours of development (0-25 h) whereas the other catalase (higher molecular weight) is accumulated preferentially in spores. Disruption of the catA gene abolished the major catalase activy present in spores without affecting the activity associated to growth.

3. Molecular and functional characterization of the specifically regulated aox gene of Penicillium chrysogenum.

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A specifically regulated gene, dependent on the physiological state and on external pH conditions, was cloned from Penicillium chrysogenum. A full length cDNA clone as well as the corresponding genomic clone of this gene were sequenced. The isolated gene shows strong homology to alcohol oxldases of methylotrophic yeasts at both the amino acid and nucleotide level. In order to investigate the function of this gene in Penicillium chysogenum the aox gene was overexpressed in Escherichia coli. However, no alcohol

oxidase activity could be measured with lysates of recombinant E. coli. Therefore the aox coding region was fused to the strong Aspergillus nidulans gpd promoter. Transformation of Penicillium chrysogenum with this construct resulted in strains that strongly overexpress the aox gene. We are currently analysing these transformants. In addition, studies to analyse thc promoter region and its regulatory signals are in progress.

4. Characterisation of creA alleles in Aspergillus nidulans.

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Carbon catabolite repression is a wide domain regulatory mechanism that, in the presence of readily metabolised carbon sources, acts to repress the expression of enzymes involved in the breakdown of alternative carbon sources. Genetical and biochemical analysis in Aspergillus nidulans has identified a number of genes involved in carbon catabolite repression. The dominance properties and the non hierarchical heterogeneity demonstrated by creA alleles suggest that this gene is a negatively acting repressor protein. The creA gene has been cloned. Theoretical translation of the DNA sequence shows the presence of a Cys2-His2 zinc finger DNA binding motif and an alanine rich region, both of which are consistent with the suggested function of CREA (Dowzer and Kelly, 1991). In addition, a comparison of the creA gene between A. nidulans and A. niger shows a small, highly acidic region and a sequence of 42 identical amino acids. This sequence shows a significant degree of similarity to a region found in RGRl - a protein involved in carbon catabolite repression in Saccharomyces cerevisiae (Drysdale et al. 1993). Using a PCR- SSCP approach, sequencing and phenotypic analysis we have characterised a number of creA alleles. The mutations fall into two broad groups. (1) Mutations disrupting the zinc finger binding domain; and (2) nonsense or frameshift mutations leading to truncation of the resulting protein. Some phenotypic characteristics are specific to the two classes of mutations. Dowzer, C. and Kelly, J. (1991) Mol. Cell. Biol. 11:5701-5709 Drysdale et al. (1993) Gene 130:241-245

5. Starvation stress and development in Aspergillus nidulans.

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Expression of the A. nidulans brlA gene plays a fundamental role in the switch from vegetative growth to asexual reproduction Using a media shifting protocol to induce submerged sporulation and brlA-lacZ as an expression marker, we have found that carbon and nitogen starvation stress induced brlA transcription to different degrees. Glucose starvation induced brlA rapidly to high levels and resulted in spore formation on reduced conidiophores, whereas nitrogen starvation induced brlA gradually to lower levels and sporulation occured to a lesser extent from more complex conidiophores. No clear qualitative differences between the two brlA transcripts were found in these starvation conditions, suggesting that the different patterns of sporulation could be explained by quantitative expression differences. Non-repressing carbon sources such as glycerol, acetate and arabinose were as effective as glucose in preventing brlA mRNA accumulation, suggesting that glucose effects on brlA expression could be explained as a response to nutrient starvation, rather than by carbon catabolite repression. When mycelia was not shifted to starvation conditions, sporulation was not observed in standard

minimal medium even after glucose was exhausted, unless medium was buffered. This and other results suggest that strong deviation from external neutral pH partally prevented brlA full induction and/or function.

6. Aflatoxin pathway gene cluster map in Aspergillus parasiticus and Aspergillus flavus.

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Aflatoxins are potent carcinogens produced by the fungi Aspergillus flavus and A. parasiticus. Aflatoxins are synthesized by condensation of acetate units; their biosynthesis is estimated to involve at least sixteen different enzymes. Genes encoding four of these enzymes including pksA, nor-1, ver-1, and omt-1, as well as a gene aflR, which regulates transcription of the pathway genes, have been cloned in our laboratories. We report here the organization of the aflatoxin pathway genes and a regulatory gene on the Aspergillus parasiticus and A. flavus chromosome. By determining overlapping mapped regions of the inserts in cosmid and lambda clones, the aflatoxin pathway genes were located and were shown to reside within a 60 kb DNA fragment in the order of pksA, nor-1, aflR, ver-1 and omt-1 in both A. parasiticus and A. flavus. This order is coincident with the order of the activities encoded by these genes in the biosynthetic pathway, with the exception of the regulatory gene, aflR, which is located between the nor-1 and ver-1 genes. Transcriptional mapping of the region between the ver-1 and the omt-1 gene revealed two additional transcripts which may be involved in the late steps of aflatoxin biosynthesis.

7. A putative recombination specific site in Penicillium chrysogenum.

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Four non-producer mutants of penicillin were analysed by Southern hybridization. All of them lack the entire penicillin biosynthetic gene cluster. By chromosome walking of the parental strains the borders of the deleted region were determined and sequenced. The sequence of the junction zone resulting from the deletion indicates that the deletion has occured at the same point in the four mutants, within the hexanucleotide TGTAAT. This short sequence also appears at the borders and the junction zone of an amplified genomic region containing the penicillin biosynthetic genes in overproducing strains. These findings support the theory that both, the deleted and the amplified region, have arised as a consequence of recombination processes induced by treatment with mutagenic agents (probably as a part of a repair mechanism for mutation damage) and that the sequence TGTAAT is a hot spot for recombination.

8. Promoter replacement studies of the cefG gene show that its expression is limiting for cephalosporin biosynthesis in Acremonium chrysogenum.

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The conversion of deacetylcephalosporin C to cephalosporin C is inefficient in most

Acremonium chrysogenum strains. The cefG gene, which encodes deacetylcephalosporin C acetyltransferase, is expressed very poorly in A. chrysogenum. Introduction of additional copies of the cefG gene with its native promoter did not produce a significant increase of the steady state level of the cefG transcript. Replacement of the native promoter by some strong promoters from other genes and other sources led to a very high steady state levels of cefG transcript and to increased deacetylcephalosporin C acetyltransferase activity in the transformants. Cephalosporin production was increased two- to three-fold in A. chrysogenum C10 transformed with constructions in which the cefG gene was expressed from some of these promoters due to a more efficient conversion of deacetylcephalosporin C to cephalosporin C, as shown by HPLC analysis of the intermediate and the final product. Promoter optimization studies may be, therefore, of great interest to increase antibiotic yields.

9. The recombinator cog in Neurospora crassa is within highly polymorphic DNA.

P. Jane Yeadon and David E. A. Catcheside, Flinders University, Adelaide, Australia.
The St Lawrence ST74-OR23-1VA and Lindegren Y8743 strains of Neurospora crassa are derived from different wild collections and have dissimilar cog alleles. The allele in Lindegren, cogLa (previously designated cog+), is a more efficient recombinator than cogS74A and cogEa (previously cog), respectively the alleles in St Lawrence and Emerson a. Restriction fragment length polymorphisms (RFLP) and sequence polymorphisms (SP) were used to map the difference between cogLa and cogS74A to a region that extends from 2.3 to 3.2 kb 3' of the his-3 coding sequence. The DNA sequence from 400 bp 3' of his-3 to 120 bp 3' of the cog-region in these strains was found to be homologous but to diverge by 3.5%. The differences include single base pair changes, short insertion/deletions, differences in the length of poly-T tracts and three longer sequences (101, 20, and 15 bp) present only in St Lawrence. Southern analysis of other laboratory strains revealed four major and several minor variants of this region. All strains examined were descendants of Lindegren A, Lindegren a, Abbott 4A and Abbott 12a and it is clear that each of these progenitors collected from the wild population had a different variant of the cog region. Sequence divergence of this degree seems remarkable, even for an intergenic region, for fully interfertile strains of a single species.

10. Genome organization and strain compatibility in Trichoderma harzianum.

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The soil fungus Trichoderma harzianum is a mycoparasitic fungus well known for its use as a biocontrol agent of phytopathogenic fungi. Among other factors, Trichoderma produces a series of antibiotics and secretes cell wall degrading enzymes. In an attempt to understand fungus-fungus interactions we are studying both the responses of Trichoderma harzianum to other fungi and to itself. Recent studies on the mechanism of action of Trichoderma spp. have led to the conclusions above mention, however, it is still unclear how does Trichoderma protect from its own lytic enzymes and antibiotics. And it is known that different species of the genus attack each other. First we established the electrophoretic karyotype of ten different T. harzianum isolates collected around the

world and localized a series of genes in the chromosomal bands. From these experiments five different karyotypes were clearly distinguishable. The localized genes always corresponded to the same band when the strains had the same karyotype. Further studies were made using RAPDs, from these data the use of five different oligonucleotides led to the same conclusions as the karyotype and gene assignment. The use of an extra set of three oligonucleotides allowed us to distinguish within isolates sharing karyotype. Confrontation experiments in different growth conditions led us to the conclusion that isolates with the same electrophoretic karyotype have compatible interactions and that those with different karyotype give in-compatible interactions. We conclude from these data, first that there is a high karyotype variation within the T. harzianum species group aggegate (Rifai), that there is a self respect signal encoded in its genome, and that the high variation in karyotype maybe due to mistaken classification because exactly the same karyotype is found in very distant places.

11. Cloning, characterization and expression of pepF and pepG, genes encoding serine carboxypeptidases from Aspergillus niger.

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Aspergillus species, and in particular A. niger, are increasingly used as hosts for the expression and secretion of large quantities of homologous and heterologous industrial enzymes. Yields of the target proteins vary considerably and one cause of low yields is recognized as being proteolytic degradation of the heterologous proteins by host proteases. The observed problems in heterologous expression in fungi combined with the industrial applications of proteases have increased the interest in fungal proteases. We have cloned a gene encoding a serine carboxypeptidase, pepF, from Aspergillus niger. The sequences were identified in a lambda genomic library using a synthetic oligodeoxyribonucleotic probe, based on the N-terminal sequence of PEPF. Nucleotide sequence data from pepF genomic and cDNA revealed that it is composed of four exons of 199, 283, 277 and 881 bp. Three introns which interrupt the coding sequence are resp. 53, 69 and 59 bp in length. The sequence of the pepF gene codes for a polypeptide of 530 amino acids. Directly downstream from the putative start codon lies a sequence that is removed by proteolytic cleavage at a monobasic site (Lys52). The cloning of another carboxypeptidase (CPDI) encoded by the pepG gene was also reported. Northern blot analysis of total cellular RNA extracted from A. niger cells indicate that the pepF gene is transcribed as a single 1.8-kb mRNA. In addition we show that the expression the pepF gene in A. niger N400 is regulated by a number of factors including nitrogen and carbon repression, specific induction and the pH of the culture medium.

12. Guest: a small inverted repeat transposable element in Neurospora crassa.

P. Jane Yeadon, Amanda H. Taylor and David E. A. Catcheside, Flinders University, Adelaide, Australia.

Amongst the sequence differences between the St Lawrence 74A and Lindegren strains is a 101 bp insert in St Lawrence 2.0 kb 3' of the histidine-3 locus in linkage group I. This insert is flanked by a 3 bp direct repeat of a sequence present in Lindegren, has terminal

inverted repeats (TIR) and shares features with several known transposable elements. At 98 bp, it may be the smallest transposable element yet found in eukaryotes. There are multiple copies of the TIR in the Neurospora genome, similar but not identical to the one sequenced. PCR amplification of Neurospora genomic DNA using 26 bp of the TIR as a single primer, gave products of discrete sizes ranging from 100 bp to about 1.3 kb, suggesting that the element isolated (Guest) may be a deletion derivative of a family of larger transposable elements. Two further iterations of Guest have been mapped in St Lawrence 74A, one to each of linkage groups III and V. Guest appears to be the second DNA intermediate transposable element reported for fungi and the first in Neurospora.

13. Novel method for differentiating populations and subpopulations of fungi.

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Often it is useful to be able to track populations of organisms, both for ecological examination of host/pathogen interactions as well as for other questions in population genetics. Other approaches to these questions have employed DNA fingerprinting techniques such as RFLP analysis, Southern hybridization, and RAPDs. These methods can be time-consuming, expensive and technically demanding. We have developed a method which is quicker, technically simple and inexpensive. The method utilizes analyses of total DNA thermal denaturation profiles. As such, it has the advantage of detecting and utilizing microheterogeneities throughout the test organisms' genomes. By modifying the parameters for analyses one can differentiate species, species hybrids, and even parental lineages. To date, this method has been used by us to distinguish Cuban crocodiles from Cuban/American hybrids and to correctly predict familial relationships for alligators and red-winged blackbirds. The anther smut, Ustilago violacea, infects flowering species in the Carnation family. It has been suggested that the 70-or-so host species define "races" of the fungus, i.e., populations or subpopulations of the fungus are delimited by which host species they are able to infect. Fourteen different sporidial isolates from a total of 5 different host species were analyzed by DNA thermal profiling. Analyses were performed using total genomic DNA, mitochondrial DNA, and purified PCR products from each strain. In many cases, the cladograms produced from such analyses reflected known population differences. This suggests that the method provides a quick and simple alernative for characterizing different populations of fungi.

14. Characterization of the arg-12 UORF reveals complex regulatory coordinaton with a gene upstream of the arg-12 UORF.

Gloria E. Turner and Richard L. Weiss. University of California, Los Angeles
Ornithine carbamoyltransferase is encoded by the arg-12 gene in Neurospora crassa. The gene has been sequenced and encodes a polypeptide of 363 amino acids that has extensive homology to the mammalian and fungal OCTase's. Primer extension analysis revealed multiple transcription starts between nucleotides -344 and -259 relative to the initiator methionine at nucleotide 1. On the arg-12 sense strand an UORF begins at nucleotide -1345 and ends at nucleotide -710; it has a putative 65bp intron. The antisense strand has an overlapping UORF that starts at nucleotide -710 and ends at nucleotide -1357; it has a putative 96bp intron. The putative introns are also overlapping. Developmental northerns probed with sense and antisense RNA from the UORF show opposite expession patterns. The antisense probe hybridized to 2 different transcripts

whose sizes are 3.6 kb and 3.0 kb. Expression of these transcripts is maximal at 8 hours. The sense probe hybridized to 3 transcripts, 3.6, 3.0 and 1.5 kb whose expression is maximal in conidia except for the 1.5 kb transcript whose expression is maximal at 8 hours; this expression, however, is much reduced relative to the antisense message levels for this time point and all time points examined. The larger transcripts are not detected at 8 hours by the sense probe. A 2.0 kb DNA fragment upstream of the UORF has been cloned. Developmental northerns and northerns using polyA RNA revealed hybridization to a 3.6 kb transcript. This mRNA is highly expressed in polyA RNA made from stationary phase mycelia. The identity of this gene and the role of the UORF in controlling expression of arg-12 and the unknown gene are being investigated.

15. A new locus for osmotic-sensitive mutants of Neurospora crassa.

Scott M. Buntin, Sara Neville Bennett, and Wayne A. Krissinger. Georgia Southern University, Statesboro, GA 30460.

SS-788, an osmotic-sensitive mutant of Neurospora crassa, was isolated following UV irradiation of wild type, 74A, conidia and a filtration-concentration procedure in minimal medium containing 3% NaCl. In contrast to typical osmotic mutants, the morphology of SS-788 resembled that of wild type. On medium containing 2% NaCl, hyphal elongation of SS-788 was reduced by about 80%, and there was no growth on medium containing 4% NaCl. Genetic analysis located the new os locus of SS-788 on LGVI, linked to ad-1 and trp-2. This is the first osmotic- sensitive mutant reported in this linkage group. It is proposed that the os locus in SS-788 be designated os-9.

16. Genetic and morphological analysis of KT-27, a new vinclozolin-resistant, osmotic-sensitive mutant of Neurospora crassa.

Regan M. Challinor, Wayne A. Krissinger, and Sara Neville Bennett. Georgia Southern University, Statesboro, GA 30460.

Several mutants which exhibited resistance to the dicarboximide fungicide, vinclozolin, were isolated. following UV irradiation of Neurospora crassa wild-type, 74A, conidia. These mutants were also found to be osmotic sensitive, consistent with previously reported work (M. Grindle. 1984. Trans. Br. Mycol. Soc. 82:635). Grindle also reported allelism of several of his vinclozolin-resistant mutants to the os-1 locus. Genetic analysis of our isolate, KT-27, also supports allelism to os-1. KT-27 exhibits altered hyphal morphology and pigmentation as compared to wild-type.

17. Isolation and characterization of UV-induced mutations affecting regulation of Neurospora arg-2.

Michael Freitag, Nelima Dighde and Matthew S. Sachs, Oregon Graduate Institute, Portland, Oregon

The N. crassa arg-2 gene encodes the small subunit of arginine-specific carbamoyl phosphate synthetase. Translational and transcriptional components are involved in negative regulation of arg-2 by arginine. A 24-codon upstream open reading frame (uORF) has been implicated in arginine-specific negative regulation. We transformed the N. crassa arg-12s pyr-3 strain with an arg-2 hph reporter gene containing the uORF. A transformant containing a single ectopically integrated copy of this reporter gene was able to grow on medium containing the antibiotic hygromycin B, but not when the

medium contained both hygromycin and arginine. Forty seven mutants were isolated following UV mutagenesis that were able to grow on media containing both hygromycin and arginine. One mutant whose phenotype genetically cosegregated with the arg-2 hph fusion gene and which showed constitutive expression of hygromycin phosphotransferase was characterized. The sequence of the fusion gene revealed a missense mutation at codon 12 of the arg-2 uORF predicted to change Asp to Asn. Other mutations altered the expression of both the fusion gene and the endogenous arg-2 gene, suggesting functional changes in one or more trans-acting factors that affect expression of both genes directly, or which have roles in determining intracellular levels of arginine.

18. Translational regulation of arg-2 and cpc-1 in response to changes in amino acid availability in Neurospora crassa.

Zongli Luo, Michael Freitag, and Matthew Sachs, Oregon Graduate Institute, Portland, Oregon

N. crassa arg-2 specifies the small subunit of arginine-specific carbamoyl phosphate synthetase, and it is the only component in the arginine biosynthetic pathway known to be negatively regulated by arginine. Moreover, arg-2 is positively regulated in response to limitation for many different amino acids through a mechanism known as cross-pathway control. Neurospora cpc-1 specifies a transcriptional activator important for cross-pathway control. Arg2p synthesis rates, Arg2p levels, arg-2 mRNA levels and the distribution of arg-2 mRNA in polysomes in wild-type N. crassa cells were examined under different conditions of amino acid availability. The amount of cpc-1 mRNA and its distribution in polysomes were also examined. Both translational and transcriptional components appeared to contribute to negative regulation of arg-2 in response to arginine and positive regulation of arg-2 and cpc-1 in response to amino acid limitation. The arg-2 and cpc-1 transcripts contain upstream open reading frames whose features suggest they have roles in translational control.

19. Demonstration of the inducible and constitutive forms of aryl hydrocarbon hydroxylase (cytochrome P450) in Neurospora crassa

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New therapeutic agents must be thoroughly investigated before approval is given for human consumption. One aspect of this research is to evaluate the manner in which drugs are metabolized oxidatively and whether the products are active. Traditionally, such information has been obtained from animal models. However, with growing concern over animal testing, acceptable alternatives are being explored. A logical approach is to utilize fungi containing cytochrome P450 enzyme systems, analogous to those found in human cells. One such microorganism is the filamentous fungus, Neurospora crassa, which we found to possess an inducible and constitutive form of the cytochrome P450 aryl hydrocarbon hydroxylase. This was demonstrated by the biosynthesis of the 3-hydroxy metabolite of Benzo(a)pyrene, in crude mycelial extracts. Enzymatic activity was determined fluorometrically in uninduced and induced 16- and 96-h mycelium. For induction, 1 mg/ml of Benzo(a)pyrene was added to the cultures at 24 h. Results of our experiments showed enzymatic activity in the uninduced 16-h cells as well as the 96-h induced mycelium. No activity was detected in 96-h uninduced cultures. N. crassa was

chosen as a model for studying oxidative biotransformation reactions because it is a genetically and biochemically well-characterized organism, and genomic and cDNA libraries are available. This will permit us to study the expression of genes encoding these enzymes, thereby facilitating direct comparisons with mammalian systems.

20. Identification of NIT4 binding sites within the nit-3 promoter.

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The expression of nit-3 and nit-6, structural genes in Neurospora crassa which encode nitrate and nitrite reductase, respectively, requires limitation of primary nitrogen sources and induction by means of nitrate. Transcriptional activation of these genes also requires the action of NIT2 and NIT4, positive regulatory proteins. NIT2 is a global acting regulatory protein, also needed for expression of a variety of other nitrogen metabolizing enzymes, while NIT4 is a pathway specific regulatory protein. NIT4 contains 1090 amino acids with a putative Cys6 zinc cluster DNA binding domain, similar to that of GAL4, found near the amino terminus. A NIT4/betaGAL fusion protein has been expressed and purified, and its DNA binding specificity determined. Two NIT4 binding sites of varying strengths in the nit-3 promoter were identified through mobility shift and DNA footprinting experiments. The stronger site contains the palindromic sequence TCCGCGGA, while the weaker site contains the related sequence TCCGTGGC. Related sequences are found in the nit-6 promoter. Further examination of this octomeric binding site and its flanking sequences is currently being done.

21. Developmental expression of Neurospora crassa HSP80.

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The heat shock response of Neurospora crassa, has been studied previously and the production of hsp70 and hsp80 transcripts, encoding two major heat shock proteins (HSPs), has been demonstrated to occur upon heat shock for 1 hour. With Northern blot analysis, we have demonstrated that not only is there an increase in the transcription of hsp70 and hsp80 under conditions of externally applied stress but an increase when the cells undergo an internal stress. Internal stress can be caused by active mycelial growth or by conidial germination. The expression of HSP80, under internal and external stress conditions was assessed by protein blot analysis using polyclonal anti-HSP80 IgG preparations and by monitoring hsp80-specific mRNA levels by Northern blot analysis. Sporulating 2-day and 4-day old cultures, subjected to heat shock, showed an increase in both the mRNA and protein levels compared to the same states that were not heat-shocked. However, the non-shocked 15-hour-old mycelial cells showed and elevated hsp80 mRNA and protein level over the heat-shocked 2 and 4-day cultures. Furthermore, there was an increase of hsp80 mRNA and protein levels in non-shocked germinating conidia. It is possible that HSP80 is required for the growth and germination, as a molecular chaperone to assist accurate protein folding and assembly.

22. The detection of a stress inducible DNA-binding protein in Neurospora crassa.

Patricia M.J. Ouimet and M. Kapoor, University of Calgary, Calgary, Alberta, Canada.

Heat shock protein (Hsp) gene promoters are typically silent until activated by various stresses especially high temperature. Induction depends upon a specific DNA sequence,

the heat-shock element (HSE). As an initial step to clarifying this activation mechanism, a Neurospora crassa factor which requires DNA binding activity upon stress induction has been identified. For our analysis, the Neurospora radiolabelled HSP70 promoter fragment (Cr27-Cr16) containing the putative HSE(s) was utilized to screen Southwestern blots of control (14 hr, 28 C) and heat-shocked (48 C, 1 hr) N. crassa mycelium. Cr27-Crl6 was found to interact specifically with a polypeptide of approximately 60 kDa in both samples but stronger binding was witnessed in heat-shocked extracts. In addition we have demonstrated that oxidative stress was able to induce binding of this protein to HSE sequences. Neurospora cells grown in varying plumbagin concentrations, an oxygen radical producing compound, were also subjected to Southwestern analysis with Cr27-Cr16 probe of hsp70 gene. Once again, the 60 kDa protein was found to bind more strongly in these samples than in control mycelium. Preliminary purification of this protein was conducted using ammonium sulfate precipitation, ion exchange chromatography and resolution on size-fractionation columns. This heat and oxidative stress activated DNA-binding protein is probably the heat shock transcription factor (HSF) of N. crassa.

24. Generation of minichromosome variability during meiosis in Glomerella graminicola.

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Pulsed-field electrophoresis and Southern hybridization analysis were employed to establish electrophoretic karyotypes and to study sequence relationships, genetic makeup and meiotic stability of minichromosomes in six isolates of Glomerella graminicola. Three to five minichromosomes ranging in size from approximately 400 kb to 1.2 Mb were present in each isolate. No two isolates had identical minichromosome profiles. Southern hybridization analysis with four minichromosome-specific clones revealed that each clone hybridized with no more than one chromosome per isolate, and in some cases failed to hybridize with any chromosome. The organization of these sequences also varies among isolates. Sequences found on the same minichromosome in one isolate were found on two minichromosomes in another isolate. Variation in minichromosomes among isolates is, therefore, the result of structural rearrangements and either sequence deletions or additions. The presence of transcriptionally active sequences residing on minichromosomes was established by the isolation of a cDNA clone which hybridized to a single minichromosome in each isolate. Karyotype analysis of tetrad progeny recovered from controlled crosses revealed nonparental chromosomes. Hybridization analysis and identification of reciprocal, nonparental homologs in tetrad progeny indicated that sequence deletions and recombination between homologous chromosomes with size polymorphisms were responsible for generation of nonparental chromosomes. We conclude that minichromosomes within G. graminicola isolates are not genetically inert. They share sequence homologies, undergo meiotic recombination resulting in the generation of novel sized chromosomes, and contain transcriptionally active sequences.

25. Homologues of aflatoxin biosynthetic genes in Aspergillus oryzae.

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Aspergillus oryzae is a non-toxigenic species used in food production and industrial enzyme production. A. oryzae is not known to produce aflatoxins but is closely related to the toxigenic species Aspergillus flavus and Aspergillus parasiticus. We have shown by Southern blotting using stringent hybridization conditions that A. oryzae contains sequences that hybridize to known structural and regulatory genes that encode enzymes in the aflatoxin biosynthetic pathway. Expression of these 'pseudogenes' did not occur under conditions known to induce aflatoxin biosynthesis in the toxigenic species. Sequences from A. oryzae have been cloned and characterized by sequence analysis. We report on the sequences obtained and discuss the possible reasons for A. oryzae being non-toxigenic.

26. Promoter elements required for arabinose induction of the beta-galactosidase (bgaS) gene from Penicillium canescens.

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A secreted beta-galactosidase from Penicilium canescens is an inducible enzyme and sugar beet pulp has been shown to be the most efficient inducer. Low molecular weight carbohydrates present in the culture fluid during fungal growth on sugar beet pulp were separated and tested for the ability to affect betaGal formation. The fraction capable of inducing the synthesis appeared to contain mostly L-arabinose. The inducing role of L-arabinose was also confirmed in transfer culture experiments. Besides beta-galactosidase, a number of other carbohydrolases were revealed in the P. canescens culture fluid upon arabinose induction, thus implying the existence of a common regulatory mechanism of expression of the whole set of genes coding for secreted proteins. To locate functional elements within the upstream region of the bgaS gene, promoter deletion analysis was initiated. A series of 5' non-coding deletion mutants were constructed and their effect on the bgaS gene expression was examined in Aspergillus nidulans, which was used as a host for gene regulation studies. The area required for arabinose induction is localized to a fragment of 300 bp, between -735 and -435 bp upstream of the ATG codon. A palindromic sequence 23 bp long containing a putative CREA binding site was observed within this fragment. Site specific mutagenesis of this motif resulted in a decreased level of the bgaS gene expression instead of expected release from carbon catabolite repression.

27. Developmental factors are necessary for the production of toxic secondary metabolites in Aspergillus nidulans.

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Aflatoxin (AF) and sterigmatocystin (ST) are biochemically related toxic secondary metabolites produced by a number of aspergilli. A putative transcription factor, aflR, containing a binuclear zinc binding domain, has been found to regulate the AF pathway of A. flavus and A. parasiticus. Using a construct which places aflR under the control of an inducible promoter, we have previously shown that expression of aflR is sufficient to

induce the expression of genes encoding enzymes necessary for the biosynthesis of ST in A. nidulans thus indicating that aflR is functionally conserved between Aspergillus spp. A number of A. nidulans mutants defective in development have been described that do not conidiate properly, and produce no or little ST. To determine if there is a link between development and secondary metabolism, we crossed the inducible aflR construct into the developmental mutants. This allows the induction of the aflR gene to test whether or not it is sufficient for the induction of pathway genes. Preliminary evidence indicates that the expression of aflR is sufficient to induce pathway genes in some, but not all, of the developmental mutants. This result suggests that the role of aflR in activating ST biosynthesis requires elements that are also needed for development.

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33. On the characterization of complex I from Neurospora mitochondria.

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Complex I is a multi-subunit structure of the inner mitochondrial membrane that couples electron transfer from NADH to ubiquinone with proton translocation from the matrix to the inter membrane space of the organelle. The enzyme is assembled from many nuclear-coded and a few mitochondrially-synthesized polypeptides. We are concentrating in the disruption of specific complex I subunits, by the generation of repeat-induced point mutations in their genes, to study several aspects of the biogenesis and function of the enzyme. As a step toward the isolation of mutants, two new genes were cloned and mapped in the Neurospora crassa genome by RFLP analysis. Several mutants have been obtained and we will describe the strategy that is being used to induce novel mutants. In addition, an analysis of complex I assembly has been performed in the stopper mutants of Neurospora. In some strains, assembly of complex I is not detected and the peripheral arm of the enzyme is accumulating.

34. Expression and secretion of human antibody light and heavy chains in Neurospora crassa.

Stephen Buczynski, David Schneck, Doug Vann, Elie Kato, and W. Dorsey Stuart, University of Hawaii, Office of Technology Transfer and Economic Development /Neugenesis Corp. (Joint Project)

A proprietary strain of N. crassa has been transformed by electroporation with plasmid vectors containing cDNAs for either a human kappa light chain or a human gamma heavy chain. The expression vectors contained the promoter and signal sequence of the gla-1 gene of N. crassa fused in frame with the cDNA of either antibody chain followed by the gla-1 terminator region. Prototrophic or antibiotic resistant co-transformants were screened for secretion of individual antibody chains by ELISA. Stable homokaryotic transformants were then grown in a liquid culture medium optimized for high expression of the gla-1 promoter and low protease secretion. Extracellular media from production cultures was harvested. The heterologous protein product was fractionated using either cation exchange or affinity chromatography. The engineered protein product was

validated by ELISA and Western blot methods. DNA integration into the host cells was characterized by Southern blot analysis. Current production levels are in the nanograms per milliter level for both kappa and gamma. Increased production levels will be achieved by designing upgraded expression cassettes, mutagenesis and selection for higher producing strains. In addition, strategies are being developed for using this system to produce intact human IgG antibody molecules.

35. Secretion of Trichoderma reesei beta-glucosidase by Saccharomyces cerevisiae.

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An intronless form of the bgl1 gene encoding an extracellular b-glucosidase from Trichoderma reesei was expressed in the yeast Saccharomyces cerevisiae under the control of the yeast GAL1 promoter. Transformation of a yeast strain with this vector resulted in transformants that produce and secrete active beta-glucosidase into the growth medium. Additionally, active recombinant b-glucosidase protein was shown to be localized predominantly in the periplasmic space by using a p-nitrophenyl beta-D-glycoside hydrolysis assay against fractionated yeast cells. The apparent size of the recombinant enzyme was 10-15 kDa larger than that of the native form. Treatment of the recombinant beta-glucosidase with endoglycosidase-H indicated the apparent increase in size is due to N-linked glycosylation.

36. sepB, an Aspergillus nidulans gene that regulates the initiation of cytokinesis.

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We have undertaken a combined genetic and molecular analysis of septum formation in Aspergillus nidulans to determine how septa are assembled and to elucidate the mechanisms that spatially and temporally coordinate this assembly with other cell cycle events. Temperature-sensitive sepB mutants are members of a novel class of A. nidulans mutants that appear to be specifically defective in the coordination of septum formation with nuclear division. At restrictive temperature, sepB mutants complete the first three rounds of nuclear division but arrest prior to initiating septum formation. Although these mitoses occur with kinetics similar to wild-type, the resulting nuclei exhibit an aberrant morphology, appearing elongated and multi-lobed. In addition, a colony sectoring assay was utilized to demonstrate that sepB mutants exhibit defects in chromosome segregation. These results suggest that sepB functions to maintain the fidelity of chromosomal DNA metabolism. Results from temperature shift experiments demonstrate that sepB functions early in septum formation. Moreover, failure to perform this function leads to the cessation of cell growth and nuclear division as well as the rapid onset of lethality. Molecular analysis of the sepB gene reveals that it is essential and possesses homology to a Saccharomyces cerevisiae gene involved in chromosomal DNA metabolism. We propose that the sepB gene product is involved in maintaining the fidelity of chromosome segregation while playing an essential role regulating the initiation of cytokinesis.

37. Translation, sexual reproduction and longevity in Podospora anserina.

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Mutations in components of the translation machinery modifying the accuracy of the decoding process have numerous phenotypes affecting various aspects of the life of P. anserina, especially life span. It is not clear what is responsible for the drastic longevity changes in the strains carrying one such mutation. We have undertaken an extensive analysis of high fidelity mutations (antisuppressors that antagonize informational suppressors) in the translation elongation factor EF-1a encoded by the AS4 gene. Antisuppressive properties of the AS4 mutations are highly variable depending on the suppressor used and the target mutation suppressed, precluding any classification on this basis. On the opposite, vegetative and reproductive abilities of the mutants are inversely correlated with their longevities. The mutants also present an additional inability to eject spores out of the perithecia. Sequences of the AS4 mutations reveal structural modifications similar to the yeast EF-1a suppressor mutations. The analysis suggests that a global modification of translation, and not the production of more accurate proteins, is responsible for the life span extension of the mutant strains. We are now investigating the biochemical parameters of translation altered in these mutants and we have started an analysis of the other accuracy genes, i.e. the omnipotent suppressor loci su1 and su2 and the genes coding for ribosomal proteins.

38. An estimation of the number of nuclear genes interfering with the senescence syndrome of Podospora anserina.

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Vegetative growth of P. anserina is accompanied by modifications of the mitochondrial DNA, leading to the death of the apical cells (senescence). The kinetics of modification is under the genetic influences of both mitochondrial and nuclear genomes. In order to estimate the number of nuclear genes involved in the control of life span, longevities of mutant strains were measured. Some of the mutants used have already been obtained on several other criteria unrelated to senescence, allowing us to uncover relations between various physiologic processes and mitochondrial DNA stability. The other mutants were constructed by insertional mutagenesis with a vector which integrated randomly and did not modify longevity by itself, allowing us to precisely estimate the proportion of the genome involved in life span control. Preliminary results suggest that life span is affected by modification of many processes. Indeed, mutations in some amino-acid biosynthetic pathways, in the cytoplasmic translation machinery and many mutations altering the morphology do modify life span. Consequently, a very large number of genes seem to be involved: among the random insertion mutants, an average of about 5% have a modified longevity. Taking in account that half of the genome is non coding and that our system can detect only a subset of the genes controlling longevity, more than 10% of the genes are involved in life span control in P. anserina.

39. Isolation and characterization of an Achlya ambisexualis cDNA and expression of a Hsp60 transcript population during antheridiol-induced differentiation.

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Previous and ongoing studies from this laboratory have shown that several different Hsp70 and Hsp90 chaperone/heat shock proteins and their mRNAs are up-regulated during antheridiol-induced hyphal differentiation in the oomycete Achyla ambisexualis. Hsp60 (GroEL) is a chaperone which mediates protein folding under normal physiological conditions, within,the mitochondrial matrix. This chaperone is also a heat shock protein. We have isolated a cDNA encoding Achlya Hsp60 and characterized the expression of Hsp60 transcripts and protein during antheridiol-induced hyphal differentiation. The levels of both were found to increase markedly with heat shock and with hormone-treatment of the mycelium. These results suggest a role for mitochondria during steroid hormone- induced hyphal branching and differentiation. (Supported by NSERC)

40. Cloning and characterization of Aspergillus nidulans cysB and cysC genes involved in cysteine synthesis.

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In A. nidulans three genes, cysB, cysC and cysE have been identified which control the last step in cysteine synthesis. The wild type cysB, coding for cysteine synthase, was cloned by complementation of a cysB mutation using our own cosmid gene library. The gene and its cDNA copies were sequenced revealing one intron at 5' end. The amino acid sequence of the enzyme shows a considerable similarity to cysteine synthases from E. coli and plants. Cysteine synthase activity in some transformants was found several-fold higher than in the wild type stains and correlated with a high resistance to sulfide. Studies of cysB expression are in progress. The cysC gene was localized on cosmid W5C10 from pWE15/pLORIST2 library from FGSC, by complementation of cysC- mutation. The transformants, in contrast to cysB+ ones, show no significant difference in the cysteine synthase level as compared to the wild type. The 5kb EcoRI/EcoRI fragment of the insert, caring the cysC gene, was subcloned. Preliminary data on the sequence analysis was presented.

41. Effects of mislocalization of ornithine transcarbamylase from the mitochondria to the cytosol in Neurospora crassa.

Terri L. Moulds and Richard L. Weiss. University of California, Los Angeles.

Regulation of metabolic pathways is accomplished in various ways. The role of compartmentation in regulation of the arginine biosynthetic pathway in Neurospora crassa is being investigated using ornithine transcarbamylase (OTC) as a model system. OTC catalyzes the formation of citrulline from ornithine and carbamyl phosphate in the mitochondria. Both substrates can cross the mitochondrial membrane and OTC's location is different in closely related organisms. Thus, the location of OTC may affect more than the arginine biosynthetic pathway. To mislocate OTC to the cytoplasm, a plasmid containing the coding region but lacking the sequence which encodes the mitochondrial targeting sequence was transformed into an arg-12 mutant that makes a truncated OTC. The transformants are arginine prototrophs but have reduced growth rates. Southern analysis indicated ectopic integration of the transformed DNA. OTC activity is found only in the cytoplasm but the specific activity is lower than wild type. The

"mislocalization" construct has also been introduced into a strain that has a disruption in the arg-12 locus. This recipient strain has no OTC activity and no detectable protein in the mitochondria. The effect of a cytoplasmic OTC in this and other mutational backgrounds is being investigated to determine the role of compartmentation in the control of amino acid metabolism.

42. The processing pathway of a polyprotein precursor of two mitochondrial enzymes in Neurospora crassa.

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In Neurospora, the mitochondrial arginine biosynthetic enzymes N-acetylglutamate kinase (AGK) and N-acetyl-g-glutamyl-phosphate reductase (AGPR) are made from the cleavage of a 96 kDa cytosolic polyprotein precursor. The precursor consists of a leader peptide followed by the two protein domains which are separated by a connector region. In order to identify sites of cleavage, elements involved in recognition by the processing protease, and the function of the polyprotein precursor, we have introduced several mutations in the region that connect the two protein domains. Processing has been analyzed by in vitro import assays with purified mitochondria and radiolabeled precursor. Analysis of processing and protein function in vivo has been performed by transformation of mutant precursors into AGK-AGPR- strains. We found a consensus arginine at position -2 from the cleavage site of the leader and at positions -2 and -3 from the N-terminal amino acid of the distal AGPR in the connector region of the polyprotein. This suggested that the same protease(s) involved in cleavage of the leader sequence may be involved in cleavage of the precursor into two mature proteins. Processing of a precursor in which arginine residues at positions -2 and -3 were changed to G and P identified a second processing site since processing yielded a wild type AGK and a larger form of the distal AGPR. Both proteins were functional as they complemented an AGK-AGPR- mutant. The mutant AGPR protein was microsequenced and the alternative cleavage site in the precursor was identified. This second cleavage site is located 20 amino acids upstream from the N-terminus of the AGPR and has an arginine residue located 3 residues upstream from the site of processing. Mutations that replace this arginine with a glycine or a proline have been obtained and its role on processing is being analyzed. Comparison of amino acid sequences in the vicinity of wild type and alternative processing sites revealed the presence of a threonine residue 2-3 amino acids downstream of the cleavage sites. The role of this residue on the mechanism of proteolytic cleavage is currently under investigation.

43. Pseudoreversion analysis of bimD6 in Aspergillus nidulans identifies a gene coding for a chromosome scaffold protein.

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A pseudoreversion analysis of the bimD6 mutation was undertaken to identify additional genes that function in the bimD pathway. Seven extragenic cold sensitive cs- suppressors of bimD6 heat sensitivity hs- were identified among 1505 revertant colonies. The cs- phenotype of all the suppressors was recessive and they fell into six complementation groups. Genetic mapping of the suppressor mutations has led us to conclude that we have identified three and possibly four extragenic suppressor genes which exhibit complex

genetic interactions. These genetic interactions include both linked complementation and unlinked noncomplementation, suggestive of physical associations. We have cloned one of the suppressor genes sudA by complementation of its recessive cs- phenotype. Sequence analysis of sudA has shown that it is a member of a rapidly growing family of proteins termed DA-box proteins because of conserved aspartate and alanine residues found in their carboxyl terminal globular domain. Structurally DA-box proteins are very similar and have two globular domains separated by a region of putative coiled-coil sequence. DA-box proteins function in chromosome condensation, segregation and gene regulation. One member of the family, dpy-27 of C. elegans, is involved in X chromosome compensation and therefore has an apparent chromosome specific function. These results suggest that bimD functions in chromosome segregation and structure in co-operation with DA-box proteins. Our suppressor genetic analysis suggests that there are two and possibly three other genes that also function in this pathway with bimD and sudA.

44. Hsp30-defective mutants of Neurospora crassa show conditional intolerance for high temperature.

Nora Plesofsky-Vig and Robert Brambl, The University of Minnesota, Saint Paul

The alpha-crystallin-related heat shock proteins are produced by all eukaryotes, but the role of these proteins in thermoprotection remains unclear. To investigate the function of one of these proteins, we disrupted expression of the single-copy hsp30 gene of Neurospora crassa, using repeat-induced point mutagenesis, and we generated and characterized mutant strains that were deficient in hsp30 synthesis. These strains could grow at high temperature and they acquired thermotolerance from a heat shock. However, the hsp30-defective strains proved to be extremely sensitive to the combined stresses of high temperature and carbohydrate limitation, enforced by the addition of a non-metabolizable glucose analogue. Under these conditions, their survival was reduced by 90% compared with wild- type cells. This sensitive phenotype was reversed by reintroduction of a functional hsp30 gene into the mutant strains. The mutant cells contained mitochondria from which a 22 kDa protein was readily extracted with detergents, in contrast to its retention by the mitochondria of wild-type cells. Antibodies against hsp30 coimmunoprecipitated a protein also of approximately 22 kDa from wild-type cells. Results of this study suggest that hsp30 may be important for efficient carbohydrate utilization during high temperature stress and that it may interact with other mitochondrial membrane proteins and function as a protein chaperone.

45. Cytochrome c oxidase in Neurospora crassa contains myristic acid covalently linked to subunit 1.

Alexei O. Vassilev, Nora Plesofsky-Vig, and Robert Brambl, The University of Minnesota, Saint Paul

Radiolabel from [3H]myristic acid was incorporated by Neurospora crassa into the core catalytic subunit 1 of cytochrome c oxidase, as indicated by immunoprecipitation. This modification of the subunit, which was specific for myristic acid, represents an uncommon type of myristoylation through an amide linkage at an internal lysine, rather than an N-terminal glycine. The [3H]myristate, which was chemically recovered from the radiolabeled subunit peptide, modified an invariant Lys324, based upon analyses of

proteolysis products. This myristoylated lysine is found within one of the predicted transmembrane helices of subunit 1 and could contribute to the environment of the active site of the enzyme. The myristate was identified by mass spectrometry as a component of mature subunit 1 of a catalytically active, purified enzyme. This is the first identification of a mitochondrial inner-membrane protein that is post-translationally modified by a fatty acylation.

46. Cloning and characterization of a functional homologue of the S. cerevisiae sar1 gene from Aspergillus niger.

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To initiate research towards a systematic analysis of the mechanism of protein targeting and secretion in filamentous fungi, the cloning of various genes encoding secretion-related GTP-binding proteins was undertaken. Cloning of these genes was carried out using heterologous hybridisation with cloned S. cerevisiae / pombe genes as probes. The successful cloning of the SAR1-related A. niger sarA gene is described. Further characterisation of the cloned gene was carried out by functional complementation of S. cerevisiae sar1 and sec12 mutants with a full length cDNA copy of the sarA gene in a yeast expression vector. Using gene-replacement strategies and a (conditional) mutant version of the cloned gene, A. niger secretion mutants will be generated.

47. Repressible phosphate/cation symporters in Neurospora crassa.

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The filamentous fungus Neurospora crassa posseses two non-homologous, high-affinity, phosphate permeases, PHO-4 and PHO-5. We have isolated separate null mutants of these permeases which has allowed us to study the remaining active transporter in vivo in terms of phosphate uptake and sensitivity to inhibitors. The specificity for the co-transported cation differs for PHO-4 and PHO-5, suggesting that these permeases employ different mechanisms for phosphate translocation. Phosphate uptake by PHO-4 is stimulated 85-fold by the addition of Na+, which supports the idea that PHO-4 is a Na+/phosphate symporter. PHO-5 is unaffected by Na+ concentration, but is much more sensitive to elevated pH than is PHO-4. Presumably, PHO-5 is a H+/phosphate symporter. Na+- coupled symport is usually associated with animal cells. The finding of such a system in a filamentous fungus is in harmony with the idea that the fungal and animal kingdoms are more closely related to each other than either is to the plant kingdom.

48. Extragenic suppressors of the nudAl cytoplasmic dynein mutation that blocks nuclear migration in Aspergillus nidulans.

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Cytoplasmic dynein is a larqe molecular weight protein complex that functions as a microtubule dependent, negative end directed, "motor". Mutations in nudA which encodes the heavy chain of cytoplasmic dynein, inhibit nuclear migration in A. nidulans. We isolated and characterized extragenic suppressors of the nudAl mutation preparatory to the identification of other protein that interact with the cytoplasmic dynein heavy chain.

Genetic analysis of 19 revertants has defined at least 5 extragenic suppressors of nudAl (snaA-E). All the sna mutations, except one, were recessive in diploids homozygous for nudAl and heterozygous for sna mutations. To characterize the nuclear migration phenotype in the sna mutants, conidia of one representative of each complementation group were germinated, fixed and stained with DAPI. The sna mutants display partial suppression of the nudA1 nuclear migration defect. The conidiophore morphology appeared abnormal in all the sna mutants except snaB76, and is linked to the extragenic suppressor mutation.

49. Search for gene(s) that biodegrade TNT in Phanerochaete chrysosporium.

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It is known that the white rot fungus Phanerochaete chrysosporium has the ability to degrade TNT (2,4,6- trinitrotoluene) (Fernando et al.: Appl. Microbiol. 56:1666-1674, 1990) but there is no report of actual isolation of this gene(s). The search for these gene(s) in this fungus responsible for TNT biodegradation has been an on going study in our laboratory. Preliminary studies indicate that this fungus can degrade TNT comes from growth studies using differential media. The fungus was grown in low starvation dose of five carbon source which consist of glucose, verathal alcohol, ammonium tartrate, sodium acetate, and tween-80 which act as cometabolites and 25 ppm TNT. Recently our laboratory has isolated TNT gene sequence from the bacterium Pseudomonas aeruginosa strain (Dutta et al., 1994, unpublished). We are currently performing hybridization studies by use of dot blots and southern blots for evidence of TNT degrading gene(s) present in P. chrysosporium. We are developing two strategies of approaches in going about isolating the gene: hybridization studies using known TNT gene probe and construction of cDNA library which are ongoing projects in our laboratory. (Supported in part by the U.S. Department of Army).

50. Isolation and characterization of monomorphic mutants of Ustilago maydis

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U. maydis is a basidiomycete responsible for the corn smut or "huitlacoche". The fungus has a saprophytic phase, growing as haploid, yeast-like cells (sporidia). Sporidia of compatible mating type are able to fuse to form an invassive dikaryotic mycelium. We have developed conditions which promote the dimorphic transition of haploid cells in vitro. Using this procedure for selection we have been able to isolate about 20 mutants which are unable to grow as mycelium under our experimental conditions. These mutants have been characterized based on their cell and colonial morphology in different media. Most of the mutants were found to be recessive when crossed with the wild type, bE or bW cells of the opposite sex. By means of reciprocal crosses among mutants of opposite mating type, strains have been allocated into at least four complementation groups. In addition, mutants have been characterized based on their pathogenic reaction. All mutants recessive for morphogenesis in vitro resulted recessive in their pathogenic behavior. Non-complementing mating pairs of mutants were non-pathogenic when inoculated into corn seedlings. Accordingly, these mutants define genes involved in mycelial growth and pathogenicity other than b.

51. The aglF gene encoding a secreted alpha-galactosidase in Aspergillus niger.

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Filamentous fungi of the genus Aspergillus are widely used for the production of fermented foods, organic acids and enzymes. Due to their capacity to secrete large amounts of proteins these organisms are often chosen for the production and secretion of homologous and heterologous proteins. However, not much is known about the secretion machinery in filamentous fungi. We plan to use alpha-galactosidases as reporters to study secretion in Aspergillus niger. A. niger has been shown to have at least six different enzymes with alpha-galactosidase activity. Depending on growth conditions most of the alpha-galactosidase activity is found in the growth medium. Two genes (aglA and aglN) encoding proteins with alpha-galactosidase activity were previously cloned from A. niger. aglA has sequence homology to other eucaryotic alpha-galactosidase encoding genes, whereas aglN shows homology to an alpha-galactosidase encoding gene from E. coli. We have now cloned the gene (aglF) responsible for one of the extracellular alpha-galactosidase activities as well as part of an additional gene likely to be encoding another alpha-galactosidase. The DNA sequence of aglF shows homology to the previously cloned aglA gene as well as other eucaryotic alpha-galactosidase encoding genes. CHEF/Southern analysis assigned the gene to A. niger chromosome II. Some data on the expression of the gene was be presented.

52. Growth pulses in Aspergillus niger wild type and an apical branching mutant.

Cristina G. Reynaga-Peña and Salomon Bartnicki-Garcia. University of California, Riverside.
Video enhanced microscopy made it possible to make precise measurements of fungal growth and to discover that fungal hyphae do not elongate steadily but grow in alternating pulses of fast and slow growth (1). We have made a detailed analysis of pulsed growth in Aspergillus niger wild type strain, and a temperature-sensitive mutant capable of apical branching at the restrictive temperature. Hyphae from either strain showed pulses of similar frequency (9- 11 pulses per min) despite substantial differences in elongation rate. The apical branching mutant allowed us to analyze growth pulses in adjoining branches from the same hypha. The pulses in these closely connected hyphal branches were of similar frequency but were not synchronous (fast and slow growth periods did not occur simultaneously). The similarity in pulse frequency suggests that hyphal growth is largely regulated by a mechanism common to all hyphae in a mycelium, while the lack of pulse synchrony suggests a certain degree of growth independence for each hypha. Presumably, the final events in the discharge of wall-building vesicles responsible for apical growth is controlled locally at each growing point. (1) Lopez-Franco, R., S. Bartnicki-Garcia and C.E. Bracker. 1994 Proc. Natl. Acad. Sci. USA. 91:12228-12232.

53. Heterologous expression of the Fusarium solani pisi cutinase gene in Aspergillus awamori.

I.A. van Gemeren(1), A. Beijersbergen(2), W. Musters(2), R. Gouka(3), C.A.M.J.J. van den Hondel(3), C.T. Verrips(1, 2). (1)Department of Molecular and Cellular Biology University of Utrecht NL. (2) Unilever Research Laboratory Vlaardingen NL. (3)TNO Nutrition and Food Research Rijswijk NL.

Filamentous fungi are used as hosts for the commercial production of heterologous proteins because they have an enormous capacity for secretion. The production levels of many heterologous proteins are however rather low compared with the high levels which are obtained with homologous proteins such as glucoamylase. This study has been started to sort out the bottlenecks in the production of heterologous proteins and subsequently to identify the factors involved in these rate-limiting pathways. Therefore the effect of different pro- and pre- sequences and the presence of multiple copies of the gene on the production of heterologous proteins is studied. The cutinase protein from Fusarium solani pisi is used as a model for the production of heterologous proteins. A synthetic copy of the cutinase cDNA was constructed and expressed under the control of the endoxylanase II expression signals from A. awamori. Four different constructs were used to test the effect of different pre- and pro-sequences. A single copy of these expression cassettes was integrated at the pyrG locus of A. awamori. Shake flask induction experiments revealed that the pre-sequences used were equally efficient in the production of extracellular cutinase. The absence of a pro-sequence however resulted in a two-fold increase in extracellular cutinase. To study the effect of multi copy gene expression one of the constructs was integrated in multiple copies into the genome of A. awamori. There was no linear correlation between copy number and extracellular cutinase production but the amount of active enzyme produced correlated with the level of cutinase specific mRNA. These data and the fact that a relatively small amount of cutinase was found inside the cell suggest that there is no limitation in the secretion of this protein by A. awamori.

54. Plasma Membrane H+-ATPase in differentiation of Neurospora.

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N. crassa hyphae were found to be differentiated in respect of the membrane potential, input resistance and electrical coupling between adjacent cells. Energetic cooperation via ionic fluxes through septal pores was revealed in 3-4 apical hyphal cells. The functioning of such cell ensembles appears to be under genetic control. Transport processes of plasma membranes of the cells composing fungal hyphae are influenced by blue light which also controls gene expression during differentiation of reproductive structures Light-induced membrane electrogenesis and photoinduced gene expression are probably controlled by a common photoregulating mechanism. Plasma membrane hyperpolarization in the illuminated hyphal cells appears to be energy-dependent and thus connected with the functioning of H+-ATPase. As in the case of gene expression, it is apparently mediated by cAMP. Except that membrane electrogenesis seems not to be a necessary connecting transduction link from the photoreceptor to genome. Synchronization of the reactions of individual hyphal segments showing a profound electrophysiological heterogeneity seems to be the functional signifigance of plasma membrane electrical responses to light.

55. Characterization of a class II type hydrophobin of Trichoderma reesei.

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Finland.
We have earlier reported the isolation of T. reesei genes highly expressed on glucose-containing media. One of the genes isolated has been identified as encoding a novel hydrophobin, hfb1. It is a small hydrophobic protein of 97 amino acids with a single sequence and eight cysteine residues arranged in a conserved pattern. The hydrophobin of T. reesei shows strongest similarity to cryparin of Cryphonectria parasitica (59%) and cerato-ulmin of Ophiostoma ulmi (52%) both acting as wilt toxins causing pathogenicity of these fungi. It belongs to the class II type of hydrophobins (J. Wessels, pers. comm.). We have also detected a hydrophobin mRNA in T. harzianum used in biocontrol of plant pathogenic fungi by using the T. reesei hfb1 as a probe in Northern analysis. The HFB1 protein of T. reesei was isolated from culture medium by precipitation induced upon freezing and from mycelium based on its SDS-insolubility and further dissolution in TFA. The isolated proteins reacted with polyclonal antibodies obtained against the HFB1 protein expressed in E. coli and where confirmed as the product of the hfb1 gene by N-terminal sequencing.

56. A new role in pseudohyphal growth. An Aspergillus nidulans abaA homologue?

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A typical feature of many pathogenic fungi is their ability to interconvert between a yeast and filamentous growth pattern. A characteristic of such dimorphic nature is the production of morphologically distinct cell types through alterations in the polarities and patterns of cell divisions. Aspergillus nidulans and Saccharomyces cerevisiae are providing insights into the mechanisms controlling such morphogenesis in that as well as offering tractable genetic sytems they both undergo a yeast like filament interconversion. Using these two organisms as tools to study this biological phenomenon, we are investigating the possibility of analogous developmental pathways being shared by these apparently morphologically divergent organisms. Forced expression of the A. nidulans developmental transcription activator gene abaA in S. cerevisiae results in enhanced filamentous growth of a diploid strain, which suggested the existence of a similar developmental pathway in yeast as well as the presence of an AbaA homologous protein. To further investigate such protein we constructed null mutants TEC1, whose gene product has been suggested to share a DNA binding domain with AbaA, was thus an attractive candidate for such a protein. By disrupting this gene and investigating the ability of a homozygous tec1/tec1 strain to form pseudohyphae we determined that TEC1 is necessary for such morphology. This would suggest that a new pathway responsible for this growth pattern has been uncovered in S. cerevisiae, and it is anticipated that this may be the analogous pathway involved in the A. nidulans developmental process.

57. Drug efflux transporters, determinants of fungal pentamidine susceptibility.

Yi Li, Gabriele Ludewig, and Chuck Staben. University of Kentucky.
Pentamidine inhibits the growth of many taxonomically diverse fungi during respiratory growth. Inhibition occurs only at much higher pentamidine concentrations during fermentative growth. The accumulation of pentamidine by fungi appeared to determine this pattern of inhibition. The facultatively aerobic fungi that we tested, Saccharomyces cerevisiae and Candida albicans, accumulated pentamidine only when grown on

nonfermentable carbon sources. An obligate aerobe, Dipodiascus uninucleatus, accumulated pentamidine on all carbon sources. At least one fungus, Rhodotorula mucilaginosa, with a high resistance to pentamidine did not accumulate pentamidine from the medium. Two of the pentamidine resistant mutants of S. cerevisiae that we have isolated showed different patterns of pentamidine accumulation. One mutant (YLS4) failed to accumulate pentamidine, a second mutant (YLS2) accumulated normal amounts of pentamidine. The mutation responsible for resistance in mutant YL54 was in the SGE1 gene. SGE1 encodes a member of the bacterial multidrug resistance efflux protein family. Disruption of the SCE1 gene increased the cell's susceptibility to pentamidine. These results indicated that drug transport is a major determinant of pentamidine susceptibility in fungi and that specific efflux transporters have a role in normal susceptibility as well as in resistance mechanisms.

58. Cryptococcus neoformans can use inositol as an energy source.

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Cryptococcus neoformans is an encapsulated fungi that infects immunocompromised hosts. Unlike the systemic infections with most fungal pathogens, C. neoformans infections usually localize to the central nervous system (CNS), a region of high inositol concentration. One distinguishing trait of C. neoformans is its capacity to use inositol, a key cellular metabolite, as a sole carbon source. Most other microbes cannot use inositol as a source of energy for growth. The first step of inositol catabolism, in higher eukaryotes, is the conversion of inositol to glucuronic acid. This reaction is catalyzed by inositol oxygenase. Inositol oxygenase activity has been demonstrated in crude lysates of C. neoformans. Moreover, this enzyme has been shown to be regulated in response to the carbon source in the growth media. Growth of C. neoformans in media with inositol instead of glucose or glucuronic acid as the carbon source results in higher specific activity of inositol oxygenase. In addition, inositol oxygenase specific activity appears to increase immediately after a shift of carbon source from glucose to inositol. Furthermore, the activity of this enzyme appears to decrease as cells enter stationary phase. To dissect inositol metabolism in C. neoformans, mutant strains were isolated that grow when glucose is provided as the carbon source but do not grow in media with inositol as the carbon source. Studies of the inositol catabolic pathway in C. neoformans will contribute to understanding overall metabolism of this organism, and may provide insight into the biochemical basis of its localization and pathogenicity.

59. Binding of fibrinogen to rodletless Aspergillus nidulans is impaired.

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Human fibrinogen is known to bind to A. fumigatus conidia, and there is evidence for specific fibrinogen receptors on their surfaces. We wished to study the effect of an intact hydrophobin rodlet layer on fibrinogen binding to A. nidulans conidia. We therefore compared quantitative binding of l25I labeled fibrinogen to the following isolates: FGSC 26 (wild-type, intact hydrophobin rodlets), RMS025 (rodletless), and Tx 5 (Rod A+, RMS025 transformant). Incubation mixtures contained 2 x 10(7) conidia and l25I-

fibrinogen (100,000 cpm). Binding results were as follows:

A. nidulans Isolate	125I-fibrinogen bound, cpm (Mean +/- SEM. n=3)
FGSC 26	6,327 +/- 291
RMS025	1,643 +/- 32
Tx5	3,562 +/- 82

Thus bindinq of 125I-fibrinogen to the rodletless strain, RMS025, was significantly reduced compared to FGSC 26 and Tx 5 ($p < 0.05$, Students paired t-test). These data suggest that an intact hydrophobin rodlet layer is required for optimal fibrinogen binding.

60. Cloning and characterization of Aspergillus nidulans genes coding for homocysteine synthesizing enzymes.

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In A. nidulans there are two enzymes which synthesise homocysteine: cystathionine beta-lyase and homocysteine synthase coded for by metG and cysD genes, respectively. The first enzyme belongs to the main pathway of homocysteine synthesis while the second one represents the alternative pathway of methionine and cysteine synthesis, which seems to occur only in fungi and yeast. Both genes have been cloned by complementation of appropriate mutants using a plasmid gene library and have been sequenced. The metG gene, the first eukaryotic cystathionine beta-lyase gene cloned, contains an intron sequence at its 5' end and codes for a polypeptyde of 407 amino acids. In the cysD gene, five introns were found and their positions were identified through the analysis of appropriate cDNA fragments synthesized by PCR. A 437 amino acid sequence of homocysteine synthase deduced from DNA sequence shows 63% similarity with its S. cerevisiae counterpart. Both genes show homology to the family of genes coding for transsulfuration enzymes, such as human, rat, and yeast cystathionine g-lyases, yeast homocysteine synthase, and both the cystathionine gamma-synthase and cystathionine beta-lyase from E. coli.

61. Septation and nuclear distribution in Aspergillus nidulans.

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The intercalary compartments of Aspergillus nidulans hyphae maintain a uniform size of approximately 395 um (Trinci and Morris, 1979). This size is determined by the positioning of septa during cytokinesis. In many organisms the position of the dividing nucleus determines the location of cytokinesis. Thus we investigated the role of nuclear positioning in determining septal placement in A. nidulans. Nuclear positioning was altered by growing three temperature sensitive nuclear distribution mutants, nudA, nudC and nudF, at semi-permissive temperature (39 C). Nud+ strains maintained an evenly spaced distribution of nuclei when grown at this temperature. In contrast, nuclei in the nud- strains clustered throughout the length of the hyphae. The nud- strains also displayed aberrant septal positioning. Although each nud- strain maintained an overall average intercalary compartment length close to 395 um, individual lengths varied considerably from this average, causing nud- intercalary lengths to differ significantly from nud+ intercalary lengths. An experiment following septal kinetics demonstrated that

the mechanics of septation is normal in nud strains, suggesting that none of the nud gene products are directly involved in septation. Examination of double mutants made between nud- and sep- (septation deficient temperature sensitive mutants) revealed nuclear clumping in the absence of septa at 39 C. We conclude that nuclear distribution affects the process of septation in A. nidulans.

62. Why search for integrin homologs in fungi?

Susan G. W. Kaminskyj and John E. Hamer, Purdue University, West Lafayette, IN 47907

Fungi are extolled as model organisms for studying the structure and function of metazoan eukaryotes, such as ourselves. As well, features of animal cells have been used successfully to predict homologous structures and/or functions in fungi. Cells in both systems appear to be regulated by protein components in the substrate to which the cell is attached: the cell wall and extracellular matrix, respectively. Consistent with this, nonstructural fungal cell wall components vary with developmental stage. Cytoskeleton-substrate attachment and signalling proteins, such as integrins, are well characterized in animal cells where integrin-matrix protein docking regulates cell form and proliferation. Integrin and matrix protein homologs in fungi and oomycetes have been identified by immunological crossreactivity and by inhibiting normal cell function with exogenous peptides containing integrin-matrix protein binding sequences. Integrins have recently been implicated in host cell attachment and pathogenesis by Candida, so these proteins' role in fungal biology is also medically relevant. However, our present understanding of the roles and regulation of integrins in fungi is rudimentary and wants first for their cloning and molecular characterization. Aspergillus nidulans contains a 156 kDa protein which crossreacts with antiserum to a conserved cytoplasmic beta-integrin domain on western blots. Using immunofluorescence, this antiserum stains peripheral patches. We are using this crossreactivity to attempt cloning a fungal integrin homolog from cDNA expression libraries.

Posters III: Plant and Animal Fungal Pathogenesis

1. A non pathogenic mutant of Colletotrichum magna is deficient in extracellular secretion of pectate lyase.

C. Wattad, S. Freeman, A. Dinoor and D. Prusky. Volcani Center, Bet Dagan, Israel.

A non pathogenic strain of Colletotrichum magna (path-1) was recently isolated. Unlike the C. gloeosporioides and the C. magna, wt isolate, path-1 did not cause disease symptoms in either the pericarp nor the mesocarp of avocado fruit. Isoelectric focusing of culture filtrate of Colletotrichum strains revealed the presence of PL and PG with no significant activity in Path-l. DNA and RNA analysis of the three Colletotrichum isolates hybridized to a pel probe from C. gloeosporiodes indicating that at the DNA level no deletion in the pe1 gene was evident and at the mRNA level similar expression levels were found. However, when PL antibodies were cross-reacted with cell lysate of the Colletotrichum strains, PL accumulated in the non pathogenic mutant suggesting that differential pathogenicity accounts for malfunction in extracellular transport of PL protein.

2. Cloning and characterization of a "tomatinase" gene from Botrytis cinerea.

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Saponin-detoxifying enzymes occur in several plant pathogenic fungi and have been shown to determine host-specificity e.g. in Gaeumannomyces graminis (Osbourn et al. 1994, Bowyer et al. 1995). Using the tomatinase gene of Septoria lycopersici as a probe (A. E. Osbourn, pers. commun.) we isolated a corresponding gene from a genomic lambda library of B. cinerea. The putative tomatinase gene (toml) shows significant homology with the probe (up to 60% at aminoacid level) and with the tomatinase gene of Gaeumannomyces graminis. Sequences homologous to tom1 were present in all field isolates of B. cinerea tested so far, though not all of the strains show tomatinase activity. The role of the "tomatinase" in pathogenicity of B. cinerea is under investigation.
Osbourn A., Bowyer P., Bryan G., Lunnes P, Clarke B., Daniels M.J.: in Advances in Molecular Genetics of Plant- Microbe Interaktions, Vol. 3:215-221 (1994); Bowyer P., Clarke B., Lunnes P., Daniels M.J., Osbourn, A., Science (in press)

3. Purification and characterization of two enzymes, a bifunctional beta-xylosidase/alpha- arabanosidase and an alpha-arabanosidase, from the maize fungal pathogen Cochliobolus carbonum.

Richard F. Ransom and Jonathan D. Walton, D.O.E. Plant Research Lab., Michigan State Univ., East Lansing, MI 48824-1312.
Two enzymes, an alpha-L-arabinosidase and a bifunctional beta-D-xylosidase/alpha- L arabinosidase secreted by C. carbonum grown on medium supplemented with corn cell walls, were purified to homogeneity from culture filtrates . The Mr Of the alpha-L-arabanosidase (CCAR) was 30-32 kD and the Mr of the bifunctional beta-D-xylosidase/alpha-L-arabanosidase (CCXA) was 42-44 kD. As is found with most other secreted cell-wall-degrading enzymes, CCAR and CCXA are catabolite repressed. The CCAR enzyme accounts for approximately 75% of the total alpha-L-arabanosidase activity, with the remainder contributed by the CCXA. Amino acid sequence of two peptides from an AspN-protease digest of CCXA showed more than 50% identity with a bifunctional beta-D-xylosidase/alpha-L- arabanosidase from Bacteriodes ovatus. Oligonucleotides derived from these sequences are being used to screen a C. carbonum genomic library, and peptide fragments of CCAR are being sequenced. The final goal of this research is to disrupt the genes encoding CCXA and CCAR to determine their role in the pathogenicity of C. carbonum on maize.

4. Cell wall degrading enzymes of fungal plant pathogens.

John S. Scott-Craig, John W. Pitkin, Patricia C. Apel, Jenifer M. Murphy, Paola Sposato, Richard F. Ransom, Holly J. Schaeffer, and Jonathan D. Walton. MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan
Cell wall degrading enzymes (CWDEs) are secreted by all phytopathogenic fungi and have been thought to play a role in the penetration and ramification by these pathogens into healthy plant tissues. In Cochliobolus carbonum genes have been cloned that encode endo-polygalacturonase, exo- polygalacturonase, three endo xylanases, cellulase, endo-beta-1,4 glucanase, exo-beta-1,3-glucanase, beta- 1,3-1,4 glucanase, and an alkaline

proteinase. Using a transformation-mediated gene disruption strategy, many of these genes have been mutated singly in pathogenic wild-type C. carbonum strains. In every case, the disruption of the CWDE gene and the concomitant loss of CWDE activity did not cause any detectable change in the pathogenicity of the mutant strain. Strains are currently being constructed containing multiple CWDE gene disruptions to assess effects on pathogenicity. Our goal is to identify all the CWDEs and their genes from C. carbonum. However, many CWDEs are difficult to assay and purify, and therefore their genes cannot be approached biochemically. To circumvent this problem, a subtracted cDNA library approach is being taken. Genes that are expressed when C. carbonum is grown on corn cell walls as carbon source but not when grown on sucrose will be identified by chemical cross-linking subtraction using the cross linking agent 2,5-diaziridinyl-1,4- benzoquinone (DZQ). The cDNAs isolated will be sequenced and putative CWDE encoding genes tested for a role in pathogenicity.

5. Characterization of a species-specific DNA probe for the in situ identification of Entomophaga maimaiga infected gypsy moth larvae.

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The zygomycete, Entomophaga maimaiga, is a member of the Entomophaga aulicae species complex and is responsible for the recent collapse of gypsy moth populations in Northeastern U.S.A. We report here the isolation of a DNA fragment cloned from E. maimaiga, which hybridized to DNA of geographically diverse E. maimaiga isolates, but did not hybridize to DNA from other entomophthoralean fungi, including other isolates of the E. aulicae species complex. Sequence analyses revealed that this species-specific DNA fragment is composed of several simple sequence motifs. The DNA sequence contained several stop codons in all six reading frames. Although weak similarity was observed with a Drosophilia LTR, no significant homology at the nucleotide or amino acid levels, was found in searches of existing databases. Quantitative dot blot hybridizations indicated that the sequence contained a repetitive element present at approximately 161 copies per E. maimaiga nucleus. The repetitive nature of this sequence proved highly useful and allowed for the detection of an E. maimaiga infection within a single gypsy moth larva. (Supported by an NSERC Canada)

6. Assessing the role of endopolygalacturonase in the pathogenicity of white-rot basidiomycete Chrondrostereum purpureum.

Yijian Tang and William E Hintz, Department of Biology, University of Victoria, Canada.

The white-rot basidiomycete Chondrostereum purpureum is being developed as a biological control agent for the suppression of unwanted hardwood species in forest renewal sites. We are investigating the role of endopolygalacturonase I (endoPG) in the pathogenicity of C. purpureum by the overexpression and/or transformation mediated disruption of the endoPG gene. EndoPG gene sequences from fungi and plants were aligned and regions of sequence homology identified in order to isolate an endoPG gene fragment from C. purpureum by PCR amplification. A putative endoPG gene fragment (214 bp) was amplified which shared significant similarities with other known endoPG gene sequences. An EMBL-3 lamda DNA Iibrary is being screened to select the full-

length gene. Overexpression and disruption of endoPG requires an efficient transformation system for C. purpureum. Transformation experiments with dominant selectable marker driven by promoters derived from ascomycetes (pAN-7 and pPS-57) have been unsuccessful. Transformation with the phleomycin resistence marker gene driven by gpd promoter of the basidiomycete Schizophyllum commune (F.H.J. Schuren, pers. com.) has apparently been successful. Analysis of the putative transformants is in process. The gpd promoter will be switched with the C. purpureum endoPG promoter in order to further increase transformation efficiency.

7. Isolation of an alfalfa gene induced by Colletotrichum trifolii during a compatible interaction using differential display.

G. M. Truesdell and M. B. Dickman, University of Nebraska-Lincoln.

We are interested in understanding how plants and their pathogens communicate at the molecular level. During a compatible interaction between a phytopathogenic fungus and its host, specific genes are induced or repressed in both organisms. Such changes in gene expression may facilitate disease development. To identify genes in either alfalfa or Colletotrichum trifolii whose regulation is altered at the time of infection, differential display was performed. Populations of mRNA expressed in alfalfa, C. trifolii and C. trifolii infected alfalfa leaves were reverse transcribed using four sets of anchored oligo(dT) primers that contain two additional bases, M and N, at their 3' ends. The resulting cDNA populations were amplified by PCR using a set of arbitrary decamers and radioactive nucleotide, and the PCR products were separated by PAGE. Numerous differentially-amplified products were isolated. Northern and Southern analysis identified one product as an alfalfa gene whose expression is induced during infection. Characterization of this gene will be discussed.

8. Genetic analysis of cultivar specificity and race evolution in the soybean pathogen, Phytophthora sojae.

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There are 12 major resistance (Rps) genes in soybean against the oomycete pathogen P. sojae, and 37 races of the pathogen. To test whether avirulence against these Rps genes is controlled by single dominant genes, we crossed three isolates of P. sojae. Since P. sojae is homothallic we used RAPDs to identify F1 hybrids from mixed cultures. The F1 hybrids were then selfed to produce F2 progeny. Avirulence was dominant or semi-dominant in the F1 progeny for all 10 Rps genes tested. RFLP and RAPD markers segregated in regular Mendelian fashion among the F2 progeny of one cross, but not of a second cross. In the first cross, avirulence against the six Rps genes tested (Rps1a, Rps1b, Rps3a, Rps3c, Rps4 and Rps6) segregated as a single Mendelian trait. Several sets of linked RFLP markers were identified including a RFLPs linked to Avr(Rps1b). Analysis of the distribution of RFLP markers and avirulence phenotypes among 48 field isolates encompassing 25 race types indicated that new races have arisen in this pathogen both by mutations (presumably in avirulence genes) and by reassortment of avirulence genes following rare outcrosses.

9. Fusarium oxysporum from cyclamens.

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Seventy-nine Fusarium isolates were recovered from cyclamen plant material and solicited from culture collections. These isolates were categorized based upon pathogenicity towards cyclamen, vegetative compatibility tests, hybridization with a repetitive DNA (fingerprint) probe, and the organization of the ribosomal intergenic spacer (IGS) sequences. Fifty-three pathogenic and 26 nonpathogenic isolates were identified. The pathogenic isolates could be subdivided into three groups. Each of these groups was limited to a single vegetative compatibility group (VCG) and all members had similar IGS organization and DNA fingerprints. The nonpathogenic isolates could be distinguished from the pathogenic isolates, and usually one another, using either the VCG or the fingerprint criteria, but not necessarily the IGS criterion.

10. A polyketide synthase (PKS) is required for fungal virulence toward Texas male sterile corn.

Ge Yang, M. Rose, O.C. Yoder and B.G. Turgeon. Dept. of Plant Pathology, Cornell Univ., Ithaca, NY.

In 1970, a new race (T) of the fungal pathogen, Cochlobolus heterostrophus appeared. Race T is highly virulent toward Texas male sterile (T) corn and differs from its progenitor, race O, at a locus (TOX1) which is responsible for production of T-toxin, a polyketide. TOX1 is associated with a complex genetic rearrangement involving a reciprocal translocation, AT-rich repeats, and an insertion. We used REMI to mutagenize and tag TOX1. Sequencing of the DNA at the insertion site and translation of a 7.8 kb ORF revealed a multifunctional PKS encoding gene with six enzymatic domains: ketoacyl-ACP synthase (KS), acyl transferase (AT), dehydrogenase (DH), enoyl reductase (ER), keto-reductase (KR), and acyl carrier protein (ACP), all with conserved motifs. The KS motif has the highest homology with other polyketide synthases. The PKS appears to be organized as a single module surrounded by highly repetitive, AT-rich DNA. Non-toxin-producing race O strains lack this gene. When the PKS-encoding gene was disrupted in race T, T toxin production and high virulence were eliminated. These results reveal, for the first time, that a PKS is required for virulence and provide a firm foundation for investigation of how a new pathogenic race arises.

11. The role of PM-toxin in pathogenesis by Mycosphaerella zeae-maydis.

Sung-Hwan Yun, B.G. Turgeon, and O.C. Yoder, Cornell University, Ithaca, NY 14853

M. zeae-maydis is a homothallic Ascomycete taxonomically unconnected to Cochliobolus heterostrophus race T. Yet both fungi are highly virulent on corn containing Texas male sterile (T) cytoplasm (but cause little damage to N-cytoplasm corn) and each produces a linear polyketide which specifically affects mitochondria of T-cytoplasm corn. Genetic analyses have shown that the C. heterostrophus race T polyketide (T-toxin) is required for high virulence to T-cytoplasm corn. To determine the role of the M. zeae-maydis polyketide (PM-toxin) in pathogenesis, the restriction enzyme mediated integration (REMI) procedure was used to produce Tox mutants via transformation of fungal protoplasts [prepared by digesting mycelium with Driselase and cellulase (each 10 mg/ml) in 0.7 M KCL and 0.2 M CaCl2, pH 6]. From 504 mitotically stable

transformants (selected for resistance to hygromycin B, blasticidin S, or benomyl), five Tox mutants were obtained, which when selfed, produced F1 and F2 progenies that were 100% Tox . These mutants were identical to wild type in all respects except that they failed to produce PM-toxin. Two of them were inoculated on T- and N-cytoplasm corn; both had drastically reduced virulence, indicating that the M. zeae-maydis polyketide, like the one from C. heterostrophus, is required for pathogenesis.

12. The host range of a plant pathogenic fungus is determined by saponin detoxification.

Paul Bowyer, Mike Daniels and Anne Osbourn, Sainsbury Laboratory, John Innes Centre, Norwich, UK.

Saponins are glycosylated steroidal compounds which occur in many plant species. The toxic effects of saponins are attributed to their ability to form complexes with membrane sterols resulting in loss of membrane integrity. Some pathogenic fungi have intrinsic resistance to these compounds as their membranes contain no sterols, whereas others produce enzymes which can enzymatically detoxify particular saponins. This implies that at least for some interactions, "saponin-saponinase" combinations may determine host range. A gene encoding a saponin-detoxifying enzyme was cloned from the cereal infecting fungus, Gaeumannomyces graminis. Fungal mutants generated by targeted gene disruption were no longer able to infect the saponin-containing host oats but retained full pathogenicity to wheat (which does not contain saponins). Thus the ability of a plant pathogenic fungus to detoxify a plant saponin can determine its host range.

13. Avenacinase-like proteins in fungi from the Gaeumannomyces-Phialophora complex.

Greg Bryan, Paul Bowyer, Mike Daniels and Anne Osbourn,. Sainsbury Lab, John Innes Institute, Colney Lane, Norwich, U.K.

Gaeumannomyces is a genus of ascomycete fungi with four known species that infect roots of grasses, cereals, or sedges. Take-all, caused by Gaeumannomyces graminis, is the most damaging root disease of wheat world-wide, and is among the most important cereal diseases in the United Kingdom. G. graminis varieties tritici, avenae, and graminis have Phialophora-like anamorphs and, together with other non-pathogenic Gaeumannomyces and Phialophora species found on cereal roots, constitute the Gaeumannomyces-Phialophora complex. cDNA encoding the saponin detoxifying enzyme avenacinase from G. graminis var. avenae, cross hybridises with DNA from other varieties of G. graminis. In addition, avenacinase antisera cross-reacts with a secreted protein with a similar molecular weight to avenacinase from these same G. graminis isolates. We have shown that these avenacinase-like proteins (ALPs) have very similar physiochemical and immunological properties to avenacinase, but unlike avenacinase, they cannot effectively deglucosylate avenacin. Cloning and DNA sequence comparisons of ALPs with avenacinase and another saponin detoxifying enzyme, tomatinase (from Septoria lycopersici) will be presented. Gene disruption experiments are in progress, and the effects of ALP gene disruption on host range and pathogenicity will be assessed.

14. Saponin detoxification by plant pathogenic fungi.

Anne Osbourn, Paul Bowyer, Greg Bryan, Patricia Lunness, Belinda Clarke and Michael Daniels, Sainsbury Lab, Norwich, UK.

Saponins occur in many plant species, and because of their antifungal properties they have been implicated as pre-formed determinants of resistance to fungal attack. Some fungi produce enzymes which remove sugars from saponins, to give molecules which are less fungitoxic. Mutants of the cereal-infecting fungus Gaeumannomyces graminis var. avenae which do not produce the saponin glucosyl hydrolase avenacinase can no longer infect the saponin-containing host oats (but are still fully pathogenic to wheat, which does not contain saponins) (see accompanying poster by Bowyer et al). Southern blots using avenacinase cDNA as a probe revealed cross-hybridising DNA sequences in a number of other phytopathogenic fungi, suggesting that enzymes related to avenacinase may be widespread. We have demonstrated for one of these fungi (the tomato pathogen, Septoria lycopersici), that the cross-hybridising DNA in this fungus also encodes a saponin detoxifying enzyme (in this case tomatinase). While avenacinase and tomatinase are clearly related, the relative activities of these enzymes towards avenacin and tomatine reflect the host specificity of the fungi from which they originate. Structure/function analysis of these two highly conserved saponin glucosyl hydrolases should allow us to identify the regions of the enzymes which are important for activity and for substrate specificity, and to design inhibitors of enzyme action which may have significance for crop protection strategies. The occurrence of DNA sequences which hybridise to avenacinase cDNA in genomic DNA of other phytopathogenic fungi suggests that saponin-saponinase combinations may be more important in determining host range than has previously been appreciated.

15. Mapping of avirulence genes in the rust fungus, Puccinia graminis.

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Extensive genetic and physiological studies of cereals rust diseases have provided the foundation for many of our current concepts about host-parasite interactions. However, we know very little about the molecular biology of rust, in part due to the obligate nature of these pathogens. As a model system for rust fungi we have chosen to study the wheat stem rust fungus, Puccinia graminis f.sp. tritici. A genetic mapping population has been generated by crossing two North American isolates and selfing a single F1 progeny. In this F2 population we have selected ten avirulence genes (avr6, avr8a, avr9a, avr9d, avr10, avr21, avr28, avr30, avrflk, and avrU) for further study. Seven of these genes segregate as single dominant genes, while one (avr10) appears to be co-dominant and one (avr9d) recessive. Avrflk is segregating with a 13:3 ratio, indicating that two genes may be involved. We are currently using RAPDs and bulked segregant analysis to identify genetic markers closely linked to these genes in order to isolate them by map-based cloning.

16. 1,3-beta-glucanase and xylanase from Phytophthora parasitica: enzyme characterization and gene cloning.

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Phytophthora parasitica produces a number of extracellular polysaccharide-hydrolyzing

enzymes. Some of these enzymes are putatively involved in pathogenesis: xylanase (XYL) is believed to facilitate the penetration of the cell wall of host plants and 1,3-beta-glucanase (GLU) is assumed to participate in overcoming a callose barrier built by the host in a defence reaction against the pathogen. Zymogram analysis of isoelectric focusing gels revealed two forms of GLU with pI around 5.0 and four forms of XYL with pI around 9.0. GLU isoenzymes were further resolved into four bands on native PAGE gels. Two forms of XYL with Mrs of 50 kD and 70 kD were identified by activity staining of SDS gels after renaturation. Xylanase from culture filtrate was partially purified by ion exchange chromatography. A cDNA library of P. parasitica was constructed in lambda ZAP and screened for activity of fusion proteins (Karlovsky, P. and Wolf, G.A. (1993) Meth. Mol. Cell. Biol. 4:40-45). Clones encoding GLU and XYL were isolated, converted into plasmids and expressed in E. coli. Sequences of protein products deduced from nucleotide sequences of cDNA clones were compared with sequences of known GLU and XYL enzymes.

17. Rapid simultaneous identification of virulence genes.

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Transposon mutagenesis has been used to identify bacterial virulence genes by testing individual mutants for altered virulence in living plants and animals, but comprehensive screening of genomes for virulence genes has not been possible because of the inability to identify mutants with attenuated virulence within pools of mutagenized bacteria, and the impracticability of separately assessing the virulence of each one of the few thousand mutants necessary to screen a bacterial genome. To circumvent this problem, we developed a novel transposon mutagenesis system in which each mutant is tagged with a unique DNA sequence. We used Salmonella typhimurium to illustrate the method because it has excellent molecular genetics, and the disease it causes in mice closely resembles typhoid fever. We show that it is possible to identify mutants with reduced virulence simultaneously, by comparing the identity of bacterial cells recovered from spleens of animals with those of a complex pool of mutant cells representing the inoculum. Screening of 1000 mutants resulted in the identification of thirty genes affecting virulence. These were either previously identified S. typhimurium virulence genes, homologues of known genes (including virulence genes) of S. typhimurium and other bacteria, or genes without similarity to entries in DNA databases. The screening system should permit the rapid isolation of virulence genes of other bacterial pathogens, and may be applicable to fungal pathogens, using the restriction enzyme mediated integration (REMI) procedure to generate random insertional mutations.

18. Lineage structure, avirulence locus polymorphism and the organization of pathotype diversity in the rice blast fungus.

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The rice blast fungus, Pyricularia grisea (Magnaporthe grisea), exhibits high levels of pathotype polymorphism that have handicapped efforts to breed durably resistant rice cultivars. Homologous- DNA fingerprints indicate that pathogen diversity is typically

organized in historically distinct genetic lineages. Each lineage has a limited cultivar range although most express multiple pathotypes that are varied combinations of lineage-wide compatibilities. Lineages are thus marked as expressing particular, historically conserved avirulences. Complementary evidence of this organization is now provided by lineage-specific RFLPs associated with P. grisea avirulence gene probes and by wholesale lineage incompatibility with single gene resistances in near-isogenic testers. Based on these features we have identified and are evaluating particular resistance gene combinations that should exclude all of the resident lineages (based on their current pathotype repertoire) in various rice-growing regions.

19. Analysis of RAPDs by graphic display reveals genetic relationships among Australian and American genotypes of bean rust.

D.J. Maclean, K.S. Braithwaite, J.A.G. Irwin, J.M. Manners and J.V. Groth. Cooperative Research Centre for Tropical Plant Pathology, University of Queensland, Australia, and Department of Plant Pathology, University of Minnesota, St. Paul (JV Groth).

Over 200 race phenotypes of the bean rust fungus Uromyces appendiculatus (Pers.) Unger var. appendiculatus, have been recorded worldwide. This study assessed genetic diversity of bean rust in Australia in relation to the Americas. Initially, phenetic analysis of 12 representative Australian isolates using RFLPs (10 cDNA probes) and RAPDs (10 decanucleotide PCR primers) revealed three clusters of isolates designated A, B, and AB. Collation of polymorphic bands characteristic of each cluster ("Graphic Display") showed that AB exhibited most bands characteristic of A and B, but gave no characteristic bands of its own, suggesting that AB isolates arose from hybridization between genotypes A and B. Further analysis using RAPDs (10 primers) compared 5 representative American isolates with an extended set of 42 Australian isolates. One American isolate clustered with the Australian isolates of genotype B, while the other four American isolates formed a separate cluster (C). Although genotypes A and B were phenetically closer to each other than genotype C, Graphic Display revealed a group of polymorphic RAPD markers common to genotypes A and C but absent from B, consistent with A being derived by recombination between progenitors of B and C. Results are discussed in relation to the derivation of current genotypes from disparate centers of origin in the Americas.

20. Molecular analysis of pathogenicity genes from the plant pathogenic fungus Glomerella cingulata.

Matthew D. Templeton, Joanna K. Bowen, Sarah Jack, Erik H.A. Rikkerink, Patrick A. Sullivan*, Molecular Genetics Group, Horticulture and Food Research Institute, Mt. Albert Research Centre, Auckland, and *Department of Biochemistry, University of Otago, Dunedin, New Zealand.*

Glomerella cingulata causes disease on a wide variety of fruit crops. The maceration of fruit tissue is caused by the action of an array of pectinolytic and other hydrolytic enzymes secreted by the pathogen. We are using gene disruption as a tool for determining the importance of these enzymes in pathogenicity. Oligonucleotides were designed to conserved amino acid domains from pectin and pectate lyases and used to amplify G. cingulata genomic DNA. Three clones with homology to pectin lyase and one with homology to pectate lyase were identified. One of the pectin lyases designated pnlA was

cloned and sequenced. Two gene-disruption vectors were constructed, a replacement and a truncation-disruption, and used to inactivate the pnlA locus. Only transformation with the truncation-disruption vector gave single-copy integrations at the desired locus. Disruption of pnlA was shown using Southern analysis. The pnlA transcript was not detected on Northern gels and PNLA could not be detected by activity staining IEF gels. Although PNLA was the most active isozyme secreted in vitro, the disruption of the pnlA locus had no effect on pathogenicity of G. cingulata on apple or capsicum. Progress on the analysis of other pathogenicity factors such as the secreted aspartic protease was also presented.

21. Host-associated genetic differentiation in Erysiphe cichoracearum: a mixture of diffuse cospeciation and colonization.

Kurt A. Zeller and Morris Levy. Purdue University, West Lafayette, IN 47907, USA.
The powdery mildew species, Erysiphe cichoracearum, is reported to infect more than 300 hosts from among 8 plant families. Evidence suggests, however, that the species is a complex of host-specialized biotypes. Our analyses of this species with RFLP's of amplified ribosomal DNA (rDNA) segments demonstrate the presence of several, distinct rDNA haplotypes among biotypes of E. cichoracearum. Samples with identical rDNA haplotypes were always collected from the same host family. However, multiple, distinct haplotypes were recovered from sunflower family biotypes. rDNA haplotypes differences also clearly distinguished samples from the morphologically similar species, E. galeopsidis, and from the mildew genus Sphaerotheca. Comparative phylogenetic analyses suggest that host associations of these obligate parasites are derived from a mixture of diffuse cospeciations at the host familial level and colonizations of different host families. Thus, there is only limited support for Fahrenholz's rule, that parasite-host associations evolve by cospeciation in a species-for-species manner. However, the specificity of individual rDNA types suggests that related hosts share basic compatibility with only a subset of these powdery mildews. The evolutionary consequences of interactions between trophic dependence and dispersal in fungal plant pathogens will also be discussed.

22. The role of polygalacturonases in the interaction between Penicillium olsonii and Arabidopsis thaliana

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The possible involvement of fungal pectin degrading enzymes in the pathogenicity of Penicillium olsonii to Arabidopsis thaliana is examined. Two fungal polygalacturonases can be induced with pectin as sole carbon source in vitro. Both enzymes are also produced by the fungus in vivo during plant infection. From known sequences of fungal PGs, oligonucleotides have been constructed which lead to the amplification of a 586 bp fragment by PCR. This PCR fragment was cloned into a vector for transformation mediated direct gene disruption. Of 80 transformants, 4 were reduced in PG activity. Southern hybridization and enzyme analysis demonstrated the disruption of one PG gene. The vector also contained the GUS gene under the control of a constitutively transcribed fungal promotor. GUS- positive transformants with unchanged PG activity compared with PG-reduced mutants showed no difference in colonizing leaves during the first six

days of infection. Investigations of later infection stages and the cloning of the second PG gene to construct a PG-deficient Penicillium are planned.

23. Cutinase is produced during infection of pea by Nectria haematococca but has no influence on virulence.

Frank Hannemann and Wilhelm Schafer. Institut fur Genbiologische Forschung, Ihnestr. 63, 14195 Berlin, Germany.

A new infection assay was established for the pathogen-plant system Nectria haematococca garden pea. Intact plant seedlings were inoculated with fungal mycelium and the infection process was assayed over a time period of 14 days. A new transformation system was established and a cutinase- deficient transformant was transformed to express the beta-glucuronidase gene. Fungal growth and vitality of cutinase-deficient and cutinase-producing strains was monitored on infected plants by measuring beta-glucuronidase production. Additionally, general esterase and cutinase activity was analysed during the infection process. Cutinase-deficient and cutinase-producing strains generated the same infection symptoms and comparable beta-glucuronidase activities. Cutinase activity was detectable in epicotyls as well as roots infected with Nectria haematococca wild type. No cutinase activity was observed during plant infection with the cutinase-deficient mutant. For the interaction of N. haematococca with pea, we draw the following conclusions: 1. Cutinase is neither essential for pathogenicity nor involved in virulence. 2. It is a rather unspecific enzyme that is found also during root colonization. 3. No additional fungal cutinase is detectable during plant infection.

24. Regulation of the nor-1 gene involved in aflatoxin biosynthesis in Aspergillus parasiticus.

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The phytopathogenic fungus, Aspergillus parasiticus, produces the toxic secondary metabolite aflatoxin. Colonization of peanuts, cotton, corn and other crops by the fungus may result in aflatoxin contaminated food and feed supplies. The first stable intermediate in the aflatoxin biosynthetic pathway is the decaketide-derived norsolorinic acid, which is converted to averufin via one or more proposed alternative pathways. One of the enzymes involved in this conversion is the nor-1 gene. Coordinate expression of the nor-1 gene and several other genes associated with aflatoxin biosynthesis indicates that these genes are similarly regulated. Analysis of the promoter regions of these genes should provide some insight into the mechanism of this regulation. Proteins were isolated from nuclei of A. parasiticus grown for 48 hrs in either aflatoxin inducing (GMS) or noninducing (PMS) medium and Mobility Shift DNA Binding assays were performed on a 224bp region upstream of the transcript initiation site. Two sites were identified that bound proteins unique to the GMS cultures. An understanding of the biosynthesis and regulation of aflatoxin production will be useful in developing novel control methods for this pathogen and in clarifying the role of aflatoxin in the biology and pathogenicity of the fungus.

25. A central role of salicylic acid in plant disease resistance.

Bernard Vernooij, Terrence Delaney, Scott Uknes, Leslie Friedrich, Kris Weymann,

David Negrotto, Thomas Gaffney, Manuella Gut-Rella, Helmut Kessmann, Eric Ward and John Ryals. Ciba Geigy, Agricultural Biotech, Research Triangle Park, NC 27709.
Transgenic tobacco and Arabidopsis thaliana expressing the bacterial enzyme salicylate hydroxylase cannot accumulate salicylic acid (SA). This defect not only makes the plants unable to induce systemic acquired resistance, but also leads to increased susceptibility to viral, fungal and bacterial pathogens. The enhanced susceptibility extends even to host-pathogen combinations that would normally result in genetic resistance. Therefore, SA accumulation is essential for the expression of multiple modes of plant disease resistance.

26. Genetics of virulence in Phytophthora sojae.

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Phytophthora sojae belongs to the Oomycetes which are characterized by gametangial meiosis, thus having a diploid somatic phase, which contrasts with the majority of the true fungi which are haploid for most of their life cycle. Until recently the genetics of virulence/avirulence in P. sojae was considered intractable due to its homothallic nature. A race 1 and a race 7 isolate were co-cultured in vitro and, using RAPD markers, ten hybrids were identified among 354 oospores analyzed. One F1 hybrid was allowed to self fertilize and produce an F2 population of 247 individuals. Fifty-three F2 individuals were selected at random for genetic analysis. A genetic linkage map has been constructed from this cross consisting of 15 major linkage groups and ten small linkage groups using 233 RAPD markers, 30 dominant RFLP markers, 10 co-dominant RFLP markers and four avirulence genes. Segregation of virulence against soybean resistance genes Rps1a, 3a and 5 revealed that the avirulence genes Avr1a, 3a and 5 were dominant to virulence. Avirulence against these three resistance genes appeared to be conditioned by one locus for Avr1a and two independent, complementary dominant loci for both Avr3a and Avr5. Segregation of virulence against Rps6 was in the ratio of 1:2:1 (avirulent : mixed reaction : virulent), suggesting a semi-dominant allele at a single locus.

27. Extraordinary heterogeneity in the ribosomal non-transcribed spacer region of Acremonium.

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Acremonium species are a group of asexual filamentous Ascomycetes that form mutualistic associations with grasses of the Pooideae sub-family (Scott and Schardl, 1993). The ubiquitous pasture grass Lolium perenne (perennial ryegrass) forms associations with Acremonium spp. from two taxonomic groupings: LpTG-1 (=A. lolii) and LpTG-2. Recent work has shown that isolates of LpTG- l are haploid whereas isolates from LpTG-2 are heteroploid, and probably derived from inter specific hybridization event(s) between the ryegrass choke pathogen, Epichloe typhina, and LpTG-l (Schardl et al . 1994). Characterization of the ribosomal region (rDNA) of an LpTG-2 Acremonium isolate revealed an extraordinary amount of length heterogeneity in the non-transcribed spacer (NTS) region. Single spore purification showed that this heterogeneity is intragenomic. The NTS pattern is largely conserved through single sporing, but some distinct length differences were observed. The extreme level of heterogeneity found in the NTS region is not found in the two putative parental species -

LpTG- 1 and E. typhina, and it appears to be specific to this region of the genome. Sequencing has shown that the NTS contains two closely-related sub-repeats, one 107bp in length and the other 120bp in length. These repeats are arranged tandemly with the organization of the two repeat classes showing no obvious pattern. We propose that the heterogeneity found in the LpTG-2 NTS region is the result of an unequal recombination mechanism, possibly in the register of these sub-NTS repeats. The appearance of this heterogeneity apparently as a consequence of hybridization has implications for the study of control of homogeneity in tandemly repeated genes.

28. Disruption of Mak1 and structural analysis of the Mak1 containing chromosome in Nectria haematococca.

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Nectria haematococca is a pathogen on a variety of host plants. We are studying its interaction with chickpea (Cicer arietinum). Our interests are focused on determining: 1) If Mak1, a gene that detoxifies the chickpea phytoalexin maackiain, is important for pathogenicity on chickpea; and 2) If there are hotspots for chromosome breakage during meiosis on the B-chromosome that contains Mak1. Mak1 was recently cloned from a chromosome-specific cosmid library. We replaced an internal 400bp fragment of Mak1 with a hygromycin B expression cassette and are using this construct to create a transformation mediated gene disruption in N. haematococca. The pathogenicity of transformants with a successful gene replacement will be tested in a virulence assay on chickpea. In previous genetic crosses it was found that in isolates that lost part of the Mak1 chromosome, the remaining fragments of the chromosome were most often 0.9-1.0 megabases in size (Miao et al. 1991 Science 254:1773- 1776). This result suggests that specific regions of the chromosome are particularly susceptible to breakage during meiosis. We are currently creating a contiguous cosmid map of the Mak1 chromosome using a procedure called sampling without replacement. The chromosome-specific library will be probed with a variety of deleted forms of the Mak1 chromosome to identify the breakage points generated during meiosis. Progress towards these goals will be presented.

29. A preliminary intraspecific phylogeny of Fusarium oxysporum based on the partial sequence of the intergenic spacer (IGS) region of the rDNA.

D.J. Appel and T. R. Gordon. University of Califronia, Berkeley

Using PCR, we amplified and sequenced 1,000bp of the 5' end of the intergenic spacer (IGS) in 15 isolates of Fusarium oxysporum and one isolate of F. subglutinans. The IGS region was chosen for sequence analysis because RFLPs revealed variation within F. oxysporum. Isolates were selected based on the genetic markers, virulence, race, vegetative compatibility group (VCG), mitochondrial DNA (mtDNA) haplotypes, IGS haplotypes, and DNA fingerprint, to represent the diversity in our collection. The objective of this research was to clarify the origin of virulence within F. oxysporum, the evolution of the different races of F. o. melonis, and the relationship between pathogenic and nonpathogenic strains. Parsimony analysis of the partial IGS sequence data identified a phylogenetic tree with highly significant branches. The two F. o. melonis VCGs, 0131 and 0134, were separated into distinct lineages closely related to F. o. cubense. Race was not distinguished by IGS sequence differences within the pathogen VCGs, except for one

isolate. This race 1 isolate was first thought to be a new race in VCG 0131, but it was also associated with the same mtDNA and IGS haplotypes, and DNA fingerprint as isolates in VCG 0134. Two IGS sequence types were found in this isolate, representing both VCG 0131 and 0134, suggesting a somatic interaction or recombination event occurred between F. o. melonis, VCG 0131 and 0134. Nonpathogens that were vegetatively compatible with the pathogen, were not closely related to the pathogen and do not appear to be potential sources of new pathogenic races.

30. Tri10: A new gene in the trichothecene gene cluster in Fusarium sporotrichioides.

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Fusarium sporotrichioides produces a spectrum of trichothecenes with the primary metabolite being T-2 toxin. Genetically, the synthesis of these sequiterpenoid mycotoxins is determined, in part, by pathway genes that are organized in a large gene cluster. Preliminary DNA sequence information indicated the presence of two small open reading frames, ORF-1 and ORF-2, downstream from the Tri5 gene which encodes the pathway enzyme, trichodiene synthase. Northern analyses conducted with DNA probes within and/or overlapping these two ORFs demonstrated that each probe hybridized to a transcript approximately 1400 bases in length. A pair of primers within ORF-2 has also yielded a PCR product of identical size from both genomic DNA and DNA prepared from a cDNA library. In addition, the pattern of expression of this transcript parallels that of the other known pathway genes located in the trichothecene gene cluster. These results indicate that we have identified a new gene, Tri10, within the trichothecene gene cluster. Experiments are underway to determine the complete sequence of the Tri10 cDNA and to investigate the function of this gene via gene disruption.

31. Pneumocystis carinii strain and species dynamics in natural and induced infections monitored by electrophoretic karyotyping.

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Pneumocystis are a group of eukaryotic organisms that cause a lethal pneumonia in immunocompromised mammalian hosts. Gene sequence data show they are phylogenetically related to fungi. Two putative species of rat-derived Pneumocystis carinii, P. carinii carinii and P. carinii rattus (previously called prototype and variant) were defined by several molecular genetic criteria. At least 8 forms of P. c. carinii could be distinguished on the basis of electrophoretic karyotype patterns. Although P. c. carinii and P. c. rattus were found as co-infections within rat lungs, co- infections with 2 forms of P. c. carinii have not been observed. Monitoring of the natural infection in commercial rat colonies by electrophoretic karyotyping, hybridization with repetitive and single gene probes, and by gene sequencing showed that a single karyotype form of P. c. carinii could remain stable in a colony 3 years or more if housing conditions were unperturbed. In colonies where rats with co-infections of the 2 species were introduced, the numbers of rats harboring co-infections were variable. Direct co-inoculation of mixtures of equal numbers of 2 defined P. c. carinii forms into immunosuppressed rats produced infections of only a single karyotype form. A delay of 10 days or 20 days between inoculations resulted in infections bearing the karyotype of the form first inoculated and in some cases,

a significant reduction in organism burden. These results indicate that not all forms of P. c. carinii are compatible in vivo and suggest that mechanisms of interference may be operational.

32. MPG1 hydrophobin and development in Magnaporthe grisea.

Janna Beckerman and Daniel Ebbole. Texas A&M University.
Upon germination on a hydrophobic substrate, conidia of the rice blast fungus (Magnaporthe grisea) form infection structures called appressoria that allow direct penetration of plant cells. We are examining expression of a hydrophobin of M. grisea named MPG1 (Magnaporthe Pathogenicity Gene 1). MPG1 was so named because mutant strains are no longer able to efficiently form appressoria and are therefore less pathogenic than wild-type. We hypothesize that this protein plays a role in recognition of surface hydrophobicity. Expression of this gene is observed during the early stages of infection of rice plants and in aerial hyphae and conidiophores. It has been reported that nutrients do not affect development of appressoria. However, we have found that nutrient conditions that repress MPG1 expression in liquid culture also strongly inhibit appressorium formation on hydrophobic surfaces. Exogenous application of cAMP results in restoration of appressorium formation and induces MPG1 expression in otherwise repressing growth medium. The data suggest a relationship between nutritional status, hydrophobic surface recognition, and cAMP signalling in appressorium formation.

33. Electrophoretic karyotypic variation in the genus Pythium.

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Significant variation is observed in electrophoretic karyotypes in the genus Pythium. While isolates of a single species tend to have a similar distribution of chromosome sizes, differences in the number of chromosomal bands and their individual sizes were observed. While some morphologically similar species share a common range in size distribution, this was not observed for all species comparisons made. One mechanism which appears to contribute to intraspecific polymorphism in karyotypes is meiotic instability, however, depending on the species investigated different types of polymorphisms were generated. For the homothallic species P. oligandrum, the only polymorphism detected was the loss of a putative supernumerary chromosome in 3.5% of the progeny. While karyotypes of progeny of the homothallic species P. spinosum were nearly identical to the parental isolate, 60% of the isolates were polymorphic for 1-2 chromosomal bands. In contrast to the results with the homothallic species, significant levels of meiotic instability were detected in the heterothallic species P. sylvaticum; in total, 80% of the progeny chromosomal bands were nonparental in size or location of specific coding regions. The contributions of mitotic instability on the generation of karyotypic polymorphisms also will be discussed.

34. Glume blotch of wheat: Adaptation of the pathogen to varieties with quantitative resistance.

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The fungal pathogen Stagonospora nodorum (syn. Septoria nodorum) causes glume

blotch of wheat. The aim of our research is to investigate variation in field populations of S. nodorum and, in particular, to determine how this variation is distributed among wheat host varieties differing in their resistance to the pathogen. RFLP markers were used to measure the amount and distribution of genetic variation in nine Swiss pathogen populations. Nine hundred isolates of S. nodorum were collected from three fields each of three varieties. DNA variation was assayed with ten probes that hybridized to single RFLP loci, one probe that showed a DNA fingerprint and one mtDNA probe. A maximum amount of genotypic diversity was found based on the DNA fingerprint. However, single locus analysis of RFLP data indicated that allele frequencies were generally very similar in all populations, including two additional populations from Texas and Oregon. There was no evidence for gametic disequilibrium in any of the populations. The results suggest a high level of sexual recombination and that the RFLP markers were selectively neutral. On an intercontinental level, the calculated gene flow (Nm>11) was strong enough to counteract genetic drift and to prevent differentiation among populations.

35. The global population genetic structure of the wheat pathogen Mycosphaerella graminicola.

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We have analyzed the genetic structure of populations of the pathogenic fungus Mycosphaerella graminicola (anamorph Septoria tritici) from wheat fields around the world. Anonymous probes that hybridized to individual RFLP loci were used to measure gene diversity, genetic identity, and evaluate the potential for gene flow among populations. Populations of the fungus are remarkably similar worldwide. Genetic diversity is distributed on a small scale in each population. All populations have a large number of different genotypes and individual RFLP loci are at gametic equilibrium, properties expected in random-mating populations. All populations share alleles at RFLP loci, often at similar frequencies. These results suggest the potential for a high degree of gene flow among populations. Yet, DNA fingerprints showed that no clones were shared among populations, so there is not a predominant clone or set of clonal lineages worldwide as has been found for other plant pathogens such as Magnaporthe grisea and Phytophthora infestans. It appears that sexual reproduction and gene flow are the dominant factors affecting the genetic structure of populations of M. graminicola worldwide.

36. Indication of population subdivision within the sorghum anthracnose fungus, Colletotrichum graminicola.

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Two populations of Colletotrichum graminicola, one from a commercial sorghum field in South Texas, the other from the sorghum disease nursery in Griffin, Georgia were analyzed for their genetic structure using seven anonymous DNA probes which hybridized to low copy fragments in each isolate. Both populations were characterized by a low number of alleles at each locus and also by a low number of genotypes retrieved from each location, indicating that this fungus exhibits much less genetic variation than

previously assumed based on virulence studies. It appears that populations of this fungus are largely clonal in sorghum populations. Upon comparison of the two populations, private alleles were found for some RFLP loci in the two populations. Shared alleles were found in both populations for other RFLP loci, but in these cases allele frequencies were often significantly different. Calculated values for Nm for most probes were less than one, indicating that gene flow is not one of the forces influencing the evolution of this fungus, at least for these two populations.

37. RAPD isolation of fingerprint probes for analysis of Colletotrichum species.

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We have been analyzing genetic diversity of isolates of Colletotrichum gloeosporioides and C. acutatum from diverse plant hosts. The availability of reliable and informative fingerprint probes would greatly facilitate analysis of phylogenetic relationships between populations of these fungi. We have used hybridization of labelled genomic DNA to screen a battery of RAPD products to identify repetitive elements from other taxa. RAPD products identified are then used to probe Southern blots of DNA from various isolats and fingerprint patterns compared. Our analyses indicate a dearth of heterodispersed, repetitive elements in these two species of Colletotrichum relative to some other plant pathogenic genera. However, different RAPD products may be readily assembled to generate useful fingerprint probes.

38. Nrs1, a repetitive element linked to pisatin demethylase genes on dispensable chromosomes of Nectria haematococca.

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We have identified a repetitive DNA sequence in T-2, an isolate of Nectria haematococca mating population VI. This repetitive family has been called Nrsl. The 2027 bp clone of the Nrsl-2 allele contains a long polyA sequence, imperfect RNA polymerase III promoter sequences, multiple inverted and direct repeats, and the potential for extensive secondary structure similar to known polymerase III transcripts and related retroelements. Ten of the 11 HindIII restriction fragments from T-2 that hybridize to Nrsl-2 segregate in a manner consistent with a 1:1 ratio for random ascospore progeny. The ten RFLP loci define three linkage groups and correspond to three chromosomes from T-2 as separated by pulse field gel electrophoresis. Three RFLP loci defined by hybridization to the gene for pisatin demethylase and located on a 1.6 Mb chromosome are genetically linked to each other and to several of the Nrsl loci. These sequences recombined despite the fact that no obvious homolog exits for the 1.6 Mb chromosome in one of the parent strains. DNA hybridization analysis, using Nrsl-2, shows homology to other Nectria isolates that are pathogenic to Pisum sativum.

39. Progress in cloning cultivar specificity gene AvrCO39 from Magnaporthe grisea

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The cultivar specificity gene AvrCO39 was mapped between marker 5-10-F and cosegregating markers CH5-120H and 1.2H on chromosome 1 of Magnaporthe grisea. RecA-Mediated Achilles' cleavage was used to determine that the physical distance between markers 5-10-F and CH5-120H is 600kb. A chromosome walk was initiated towards AvrC039 from markers 1.2H and 5-10-F. Unfortunately, both walks were curtailed before reaching AvrCO39. While walking away from 1.2H, a region of repetitive DNA was encountered which contained, among other repeats, a copy of the grasshopper retrotransposon. Within this repetitive region, it was not possible to find a single copy probe to identify an overlapping cosmid. From the other direction, it was not possible to progress more than six steps beyond marker 5-10-F, despite screening cosmid clones from a second library of more than 20 genome equivalents. Presumably the overlapping cosmid contains a sequence that is "poisonous" to E. coli. A novel method was used to overcome both of these obstacles. Achilles' cleavage was directed to markers 43-2-H and 18-2-F which were identifed while walking. This resulted in the liberation of a 280kb restriction fragment containing AvrCO39. This fragment was resolved using CHEF gel electrophoresis, digested using BamHI and then subcloned into a plasmid vector. In this manner, markers were identified that cosegregate fully with AvrCO39. Conventional chromosome walking was resumed to assemble contigs that span the entire locus. Progress in cloning AvrCO39 was reported.

40. Detecting genetic variation in a fungal population using amplified frangment length polymorphism (AFLP).

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AFLP (Amplified Fragment Length Polymorphism) analysis was originally developed by Keygene, Wageningen, as a tool for plant genome mapping. However, these new molecular markers have great potential for studying genetic variation in fungal populations. The main advantage of AFLPs is that variation can be found quickly and reliably, even in fungal species where other methods have failed to detect sufficient variation. The ascomycete Pyrenopeziza brassicae is the causal agent of light leaf spot of Bassica species. The population structure of this economically important pathogen is unknown. We present AFLP data on isolates from six geographical areas in the UK and discuss advantages of using AFLP analysis (as opposed to RAPDs, RELPs or fingerprinting methods) for population studies to answer questions on population substructure and evolution of host - parasite interactions.

41. Detection of terminal restriction fragment length polymorphisms among race 8 of Ustilago hordei and its inbreeding progeny.

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Ustilago hordei (Pers.) Lagerth is the causal agent of covered smut on barley (Hordeum vulgare) and has a physiologic race structure that obeys the gene-for-gene model. Race 8 of U. hordei is pathogenic only on the universal susceptible cultivar, Odessa. In a previous study, inbreeding of race 8 increased its pathogenicity in a stepwise progression and was speculated to have given rise to a new race, race 14, pathogenic on all

differential cultivars (Phytopath. 69:1207-1212). Inbreeding of race 8 in our laboratory failed to reproduce this increase of pathogenicity and the progeny of successive selfed generations were pathogenic on Odessa only. The electrophoretic karyotypes (EK) of a race 8 strain, F1, and F2 inbreeding progeny from our laboratory and the previous study were resolved. All populations have a similar EK with the exception of the F2 progeny from the previous study, which had an additional chromosome ca. 1100 kb. Estimates of the number of chromosomes in the race 8 strain and the inbreeding progeny were obtained by hybridization of a telomere-specific probe to Southern blotted BamHI, BglII, and EcoRI digests from CHEF fractionated chromosomal bands. The F2 progeny from the previous study had 24 chromosomes while all other populations had 23 chromosomes. Hybridization of the telomere probe to BamHI, BglII, and EcoRI digests of total genomic DNA from all populations detected RFLPs only between race 8 and the F2 progeny from the previous study. The significance of these results with regard to the relatedness of race 8 and race 14 strains of U. hordei was discussed.

42. Molecular analysis of chromosome polymorphism in Ustilago hordei by using chromosome-specific probes and a telomere repeat as markers.

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Variation in chromosome number and length have been detected by CHEF pulsed field gel electrophoresis (PFGE) in strains representing the 14 races of Ustilago hordei, the causal agent of covered smut of barley. Our objective was to determine the exact chromosome number for each race and to ascertain whether some strains are aneuploid using 2 approaches. Chromosome-specific libraries were constructed and 3 single copy probes from each library were hybridized onto Southern blots of CHEF gels containing chromosomes from strains of the 14 races. The homologous chromosomes identified were typically monosomic with length polymorphisms ranging to 100 kb, but cases of disomy were also observed in some strains. The second approach involved a telomere-specific repeat from Fusarium oxysporum, which was used as a hybridization probe onto Southern blots of restriction digests of individual chromosomes. Using this technique, 20 to 23 chromosomes were detected in 15 to 19 chromosome bands visualized by CHEF PFGE analysis in the strains representing the 14 races. The telomere-specific probe also generated specific RFLP patterns for each chromosome and allowed comparisons of homologous chromosomes. Each race of U. hordei showed a unique RFLP pattern for each of its chromosomes except for strains representing race 10 and 13. These strains had identical karyotypes and no RFLP differences.

43. Molecular approaches to distinguish physiologic "races" of Ustilago violacea.

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Ustilago violacea is a heterobasidiomycete plant pathogen that infects over 70 species of Caryophyllaceae (Pinks). Circumstantial evidence has suggested that physiological races of U. violacea can be defined according to which host species are productively infected, as individual strains of the fungus tend to be only able to infect host species from which they are isolated and such host preference varies among different U. violacea isolates. Since development of the dikaryotic and spore forms of the fungus is obligately parasitic,

the molecular bases for such "races" (if bona fide) and for their host preference are of interest. To date, there has been a paucity of genetic linkage data concerning U. violacea races. In the absence of such data, we have investigated several approaches to produce molecular "fingerprints" of U. violacea strains isolated from different host species and to further characterize the relatedness of such strains. Eighteen different sporidial strains representing 7 different races were examined for electrophoretic karyotype, RAPD-PCR profile, and by phylogenetic analysis of intron sequences in the gamma-tubulin gene. Comparison of electrophoretic karyotypes in conjunction with Southern hybridization with gamma-tubulin gene as a probe provided a measure for gauging strain relatedness. The combination of these methods with RAPD profiles identified isolates in a manner consistent with their anecdotal race designations.

44. Genetic variability in Sclerotinia trifoliorum.

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S. trifoliorum is a pathogen of forage legumes with an interesting genetic system. Within each ascus there are four large and four small ascospores. The large spores give rise to homothallic (selfing) isolates. The small spores give rise to obligate heterothallic (outcrossing) isolates. A field study was conducted to deterrnine if outcrossing occurred in two infested alfalfa fields near Ithaca, New York. Outcrossing was assessed by using a mycelial incompatibility assay to examine genetic variability between sibling ascospore isolates from individual apothecia. In field #1, which was heavily infested during the previous spring and had relatively low crop density and low numbers of apothecia, 14 of 60 apothecia were outcrossed. In field #2, which was less heavily infested in the spring and had a higher crop density and abundant apothecia, one of 80 apothecia was outcrossed. We conclude outcrossing occurs and gives rise to genetic variability. We also assessed the levels of genetic variability within the field populations by testing single ascospore progeny from the homothallic (selfing) apothecia for mycelial incompatibility. We found that 42 of 46 isolates from field #1 were mycelially incompatible with all other isolates. From field #2, 75 of 79 isolates were mycelially incompatible with all other isolates. We conclude that S. trifoliorum has multiple incompatibility genes and that field populations are genetically diverse.

45. The PWL host-species specificity gene family in the rice blast fungus.

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Poor understanding of the mechanisms underlying the specificity of interactions between fungal pathogens and plants has handicapped resistance breeding and other control strategies for these economically devastating diseases. Molecular cloning of fungal genes that determine the specificity of fungal-plant interactions now opens up an opportunity to study the biochemical and genetic basis of these important but elusive interactions. We have cloned and characterized members of the PWL gene family implicated in controlling host- species specificity in an emerging model system for fungal pathogenesis, Magnaporthe grisea. The rice blast fungus M. grisea is the cause of the major disease of this widely cultivated cereal grain. M. grisea also attacks diverse grass species, including

millets, wheat, barley, and maize. Members of the PWL gene family are highly divergent and ubiquitous in nearly all host-specific forms of M. grisea. The PWL genes have a mode of action that is analogous to that of classical avirulence genes that determine cultivar specificity. The proteins encoded by PWL genes are predicted to be low molecular weight, glycine-rich, hydrophilic, and secreted. The working model is that the proteins encoded by PWL genes are secreted by M. grisea and directly trigger host defense response.