HOST-FUNGUS PATHOGENIC INTERACTIONS

Organizers: Donald Nuss and Yigal Koltin

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Plenary Sessions *Page*

Poster Sessions

Host-Fungus Pathogenic Interactions

Keynote Address

B4-001 MECHANISMS OF FUNGAL PATHOGENESIS, William E. Timberlake, Myco Pharmaceuticals Inc., One Kendall Square, Building 300, Cambridge, MA 02139

In the past decade, gene cloning and manipulation technologies that were originally developed for model fungal systems, such as *Saccharomyces, Neurospora*, and *Aspergillus*, have been adapted to many fungal pathogens of plants and humans. Thus, it is now practical and efficient to clone genes encoding products thought to be important to the host-pathogen interaction from almost any fungal species, to disrupt or delete the gene, and to test formally the effect of its loss on pathogenicity and virulence. The resulting steady flow of data has significantly altered our perception of which proposed pathogenic functions are essential and which are dispensable, with many surprises. While reviewing several recent examples to illustrate this point, I will indicate some of the potential problems and pitfalls in interpreting data from gene disruption experiments. I will then discuss how the results can be used in the discovery of novel agents that may interfere with the disease process. I will conclude that the knowledge we have now and that we will gain in the near future provides exciting opportunities to develop new methods and strategies for modifying plant and human diseases caused by fungi.

Recognition, Attachment and Penetration I

B4-002 **CHARACTERIZATION OF OLIGOGLUCOSIDE ELICITOR-BINDING PROTEIN(S) IN SOYBEAN ROOT PLASMA MEMBRANES**, Michael G. Hahn, François Côté, Rob Alba, and Jian Yang, The University of Georgia, Complex Carbohydrate Research Center and Department of Botany, 220 Riverbend Road, Athens, Georgia, 30602-4712, USA.

Glucans isolated from the mycelial walls of the phytopathogenic oomycete *Phytophthora sojae* induce a major defense response in soybean, the biosynthesis and accumulation of anti-microbial compounds (phytoalexins). The smallest elicitor-active glucan fragment that has been fully characterized is a branched hepta-β-glucoside that induces half-maximal accumulation of phytoalexins in soybean cotyledons at a concentration of ~10 nM. Detailed structure-activity studies carried out using chemically synthesized oligosaccharides have demonstrated that all three non-reducing terminal glucosyl residues are essential for biological activity, while the reducing terminal glucosyl residue is not essential and can be modified to yield fully active biochemically useful elicitor derivatives. A detailed understanding of how the hepta-β-glucoside elicitor triggers the biosynthesis and accumulation of phytoalexins requires elucidation of the cellular signaling pathway(s) induced by this signal molecule. Our research has focused on an early step in this signal pathway, the specific recognition of the hepta-β-glucoside elicitor by binding protein(s) (putative receptors) in soybean cells. Proteinaceous binding sites for a radio-iodinated tyraminyl derivative of the hepta-β-glucoside elicitor are present in membranes prepared from all major parts of soybean seedlings. These elicitor binding proteins (EBPs) co-migrate with a plasma membrane marker (vanadate-sensitive ATPase) in linear sucrose density gradients. Binding of the radiolabeled hepta-β-glucoside elicitor was specific, reversible, saturable, and of high affinity (apparent K_d = 0.75 nM). Competitive displacement of the radio-labeled hepta-β-glucoside elicitor with a number of chemically synthesized elicitor-active and structurally related, but less active oligoglucosides demonstrated a direct correlation between the ability of an oligoglucoside to displace the labeled elicitor and its elicitor activity. Thus, the EBPs recognize the same structural elements of the hepta-β-glucoside elicitor that are essential for its phytoalexin-inducing activity, suggesting that the EBPs are physiological receptors for the elicitor. The EBPs have been solubilized using the non-ionic detergent, *n*-dodecylsucrose, and the solubilized EBPs purified on an affinity column containing immobilized hepta-β-glucoside elicitor. The *n*-dodecylsucrose-solubilized, affinity-purified EBPs retain the binding affinity (apparent K_d = 1.3 nM) for the radiolabeled elicitor and show the same specificity for elicitor-active oligoglucosides determined previously for the membrane-localized EBPs. Affinity purified EBP preparations contain multiple polypeptides and experimental evidence suggests that a high molecular weight protein complex is required for elicitor-binding activity. Current research is directed toward the identification of the polypeptides essential for elicitor-binding activity and purification of sufficient quantities of those polypeptides for detailed characterization.

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B4-003 FUNGAL CELL GROWTH AND DIFFERENTIATION: TOPOGRAPHY AND HYDROPHOBICITY CUES OF THE SUBSTRATUM, H. C. Hoch[1], A. Corrêa Jr.[1], K. C. Kuo[1], J. Lamboy[1], and R. C. Staples[2], [1]Department of Plant Pathology, Cornell University, NYSAES, Geneva, New York 14456, and [2]The Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, New York.

Fungal plant pathogens are most often dispersed by wind and rain to host surfaces as spores. Once on the plant surface the spores are triggered to germinate and grow for varying times before they prepare for ingress into the host tissues. Such entry is achieved with a variety of specialized infection structures; however, for most fungi the appressorium is developmentally the first and most important structure to be formed in preparation for host colonization. It must be positioned at an appropriate site on the host in a timely way so that subsequent infection can be assured. For fungi that cause rust diseases of plants, positioning of the appressorium is the most critical stage because invasion of the host can occur only via the stomata. Urediospores of these fungi, e.g., *Uromyces appendiculatus,* germinate and grow, directed by the leaf surface topography toward stomata where they cease growth and develop appressoria directly over the stomatal openings. The germlings recognize stomata by the architectural characteristics of the stomatal complex, viz., the cuticular 'lips' of the guard cells. Using photo- and electron beam lithography, various topographical patterns have been microfabricated in silicon or SiO_2 media to mimic topographical features of host leaves (1). For *U. appendiculatus*, the signal for appressorium initiation was determined to be an abrupt change in substratum elevation, e.g., a 0.5 μm high ridge. The region of the fungal cell thigmoresponsive to topographical signaling was determined to be located within the apical 20 μm, and only at the cell-substratum interface. Signal reception for appressorium initiation occurs within 1 min of cell contact with an inductive signal; however, the signal must be persistent for at least 20 min for the cell differentiation process to be completed which takes about 50 min (2). For optimal perception of topography the germlings must be tenuously adhered to the substratum, and hydrophobicity is an important requisite for such adhesion. Topographical signal parameters for appressorium initiation have been characterized for thirty additional species of rust fungi.

Spores of most fungi germinate upon hydration; however, certain criteria sometimes must first be met such as removal of germination self-inhibitors as in many rust fungi. *Guignardia bidwellii*, the causal agent of black rot of grape, has a unique physical requirement for germination; it must adhere to the substratum in order for germination to ensue. Later appressoria will develop. The wettability of the substratum is an important factor in determining adhesion, and hydrophobic properties are particularly favorable for spore adhesion for this fungus.

1. Hoch, H. C., Staples, R. C., Whitehead, B., Comeau, J., and Wolf, E. D. 1987. Signaling for growth orientation and cell differentiation by surface topography in *Uromyces*. Science 235:1659-1662.
2. Corrêa Jr., A., and H. C. Hoch. 1995. Identification of thigmoresponsive loci for cell differentiation in *Uromyces* germlings. Protoplasma (in press).

B4-004 BREAKING AND ENTERING - EARLY STAGES OF PATHOGENESIS LEADING TO RICE BLAST DISEASE, Richard. J. Howard, Timothy M. Bourett, Margaret A. Ferrari and Kirk J. Czymmek, DuPont Science and Engineering Laboratories, Central Research & Development, Wilmington, DE 19880-0402. We have developed and applied numerous modern techniques for cytological analysis of *Magnaporthe grisea* growth and differentiation related to early stages of interaction with the host plant. Together with physiological studies of appressorium function, our efforts provide a significant advance toward the aim of understanding the biology of this process at a molecular level. Cryo techniques have been especially advantageous, in combination with scanning (SEM) and transmission (TEM) electron microscopy, for the enhancement of ultrastructural, and cytochemical analyses including immuno- and lectino-localizations, and with light microscopy for in situ hybridizations. We have begun to examine the morphogenesis of conidiophores and conidiogenesis from diseased tissues using low voltage, field emission cryo SEM. Incipient conidiophores appear to emerge from diseased tissues through a mechanical rupture in the cuticle. Subsequent events including release of spore tip mucilage, germination, germ tube growth, and appressorium formation have been further characterized, in combination with TEM analysis of specimens prepared by freeze substitution. Spore tip mucilage, which attaches conidia to substrates, is often visible prior to separation of conidia from the conidiophore. Germination from the apical and/or basal conidial cells gives rise to a specialized tip growing cell, the germ tube, which is distinctly different from vegetative hyphae. We have begun to use lectin and antibody probes on thin sections to further characterize these important cell types. Germ tube and hyphal apices exhibit a cluster of cytoplasmic vesicles and actin, but the relative positions of the clusters are different. Both apices also exhibit cell surface specialization identified by a lower affinity for wheat germ agglutinin in a localized region of apical cell wall. Formation of an appressorium follows release of a unique mucilage from the germ tube tip. Swelling of the tip proceeds while maintaining the region of specialized cell surface, now corresponding to the area of contact with the host. Deposition of cell wall melanin signals the final stage of appressorium development. This melanin layer is required for the subsequent buildup of enormous appressorial turgor pressures that facilitate entry of the penetration peg into host cytoplasm. Typical of "higher" fungi, *Magnaporthe* apparently relies upon an as of yet poorly characterized endomembrane system for the synthesis of secretory products. Concanavalin A-binding smooth membrane cisternae probably represent Golgi-equivalent organelles, a hypothesis supported by morphological changes induced by the pharmacological agent brefeldin A.

B4-005 PHYLOGENETICS AND POPULATION GENETICS: NATURAL VARIATION AMONG PATHOGENIC FUNGI, John Taylor[1], Austin Burt[1], Deidre Carter[2], Gina Koenig[2], Vasso Koufopanou[1], and Tom White[2], [1]Department of Plant Biology, University of California, Berkeley, CA 94720 and [2]Roche Molecular Systems, Alameda, CA 94510.

Fungal evolutionary biology has advanced the study of fungal pathogens in several ways. Phylogenetic studies using ribosomal DNA sequences and chitin synthase genes have shown relationships among fungal model systems and pathogens (1), and this information now forms the basis for taxonomic searches of GenBank and Medline using the NCBI Entrez retrieval system (2). Another application of variation in nuclear small subunit rRNA nucleotide sequence has been to design identification systems for fungal pathogens (3), one of which is now widely used in clinics (4). At the other end of the evolutionary spectrum are studies documenting nucleic acid variation within fungal species. For example, studies of plant pathogens have shown the relative contribution of sexual and clonal reproduction to population structure (5) and the spread of genotypes through space and time (6). In our studies, we have defined polymorphic loci as single variable nucleotide positions in arbitrary sequences and are using them to analyze fungal natural history (7). For example, our data show that *Coccidioides immitis*, which was thought to be asexual, is recombining in nature. This surprising information should affect thinking about many phases of coccidioidomycosis, from epidemiology to the search for pathogenicity genes.

1. Taylor, J. W., B. Bowman, M. L. Berbee, and T. J. White. 1993. Fungal model organisms: phylogenetics of *Saccharomyces*, *Aspergillus* and *Neurospora*. Syst. Biol. 42:440-457. 2. Benson, D., D. J. Lipman, and J. Ostell. 1993. GenBank. Nuc. Acids Res. 21:2963-2965. 3. Bowman, B. H. 1992. Designing a PCR/probe detection system for pathogenic fungi. Clin. Immunol. News. 12:65-69. 4. Padhye, A., G. Smith, P. Standard, D. McLaughlin, and L. Kaufman. 1994. Comparative evaluation of chemiluminescent DNA probe assays and exoantigen tests for rapid identification of *Blastomyces dermatitidis* and *Coccidioides immitis*. J. of Clin. Microbiol. 32:867-870. 5. McDonald, B. A., and J. P. Martinez. 1990. DNA Restriction fragment length polymorphisms among *Mycosphaerella graminicola* (Anamorph *Septoria tritici*) isolates collected from a single wheat field. Phytopathology 80:1368-1373. 6. McDermott, J., and B. McDonald. 1993. Gene flow in plant pathosystems. Ann. Rev. Phytopath. 31:353-373. 7. Burt, A., D. A. Carter, T. J. White, and J. W. Taylor. 1994. DNA sequencing with arbitrary primer pairs. Molec. Ecol. 3:(in press).

Recognition, Attachment and Penetration II

B4-006 GENETIC CONTROL OF CANDIDAL ADHESION, William A. Fonzi, Department of Microbiology and Immunology, Georgetown University Medical Center, Washington DC.

The molecular basis of *C. albicans* adherence to host tissue is not clearly defined. Biochemical evidence suggests multiple mechanisms including lectin-like activities, integrin-related proteins, and mannan adhesins. Application of molecular genetics can provide molecular details of these various adhesins and, via reverse genetics, establish their in vivo significance in the infectious process. Adherence to intravascular structures is considered to be a critical step in the egress of *Candida spp.* from the intravascular compartment to hematogenously infected organs and thus, we have focused on the genetic determinants of adherence to vascular endothelium. Genetic determinants of endothelial adhesion were isolated by their ability to confer adhesion upon *S. cerevisiae* cells, which do not normally bind to endothelial cells. *S. cerevisiae* cells were transformed with a genomic library prepared from *C. albicans* ATCC36082 DNA. Cells expressing adherence determinants were isolated by a panning procedure based upon selective binding to monolayers of human umbilical cord endothelial cells. This panning procedure yielded clones containing two unique Candidal genomic segments which significantly increased adherence of *S. cerevisiae* cells to vascular endothelium. These genes also conferred the properties of self-aggregation and adherence to plastic, properties also innate to *C. albicans*. Preliminary biochemical characterization of one of the genes indicated that neither endothelial cell adherence nor self-aggregation of the transformed cells was inhibited by yeast mannans or common monosaccharides. Furthermore, transformed cells did not co-aggregate with plasmid control transformants. Together these data suggest a non-lectin activity involving self-self recognition that may be involved in *C. albicans* adhesion to vascular endothelium.

Postrecognition Signaling and Host Response I

B4-007 PATTERN RECOGNITION MOLECULES IN FIRST LINE HOST DEFENSE. R. Alan B. Ezekowitz[1], Neil Harris[1], Jean-Luc Dimarcq[1], ChiehYing Y. Chang[2] and Steven Sheriff[2]. [1]Department of Pediatrics, Harvard Medical School and Divisions of Hematology/Oncology and Infectious Diseases, Children's Hospital, Boston, MA and [2]Bristol Myers Squibb Pharmaceutical Research Institute, Princeton, NJ

Pattern recognition molecules play a role in first line host defense. The human mannose-binding protein and the human macrophage mannose receptor appear to distinguish the patterns of oligosaccharides expressed on the surface of fungal pathogens from host glycoproteins. Although products of distinct genes, both molecules are members of the C-type family of lectins. The mannose-binding protein circulates in serum and appears to function as an `ante-antibody'. It is considered a collectin as it has a collagen domain and a CRD. MBP assembles as an oligomer of trimers. Analysis of the carbohydrate recognition domain and the neck region of human MBP forms trimer in solution and in crystals. The structure of trimer MBP has been determined in two difference crystal forms. The neck forms an α-helical coiled-coil. The spatial arrangement of the CRD's suggest how MBP forms a recognition unit for branched oligosaccharides that form part of yeast and fungal cell walls. The mannose receptor recognizes unopsonized yeast and fungi. Bound organisms are ingested via this phagocytic receptor. Recent work suggests that *Drosophila* phagocytes are able to ingest yeast via a receptor that is distinct from the mannose receptor. This suggests that pattern recognition molecules also play a role in insect immunity.

B4-008 THE ROLE OF THE MARCKS FAMILY OF PROTEIN KINASE C SUBSTRATES IN PHAGOSOME MATURATION, Alan Aderem, The Rockefeller University, New York, NY 10021.

MARCKS is a widely distributed protein kinase C (PKC) substrate which binds both actin and calmodulin, and which has been implicated in motility, secretion, the regulation of the cell cycle, and transformation. MARCKS localizes to points of actin filament - membrane interaction and appears to regulate actin structure at the membrane, as well as actin membrane interaction. During phagocytosis in macrophages, MARCKS associates with the nascent phagosome together with actin, and remains associated with phagosomes after actin has depolymerized, and phagosome-lysosome fusion has occurred. The role of MARCKS in regulating phagocytosis and phagosome-lysosome fusion will be discussed.

MacMARCKS, a member of the MARCKS family of PKC substrates, is restricted in its expression and is strongly induced in macrophages which have interacted with bacteria. Like MARCKS, MacMARCKS also interacts with calmodulin and actin, and also appears to regulate actin plasticity at the membrane. Upon phagocytosis, MacMARCKS coated vesicles appear to fuse with phagosomes after they have been denuded of actin, and MacMARCKS remains associated with the phagosome till phagosome-lysosome fusion has occurred. The role of MacMARCKS in regulating membrane flow to the phagosome and the effect on virulence factors on this pathway will be discussed.

Postrecognition Signaling and Host Response II

B4-009 ELICITOR-MEDIATED DEFENSE GENE ACTIVATION IN INCOMPATIBLE PLANT/FUNGUS INTERACTIONS, Klaus Hahlbrock, Elke Logemann, Thorsten Nürnberger, Wendy R. Sacks, Dierk Scheel , Elmon Schmelzer and Imre E. Somssich, Max-Planck-Institut für Züchtungsforschung, Abteilung Biochemie, Carl-von-Linné-Weg 10, D-50829 Köln, Germany.

We have used suspension-cultured parsley cells (*Petroselinum crispum*) and an oligopeptide elicitor derived from a surface glycoprotein of the phytopathogenic fungus *Phytophthora megasperma* f.sp. *glycinea* to study the signalling pathway from elicitor recognition to defense gene activation. Immediately after specific binding of the elicitor by a receptor in the plasma membrane, large and transient increases in several inorganic ion fluxes (Ca^{2+}, H^+, K^+, Cl^-) and H_2O_2 formation are the first detectable plant cell responses. These are rapidly followed by transient changes in the phosphorylation status of various proteins and by the activation of numerous defense-related genes, concomitant with the inactivation of several other non-defense-related genes. A great diversity of *cis*-acting elements and *trans*-acting factors appears to be involved in elicitor-mediated gene regulation, similar to the apparently complex nature of the signal transduced intracellularly. With few exceptions, all individual defense responses analyzed in fungus-infected parsley leaves have been found to be closely mimicked in elicitor-treated, cultured parsley cells, thus validating the use of the elicitor/cell culture system as a valuable model system for these types of study.

B4-010 DISEASE RESISTANCE SIGNALS IN PLANTS, Ilya Raskin, José León, Vladimir Shulaev, and Hyung-il Lee
AgBiotech Center, Rutgers Univ., New Brunswick, NJ 08903 USA

Salicylic acid (SA) is a likely endogenous signal in the development of systemic acquired resistance in dicotyledonous plants for the following reasons:

- SA levels increase locally and systemically following inoculation
- SA increases are sufficient for the induction of PR proteins and resistance
- Environmental, developmental, and genetic changes in resistance and PR protein expression correlate with changes in tissue SA
- SA moves in the plant
- Blocking SA accumulation blocks SAR

β-*O*-D-GlucosylSA (GSA) and volatile methyl salicylate are the major metabolites of SA in tobacco. GSA accumulates rapidly in tobacco leaves treated with SA or in the immediate vicinity of TMV-induced lesions. GSA is formed by the action of a UDPglucose:SA glucosyltransferase, which is induced by the high levels of SA present in TMV-inoculated tobacco leaves. The synthesis of SA from benzoic acid in tobacco leaves is catalyzed by a benzoic acid 2-hydroxylase (BA2H). BA2H acts as a Cyt P450 monooxygenase which specifically catalyzes the 2-hydroxylation of BA to SA using atmospheric oxygen. Antibodies against SU2, a soluble Cyt P450 from *Streptomyces griseolus*, depleted the BA2H activity from soluble protein fractions and immunoprecipitated a protein putatively identified as BA2H. BA2H was also activated *in vitro* by hydrogen peroxide. Labeling endogenously produced SA with $^{18}O_2$ demonstrated that systemic increases in SA in healthy tissues can be at least partially explained by SA transport from the TMV-inoculated leaf.

Fungal Morphogenesis and Pathogenesis

B4-011 EXTRACELLULAR pH MODULATION BY COCCIDIOIDES IMMITIS, Garry T. Cole[1] and David Kruse[2], University of Texas, Austin[1] and National Institutes of Health, Bethesda.

Coccidioides immitis is an ascomycetous fungus which resides in alkaline desert soils of the southwestern United States, and is the causative agent of a respiratory disease of humans. *In vitro* growth of the saprobic (mycelial) or parasitic (spherule-endospore) phase results in progressive increase in the pH of the medium, which is due to secretion of ammonia by the fungal cells. Acidification of the defined glucose/salts medium which supports growth of the parasitic phase has been shown to stimulate endospores to sharply increase their production of ammonia. When opsonized endospores are engulfed by murine bronchoalveolar macrophages *in vitro* , they are exposed to an acidic microenvironment. Using a fluorescent, dual-emission pH indicator dye (SNARF; Molecular Probes), phagocytosed endospores have been shown to respond to this acidic microenvironment by increasing their secretion of ammonia. This may represent an important survival response of the pathogen and, therefore, ammonia production may be a significant virulence factor. Both the saprobic and parasitic phases of *C. immitis* demonstrate urease activity, which may be largely responsible for ammonia production by hydrolysis of urea. A developing hypothesis is presented that outlines a possible mechanism by which the microorganism responds to external pH and regulates ammonia production. The proposed enzymatic pathway involves the coordinated expression of arginase, ornithine decarboxylase and urease genes, and may be at least partly responsible for extracellular pH modulation by *C. immitis in vitro* and *in vivo* .

B4-012 THE CONTROL OF PATHOGENIC DEVELOPMENT IN *USTILAGO MAYDIS*. R. Bohlmann, H. Böhnert, M. Bölker, S. Génin, J. Kämper, M. Reichmann, T. Romeis, F. Schauwecker and R. Kahmann. Institut für Genetik und Mikrobiologie, Universität München, Maria-Ward-Str. 1a, D-80638 München, Germany.

In the maize pathogenic fungus *Ustilago maydis* mating and sexual development are governed by the *a* and *b* mating type loci. Haploid cells grow yeast-like and are of two alternative *a* mating types, called *a1* and *a2*. After fusion they form a new cell type, the *a1/a2* dikaryon. If the dikaryon is heterozygous for *b* as well as for *a* the yeast-like growth will cease and filamentous growth is initiated. The *a* locus encodes a pheromone based cell recognition system.

The multiallelic *b* locus is the central control locus for pathogenic development. Each allele encodes two regulatory proteins bEast and bWest which both contain a homeodomain-related sequence motif. bE and bW function only in those pairwise combinations where bE and bW from different alleles are combined. This suggested that the two proteins might interact to function as regulators for development. We have studied the interaction of the two b proteins in various combinations and present evidence for their interaction and gene activation potential. To identify genes that are regulated through the *b* locus either directly or indirectly we have isolated genes that are differentially expressed in haploid strains and in the dikaryon. Another set of genes was isolated by complementation of mutants unable to form the filamentous stage and a third set of genes was isolated by REMI mutagenesis and screening directly for non-pathogenic mutants. We shall discuss the structure of the genes isolated in the various approaches and their presumed role in pathogenicity.

Postrecognition Signaling and Pathogen Response

B4-013 STRUCTURE-FUNCTION RELATIONSHIPS OF ELICITORS OF THE TOMATO PATHOGEN *CLADOSPORIUM FULVUM*,
Pierre J.G.M. De Wit, Matthieu H.A.J. Joosten, Paul J.M.J. Vossen, Ton J. Cozijnsen, Guy Honée, Miriam Kooman-Gersmann & Ralph Vogelsang, Department of Phytopathology, Wageningen Agricultural University, Wageningen, The Netherlands.

Host genotype specificity in the interaction between the biotrophic fungal pathogen *Cladosporium fulvum* and tomato complies with the gene-for-gene model. Success or failure of infection is determined by absence or presence of complementary genes, avirulence and resistance genes, in the pathogen and the host plant, respectively. Resistance, expressed by the induction of a hypersensitive response (HR) followed by other defence responses in the host, is envisaged to be based on recognition of the pathogen, mediated through (in)direct interaction between products of avirulence genes of the pathogen (the so-called race-specific elicitors) and receptors in the host plant, the putative products of resistance genes. Here we report on isolation, characterization and biological function of the race-specific elicitors AVR4 and AVR9 of *C. fulvum* and cloning of their encoding genes. The AVR9 elicitor is a 28 amino acid peptide which induces HR in tomato plants carrying the complementary resistance gene *Cf9*. The *Avr9* gene encodes a pre-pro-protein of 63 amino acids including a signal peptide of 23 amino acids and 12 amino acids which are cleaved off by plant and/or fungal proteases leaving a mature peptide of 28 amino acids. In races of *C. fulvum* virulent on Cf9 genotypes the *Avr9* gene is absent. Transformation of the latter races with the *Avr9* gene leads to transformants which are avirulent on Cf9 genotypes. Disruption of the *avr9* gene in races of *C. fulvum* avirulent on Cf9 genotypes leads to transformants which are virulent on Cf9 genotypes. NMR studies revealed that the AVR9 elicitor is a compact globular molecule consisting of 3 antiparallel β-sheets and possibly an α-helix. Structure-function relation studies on the AVR9 elicitor revealed that by *in vitro* mutagenesis of the *Avr9* gene, elicitor molecules can be produced with HR-inducing activities which are the same higher or lower than the wild type AVR9 elicitor. The mature AVR4 elicitor is a 106 amino acid peptide. The *Avr4* gene encodes a pre-pro-protein of 135 amino acids which is processed in a similar way as the AVR9 elicitor. All strains avirulent on Cf4 genotypes contain an identical *Avr4* gene, while strains virulent on Cf4 genotypes contain single basepair changes in the coding region leading to one amino acid changes in the elicitor molecule. In one strain virulent on Cf4 genotypes a frameshift mutation had occurred. Antibodies raised against the AVR4 protein did not recognize proteins produced by the virulent alleles. This is most probably due to instability of the proteins produced by these alleles, which makes them very difficult to detect. Both avirulence genes are hardly expressed *in vitro* but are strongly expressed *in planta*. Expression occurs as soon as the fungus has penetrated the stomata which was studied with transformants of *C. fulvum* carrying promoter-GUS fusions. *In vitro* expression of the *Avr9* gene can be induced under nitrogen limiting growth conditions, while for *Avr4* no *in vitro* growth conditions inducive for its expression could be found.

B4-014 MOLECULAR STRATEGIES FUNGI USE TO INVADE THROUGH THE STRUCTURAL BARRIERS OF ANIMAL AND PLANT HOSTS,
P.E. Kolattukudy[1], Wenjin Guo[1], Cheng-Shine Hwang[1], Moshe Flaishman[1], Tatiana Sirakova[1], Ramesh Matur[1], Adam Makaryan[1] and J.D. Lee[1], Ohio State Biotechnology Center, [1]The Ohio State University, Columbus, OH 43210.

Fungal pathogens penetrate through the structural barriers in animals and plants during the invasion of their hosts. Many plant pathogens are known to undergo differentiation of their germ tubes into specialized penetration structures called appressoria. The host signals that trigger such differentiation, as well as signal transduction and some unique genes involved in this process will be discussed. How certain fungal pathogens that lay dormant, use hosts ripening hormone to time their infection will be discussed. How phytophathogens penetrate through the polyester barrier cutin and the regulation of expression of cutinase gene by plant signals will be reviewed. The promoter elements and the transcription factors that bind these elements will be discussed. The family of pectin lyase genes involved in penetration through the carbohydrate polymers and their differential regulation of expression will be reviewed. The diverse group of extracellular proteases fungi use to degrade the proteinaceous barriers in the animal hosts during infection of immunocompromised animals will be reviewed. Evidence of their involvement in pathogenesis will be discussed.

Emerging Control Strategies

B4-015 MULTIDRUG RESISTANCE IN *Candida albicans*,
Rina Ben-Yaacov[1], Jeffrey M. Becker[2], Amos Oppenheim[3], Martin Goldway[3], Ruth Schmidt[4], Weidong Jiang[4], Julie Clifford[4] and Yigal Koltin[1,4]. [1]Faculty of Life Sci., Tel Aviv Univ., Ramat Aviv, Israel 69978; [2]Dept. of Microbiology, Univ. of Tennessee, Knoxville, TN 37996; [3]Dept. of Mol. Genetics, Hebrew Univ. Medical School, Jerusalem. Israel 91010; [4]Myco Pharmaceuticals Inc., 1 Kendall Sq., Cambridge, MA 02139.

Candida albicans is not inhibited by certain drugs known to affect other fungi. The basis for this resistance in most cases is not known although it has been assumed to result from unique impermeability of this species. Recent characterization of this phenomena indicates that the source of this drug resistance may be attributed to an active mechanism similar to that of mammalian multidrug resistance. A *C. albicans* gene identified initially as imparting benomyl resistance on *Saccharomyces cerevisiae* was shown later to impart resistance to 5 additional structurally and functionally unrelated drugs. The protein encoded by this gene (designated initially as *Ben^r*) displays features common to efflux pumps that confer drug resistance in both prokaryotes and eukaryotes. However, the amino acids sequence bears no similarity to other known multidrug resistance proteins (MDRs) of the P-glycoprotein family and to the prokaryotic ABC transporter family. Nonetheless, this gene can functionally complement a *S. cerevisiae* mutant affected in the efflux pump conferring resistance to aminotriazole and 4 nitro-quinoline oxide. Gene disruption of *Ben^r* in *C. albicans* indicates that the gene is nonessential and its inactivation results in a gene dose-dependent increase in sensitivity to 3 of the 4 drugs tested. Resistance of homozygous disruptants to benomyl is not altered, suggesting that more than one mechanism contributes to the resistance to this drug. It is proposed that this gene *Ben^r* be renamed as *CaMDR1* to reflect its function. Recently, a second multidrug resistance gene in *C. albicans* was reported. The product of this gene displays close structural similarity to the mammalian P-glycoprotein. The spectrum of resistance conferred by this gene is not known as yet. Also, it is not known whether this gene confers resistance to benomyl.
Drug discovery efforts in recent years have focused on agents that will overcome MDR mechanisms. These efforts were performed without sufficient information on the biological role of MDRs. Currently the physiological role of the MDRs is beginning to unfold. These data raise the possibility that intervention in the normal function of specific MDRs of pathogens may affect their virulence. The effect of disruption of *CaMDR1* on virulence was studied. The results of these tests indicate a role for *CaMDR1* in pathogenesis and suggest a new approach for antifungal drug development.

B4-016 ENGINEERING VIRUSES TO UNDERSTAND AND CONTROL FUNGAL PATHOGENESIS, Donald L. Nuss, Roche Institute of Molecular Biology, Nutley, New Jersey, 07110

Cytoplasmically-transmissible RNA viruses of the genus *Hypovirus* cause reduced virulence (hypovirulence) in the chestnut blight fungus *Cryphonectria parasitica*, thus providing a paradigm for the use of viruses to understand and control fungal pathogenesis. The development of a full-length infectious hypovirus cDNA copy has made it possible to engineer hypovirulent *C. parasitica* strains with specific phenotypic traits and with improved biological control potential. Using an infectious synthetic hypovirus transcript, we were recently able to expand the hypovirus host range to include several fungal species not previously reported to harbor viruses and to extend virus-mediated virulence-attenuation to a new fungal taxonomic family.

Several lines of evidence indicate that hypovirus infection alters regulatory pathways involved in normal fungal gene expression. We recently obtained evidence that one way in which hypoviruses attenuate fungal virulence is by suppressing the accumulation of a key component of a G protein-linked signal transduction pathway that is required for the fungal host to mount a pathogenic response. These results further illustrate the potential utility of hypoviruses as tools for probing and manipulating signaling pathways involved in fungal pathogenesis. They also reinforce the emerging view that the role of putative virulence determinants must be considered within the broader context of the regulatory pathways that govern their elaboration at the host-pathogen interface.

B4-017 SYSTEMIC ACQUIRED RESISTANCE AS A STRATEGY FOR DISEASE CONTROL. John Ryals[1], Helmut Kessmann2, Terrance Delaney[1], Urs Neuenschwander[1], Leslie Friedrich[1], Kris Weymann[1], Kay Lawton[1]. [1]Ciba Agricultural Biotechnology Research Unit, Research Triangle Park, NC. [2]Ciba Crop Protection Division, Basel , Switzerland.

Many plants respond to a pathogen infection by inducing a broad-spectrum, systemic resistant state that is effective against many pathogens for several weeks to months. This systemic acquired resistance (SAR) may serve as the basis for future crop protection strategies. We have identified a number of genes (SAR genes) whose expression correlates well with the onset of SAR. Plants expressing these cDNA's provide significant levels of pathogen tolerance. We have previously shown that salicylic acid is required for SAR signaling. However, the role of SA appears to be in the signal transduction process, not as the translocated signal. Recently, it has been shown that salicylic acid can bind and inhibit a particular isozyme of catalase. In this case, high-levels of SA would lead to a build-up of active oxygen species. This mode of action for SA is apparently an important aspect of its role in plant pathogen interactions, however, it does not appear to be the mode of action involved in signaling SAR gene expression.

We have developed several chemicals that induce SAR when applied to plants. There is potential for some of these chemicals to be used as crop protection compounds. one of these compounds is the synthetic chemical 2,6-dichloroisonicotinic acid (INA). INA works by triggering a signal transduction pathway that is indistinguishable from the bona-fide SAR pathway. INA does not induce the accumulation of SA and it is an active inducer in plants that are unable to accumulate SA. However, it's action is blocked in Arabdiopsis by a pathway mutation that also blocks SAR. Thus INA appears to mimic the action of SA at a step downstream of SA accumulation.

We have studied the SAR signaling pathway using Arabidopsis genetics. Several mutants have been isolated that constitutively express SAR. Also, one mutant has been isolated that can no longer be induced by INA, SA or other chemical induces. The progress of understanding these mutants will be discussed.

Genome Rearrangements and Pathogenesis

B4-018 ACTIVATION OF CRYPTIC GENES AND CHROMOSOMAL ABERRATIONS, Elena P. Rustchenko and Fred Sherman, Department of Biochemistry, University of Rochester, School of Medicine and Dentistry, Rochester, New York 14642.

A systematic analysis of over 100 spontaneous colony morphology mutants of *Candida albicans* established that chromosomal alterations and patterns of carbon assimilation were unique for each strain. A number of other features were also altered in all or some of these mutants. These changes resembled the polymorphic variation found among different wild-type strains of *C. albicans*. Also, we have selected positive mutants gaining the ability to utilize either sorbose or D-arabinose, and these contained chromosomal rearrangements, with each class of positive mutants having alterations of specific chromosomes (1). These findings demonstrated for the first time that chromosomal alterations in *C. albicans* are involved in genetic variation of fundamental functions of this asexual microorganism. Our working hypothesis is that the acquired functions are due to the activation of cryptic genes, and that the chromosomal aberrations are an indirect consequence of mutational process producing the activation, because of common genetic perturbations.

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B4-019 GENES THAT DETERMINE HOST SPECIFICITY IN THE RICE BLAST FUNGUS. Barbara Valent[1], James A. Sweigard[1], Seogchan Kang[1], Leonard Farrall[1], Anne M. Carroll[1], Marc J. Orbach[1,2], and Forrest G. Chumley[1]. [1]DuPont Science and Engineering Laboratories, Wilmington, DE 19880-0402 USA; [2]Dept. of Plant Pathology, University of Arizona, Tucson, AZ 85721

The filamentous ascomycete Magnaporthe grisea causes disease on a wide variety of gramineous hosts, although individual strains of the fungus are limited to infecting one or two grass species. Some examples of host specificity appear to be due to early recognition of the potential pathogen by the host plant, leading to an effective plant defense response. We are characterizing two families of pathogen genes that appear to be involved, directly or indirectly, in producing the pathogen signal molecules that mediate recognition. Functional genes from the PWL2 gene family prevent the fungus from infecting weeping lovegrass, Eragrostis curvula. These host species specificity genes encode proteins (16,000 MW) that are rich in glycine and in hydrophilic amino acids. These proteins have putative signal peptides, suggesting that they are secreted. PWL genes reside in different genomic locations in different M. grisea strains. The PWL2 gene is genetically unstable in some strains of the rice pathogen, and this instability is due to frequent deletion of PWL2 and surrounding sequences. Avirulence genes in the second family function to prevent the rice pathogen from infecting specific genotypes of rice, Oryza sativa. Specifically, the AVR2-YAMO genes encode proteins (24,000 MW) that prevent infection of rice variety Yashiro-mochi. In some strains of the fungus, the AVR2-YAMO gene resides within 1.5 kb of the tip of a chromosome. Frequent spontaneous mutation events that occur at this telomeric AVR2-YAMO locus include deletions, point mutations and insertions. Functional AVR2-YAMO genes have been cloned from strains of the pathogen that infect Digitaria spp. or Pennisetum spp. and not rice. DNA sequences surrounding these non-telomeric AVR2-YAMO genes contain frequent deletions and rearrangements.

Emerging Antifungal Targets

B4-020 ROLE OF CHITIN IN THE VIRULENCE OF CANDIDA ALBICANS, C. Bulawa[1], D. Miller[2], K. Henry[2] and J. Becker[2], [1]Myco Pharmaceuticals, Inc., Cambridge, MA 02139 and [2]The University of Tennessee, Knoxville, TN 37996

We have performed a genetic analysis of the role of chitin, a cell wall polysaccharide, in the virulence of Candida albicans. Mutants with a five-fold reduction in chitin were obtained in two ways: 1) by selecting strains resistant to Calcofluor, a fluorescent dye that binds to chitin and inhibits growth, and 2) by disrupting *CHS3*, the C. albicans homolog of *CSD2/CAL1/DIT101/KTI2*, a Saccharomyces cerevisiae gene that is required for synthesis of ~90% of the cell wall chitin and for chitin synthase III activity. Chitin-deficient mutants have no obvious alterations in growth rate, sugar assimilation, chlamydospore formation, or germination in various media (RPMI, 50% serum, and N-acetylglucosamine-containing medium). When growing vegetatively in liquid media, the mutants tend to clump and display minor changes in morphology, especially at 37°C. Staining of cells with the fluorescent dye Calcofluor indicates that *CHS3* is required for the synthesis of the chitin rings found on the surface of yeast cells and for the synthesis of the chitin located in the lateral wall of both yeast and hyphal cells. Septa stain brightly in both yeast and hyphal cells, indicating that *CHS3* is not required for septum formation. Despite their relatively normal growth, the mutants are significantly less virulent than the parental strain in both immunocompetent and immunosuppressed mice; 10^7 chitin-deficient cells are required to produce the same rate of morbidity as 10^5 control cells. The chitin-deficient strains can colonize the organs of infected mice, suggesting that the reduced virulence of the mutants is not due to accelerated clearing. Additional studies on the interaction of the chitin-deficient mutants and host defense systems are in progress. We have begun the disruption of another gene involved in chitin synthesis, *CHS1*, the homolog of S. cerevisiae *CHS2*, a gene required for normal septation and for chitin synthase II activity. The construction and characterization of these mutants will be presented.

B4-021 GENES ASSOCIATED WITH *ASPERGILLUS* PATHOGENICITY, David W. Holden[1], Michael Hensel[1], Christoph M Tang[2], Herbert N. Arst Jr.[1]. [1]Department of Infectious Diseases and Bacteriology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 ONN, U.K., and [2]Nuffield Department of Medicine, John Radcliffe Hospital, Oxford OX3 9DU, U.K.

Aspergillus fumigatus is a filamentous saprophytic ascomycete. It has only become important medically in the last few decades, with the advent of solid organ and bone marrow transplantation and the more aggressive use of antineoplastic therapy. The inhalation of airborne conidiospores by individuals who are immunosuppressed by these procedures can result in hyphal growth and colonisation of the lung parenchyma, a condition referred to as invasive pulmonary aspergillosis (IPA). Although it is difficult to imagine that the fungus can have evolved virulence determinants specifically for growth in lung tissue, *A. fumigatus* predominates as one of the most important opportunistic pathogens out of the many hundreds of fungal species whose spores are regularly inhaled by humans. We are therefore interested in discovering what physiological characteristics contribute to its virulence. Methods to screen for fungal genes that are important for utilising nutrients in lung tissue will be described. Work is also focussed on fungal metabolic pathways that are known to be absent from mammalian cells, because if any of these are essential for fungal growth in the lung, then they would represent good targets for the design of antifungal drugs. To this end we investigated the pathogenicity of a range of auxotrophic mutants of *Aspergillus nidulans* in a murine model of IPA. The ability of strains carrying mutations in the *lysA* and *hisA* genes to cause pulmonary disease shows that lung tissue provides sufficient amounts of lysine and histidine to supplement the biosynthetic defects in the mutants, and that neither pathway is likely to provide useful antifungal targets. On the other hand, the total non-pathogenicity of strains affected in *p*-aminobenzoic acid and tryptophan biosynthesis means that specific inhibition of any steps leading to the synthesis of these compounds (including the shikimate pathway, which is common to both) would prevent growth of the fungus.

Emerging Antifungal Targets and Resistance Mechanisms

B4-022 DRUG RESISTANCE IN PATHOGENIC FUNGI, David Kerridge, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 2QW, United Kingdom.

Changes in medical practice and in diseases to which humans are exposed, have resulted in an increase in diseases caused by opportunistic fungal pathogens. The most important are, *Aspergillus fumigatus*, *Candida* spp and *Pneumocystis carinii*. There are few antifungal drugs suitable for administering to patients suffering from systemic mycoses. Drugs used clinically are the polyene macrolide antibiotic, amphotericin B, the synthetic azole derivatives, fluconazole and itraconazole and the pyrimidine analogue, 5-fluorocytosine. In the past, drug resistance, with the exception of 5-fluorocytosine, was not a clinical problem, although resistant strains of *Candida* can be obtained in the laboratory. However, with the prolonged prophylactic use of triazole drugs, this picture is changing

Amphotericin B interacts with sterol containing plasma membranes causing proton leakage, cessation of growth and cell death. Drug resistance and selectivity result from the nature of the membrane sterols. Resistance is not a clinical problem The rate limiting step in the interaction of amphotericin B with the plasma membrane is its passage though the cell wall and phenotypic changes in the wall affect the sensitivity of *C. albicans* to this antibiotic. 5-Fluorocytosine is one antifungal drug where resistance is a clinical problem. A significant number of isolates of *C.albicans* are heterozygous for the gene encoding the UMP: pyrophosphorylase. These strains can give rise to sensitive and completely resistant strains. Selectivity results, not from the loss of this enzyme, but from the loss of cytosine deaminase. Extensive use of triazole drugs has resulted in an increase in the number of reports of resistant fungi. In these isolates, resistance results from either a failure of the drug to penetrate the cell envelope (in one instance, the cell wall), or from an altered cyt P450 sterol demethylase having a lower affinity for the drug. One developing problem is the low sensitivity of species of *Candida* other than *albicans* to the azole drugs. *Candida albicans* is diploid with no sexual stage in its life cycle, and that the target is complex (amphotericin B), or that there are multiple targets (azole drugs), as a result the frequency of resistant isolates is low. Where resistance occurs, it results from modification of the target or failure to reach it.

B4-023 GLUCANOSYL TRANSFERASES OF THE FUNGAL CELL WALL, Patrick A. Sullivan[1] and Robert C. Goldman[2], [1]Biochemistry Department, University of Otago, Dunedin, New Zealand and [2]Anti-infective Research, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park,IL.

We previously (1) showed that *Candida albicans* secretes a 34kD glucanosyl transferase which catalyzes the reaction:

$$2G_n \longrightarrow G_{2n-2} + G_2 \qquad \text{and } G_y + G_n \longrightarrow G_{y+(n-2)} + G_2$$

where G_n is a β-1,3 glucan oligosaccharide of five or more residues; eg, laminaripentaose is converted to an octaose and laminaribiose. The G_8 (G_y) can participate in a second-round reaction with G_5 giving rise to a G_{11} and G_2. The transferase has also been purified from the cell wall of *Saccharomyces cerevisiae* and ^{13}C-NMR showed that the enzyme introduces β-1,6 linkages at the transferase sites (2). G_4 serves as an acceptor but not as a donor in the reaction and K_m values for the donor and acceptor sites determined with G_5 and G_4 were 45 mM and 0.4 mM respectively. At concentrations of G_5 <10 μM H_2O competes with the acceptor oligosaccharide and yields G_3 and G_2 but above 1 mM G_3 decreased to below the limits of detection.

N terminal analysis showed that the transferases from both yeasts are encoded the *BGL2* gene previously cloned from *S. cerevisiae* (3). The latter has previously been described as an endo-β-1,3 glucanase (4). The *C. albicans BGL2* codes for 309 amino acid residues and the sequence has 63.2 % identity with the deduced sequence of *S. cerevisiae*. BGL2. Possible functions for the transferase include; the covalent linking of newly synthesized glucan into the wall structure, a repair enzyme and disordering of the β-1,3 glucan fibrils by the insertion of β-1,6 kinks. It is noteworthy that an analogous xylo-glucan endo transferase recently isolated from the cell walls of plants has been ascribed a role in wall expansion (5).

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2. Yu, L., Goldman, R., Walker, G.F., Sullivan, P. and Fesik, S.W. Heteronuclear NMR studies of ^{13}C-labeled cell wall β-glucan oligosaccharides. J. Macromolecular NMR (1993) 3: 429-441.
3. Klebl, F. and Tanner, W. Molecular cloning of a cell wall exo-β-1,3 glucanase from *Saccharomyces cerevisiae*. J. Bact. (1989) 171: 6259-6264.
4. Mrsa, V., Klebl, F. and Tanner, W. Purification and characterization of the *BGL2* gene product, a cell wall endo-β-1,3 glucanase. J. Bact. (1993) 175: 2102-2106.
5. Fry, S.C. Loosening the ties. Curr. Biol. (1993) 3: 355-357.

Late Abstracts

PROCESSING AND SECRETION OF VIRALLY ENCODED ANTI-FUNGAL TOXINS IN TRANSGENIC PLANTS, Hai Kinal,[a] Chung-Mo Park,[1] James O. Berry,[1] Yigal Koltin,[2] and Jeremy A. Bruenn[1], [1] Department of Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14260, [2] Department of Molecular Microbiology and Biotechnology, Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, Israel 69978.

Ustilago maydis is a fungal pathogen of maize and is the causative agent of corn smut. Many strains of *U. maydis* contain resident persistent double-stranded RNA viruses. Some subtypes of these viruses possess distinct RNA segments that encode secreted polypeptide toxins capable of killing other susceptible strains of *U. maydis*. We show here that two of these viral toxins, the KP4 and KP6 killer toxins, are synthesized by transgenic tobacco plants containing the viral toxin cDNA under control of a CaMV promoter. Polypeptides similar in size to the KP4 toxin (as single polypeptide) and to the two components of KP6 toxin, designated α and β, were isolated from the intercellular fluid of two sets of transgenic tobacco plants. The toxin polypeptides possessed activity and specificity identical to that found in toxins secreted by *U. maydis* cells. These findings suggest that the processing and secretion of the toxin polypeptides occurred in the transgenic tobacco plants in a manner analogous to that which occurs in fungi. The normal processing and activity of the KP6 toxin in *U. maydis* requires a subtilisin-like processing protease, Kex2. The Kex2 protein is present in both animal and fungal cells, and is required for the processing of small secreted polypeptide hormones (in animal and fungal cells) and secreted toxins (in fungal cells). Our findings present the first evidence for Kex2-like processing activity in plants. The systemic production of this viral killer toxin in tobacco reveals new insights into the processing and secretion of extracellular proteins in plants and may provide a new method of engineering biological control of fungal pathogens in crop plants.

SYNTHESIS AND REGULATION OF CELL WALL ß 1, 6-GLUCAN IN *SACCHAROMYCES CEREVISIAE*, Howard Bussey, Jeffrey L.Brown and Terry Roemer, Department of Biology, McGill University, Montreal, Canada.

We have explored ß 1, 6-glucan synthesis and shown that this essential polymer is made sequentially in the yeast secretory pathway, and is a glucosyl moeity of glycoproteins. Genes whose products participate in the highly regulated synthesis of this glucan are found cytoplasmically, in the endoplasmic reticulum, in the Golgi, and at the cell surface. Recent work has focussed on two areas:

1. Characterization of the Kre6p and Skn1p proteins, integral membrane Golgi phosphoglycoproteins necessary for ß 1, 6-glucan synthesis. Consistent with their direct role in assembly of the polymer both Kre6p and Skn1p share significant similarity with known glucan binding proteins. Deletion of the yeast protein kinase homolog, *PKC1*, leads to a cell lysis defect. Kre6p is a phosphoprotein that, when overproduced, can partially suppress this *pkc1* defect and, when deleted with *pkc1*, causes synthetic lethality. These suppression and synthetic lethal interactions suggest that *PKC1* participates in cell wall assembly by regulating the synthesis of cell wall components, including ß 1, 6-glucan.
2. Studies on suppressors of mutations in *KRE9*, a gene encoding a cell wall protein involved in ß 1, 6-glucan synthesis, have identified *SKN7*, a gene encoding a functional bacterial two component system implicated in the regulation of the yeast extracellular matrix. Skn7p contains a potential "receiver motif" homologous to that found in bacterial response regulators, signal transducing effector proteins regulated by phosphorylation at conserved aspartate residue coresponding to D427 in Skn7p. Mutations at D427 can lead to diminished or enhanced activity of Skn7p function as a transcription factor, providing evidence that a receiver motif functions in regulating the activity of an effector protein in a eukaryote. Skn7p, when overproduced, also suppressed growth defects associated with a *pkc1* mutation. However, epistasis experiments indicate that Skn7p does not appear to function directly downstream of the *PKC1*-MAP kinase pathway. Rather, Skn7p may function in a two component signal transduction pathway that acts in parallel with the *PKC1* cascade to regulate growth at the cell surface.

THE ROLE OF *FKS* IN 1,3-β-D-GLUCAN SYNTHASE FROM *SACCHAROMYCES CEREVISIAE*, Paul Mazur, Walter Baginsky, Forrest Foor, Nancy Morin, Joanne Williamson, Jennifer B. Nielsen, Cynthia Bonfiglio, Cameron M. Douglas, and Myra B. Kurtz, Merck Research Laboratories, P.O. Box 2000, Rahway, NJ 07065.

1,3-β-D-glucan constitutes an important structural component of yeast and fungal cell walls, the synthesis of which presents a selective antifungal target. *FKS1* and *FKS2* have recently been cloned from *S. cerevisiae* by complementing mutations in *FKS1* confering either echinocandin resistance, or hypersensitivity to calcineurin inhibitors. The homologous pair of genes likely encode redundant components of 1,3-β-D-glucan synthase. Glucan synthase activity from *S. cerevisiae* WT, *fks1Δ*, and *fks2Δ* strains has been characterized. Glucan synthase corresponding to *FKS1* (*fks2Δ*) was 3-8 fold less sensitive to echinocandins and dihydro-papulacandin than that from *FKS2* (*fks1Δ*). No other significant biochemical differences were observed. Glucan synthase specific activity and echinocandin (L-733,560) sensitivity were determined, and Western blot analysis was performed on membrane extracts from *S. cerevisiae* WT, *fks1Δ*, *fks1-1*, *fks2Δ*, and *cnb1Δ*. *FKS1* is the predominant wild-type isoform found in cultures grown in liquid media containing glucose. Levels of *FKS2* are regulated in a calcium/calcineurin dependent manner, explaining the calcineurin inhibitor hypersensitivity of the *fks1-1* mutant. However, the expression of *FKS2* is calcineurin independent in the presence of carbon sources other than glucose. *FKS* homologs have also been identified in other organisms including pathogenic fungi and yeast (*C. albicans, C. neoformans*) by Western blots with anti-FKS antisera. Immunodepletion of membrane bound and solubilized glucan synthase activity by purified anti-FKS2 antibodies, and recovery of activity in the immunoprecipitate has been demonstrated, suggesting that *FKS* is an integral component of glucan synthase.

Recognition, Attachment and Penetration; Postrecognition Signaling

B4-100 CHARACTERIZATION AND SOLUBILIZATION OF A SPECIFIC BINDING SITE FOR CHITIN FRAGMENTS IN SUSPENSION-CULTURED TOMATO CELLS AND MICROSOMAL MEMBRANES, Karl Baureithel and Thomas Boller, Friedrich Miescher Institut, CH-Basel, Switzerland.

Tomato cells in suspension culture have been found to possess a highly sensitive perception system for chitin and chitin derivatives: chitin fragments with a degree of polymerization (DP) ≥ 4 induce a transient alkalinization of the culture medium at subnanomolar concentrations (Felix, G., Regenass, M., and Boller, T., Plant J. 4, 307-316, 1993). In order to prepare a radioactive ligand for studies of binding to potential receptor sites, a chitin fragment with DP 5 was aminated at the reducing end and coupled to *t*-butoxy-carbonyl-L-[^{35}S]methionine via an amidoglycine spacer. This radiolabeled chitin fragment exhibited specific, saturable and reversible binding to whole cells and microsomal membranes, with dissociation constants of 1.4 nM and 23 nM respectively. Binding of the radioligand was competed by chitin fragments of different DP with IC_{50} values (50% inhibition of binding) that closely paralleled the concentrations required for half-maximal biological activity, *i. e.* induction of the alkalinization response. A lipochito-oligosaccharide (Nod factor) from *Rhizobium leguminosarum* stimulated the alkalinization reponse in tomato cells half-maximally at 3nM and competed radioligand binding to the cells with an IC_{50} value of 8nM. These results demonstrate the presence of a high affinity binding site for chitin fragments on the tomato cell membrane that may function as a receptor (Baureithel, K., and Boller, T., J. Biol. Chem. 269, 17931-17938, 1994). We have now solubilized this binding site with detergents, and we are in the process of purifying it by affinity chromatography on chitin.

B4-101 A PATHOGEN-INDUCED ANTIFUNGAL PEPTIDE FROM RADISH LEAVES, Willem F. Broekaert, Franky R.G. Terras, University of Leuven, Willem de Croylaan 42, B-3001 Heverlee, Belgium

Previous work in our laboratory has led to the identification of a novel class of antifungal peptides occurring in seeds of many Brassicaceae, including radish. These peptides, which we term 'plant defensins', are 5 kDa in size and contain 4 intramolecular disulphide bridges. The plant defensins from radish seeds have previously been shown to be preferentially released during germination at amounts sufficient to suppress fungal growth in a zone around the seeds. Northern blot experiments showed that transcripts cross-hybridizing to a radish seed defensin cDNA are present at low levels in healthy leaves and accumulate in leaves infected by fungi or treated with mercury chloride. We now have purified a homolog of these seed peptides from radish leaves treated with mercuri chloride. The leaf plant defensin shares about 90 % identity with the seed plant defensins at the amino acid sequence level. The leaf plant defensin is a potent inhibitor of fungal growth in vitro and causes morphological distortions of fungal hyphae. Experiments are underway to determine which signal molecules intervene in the pathogen-induced accumulation of these antifungal peptides.

B4-102 INHIBITION OF PHENYLALANINE AMMONIA LYASE (PAL) OR CINNAMYL-ALCOHOL DEHYDROGENASE (CAD) SUPPRESSES *Ml-a*, BUT NOT *ml-o* RESISTANCE IN POWDERY MILDEW OF BARLEY, William R. Bushnell, Cereal Rust Laboratory, Agricultural Research Service, U.S. Department of Agriculture, University of Minnesota, St. Paul, MN 55108, Richard J. Zeyen, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, Timothy L.W. Carver, and M.P. Robbins, AFRC, I.G.E.R., Aberystwyth, Dyfed SY23-3EB, UK

AOPP, an inhibitor of phenylalanine ammonia lyase (PAL) and OH-PAS, an inhibitor of cinnamyl-alcohol dehydrogenase (CAD) were tested for effects on *Ml-a* and *ml-o* major gene resistances in powdery mildew of barley. The inhibitors were applied either directly to epidermal tissues dissected from coleoptiles, or through cut ends of detached leaves. With *Ml-a*, which conditions rapid hypersensitive cell death, both AOPP and OH-PAS strongly inhibited the hypersensitive reaction. AOPP was effective at 1 mM with both coleoptile tissue and leaves. OH-PAS was effective at 1-11 µM with coleoptile epidermis; at 1 mM with leaves. The reduction in the hypersensitive response in coleoptile tissue was accompanied by increase in average haustorium size and percentage of germlings that produced hyphae. Furthermore, OH-PAS was effective with coleoptile tissue when applied as late as 16 hr after inoculation, about 2 hr before the beginning of hypersensitive cell death. The results strongly indicate that CAD activity has a direct role in hypersensitive resistance, and suggest that cinnamic acid products of CAD are required. In contrast, *ml-o* resistance, which is expressed by failure of the fungus to penetrate host cells and form haustoria, was unaffected by OH-PAS or AOPP. Autofluorescence localized at attack sites was reduced, but this had no effect on penetration as judged from rates of haustorium formation. These results indicate that *ml-o* resistance does not require PAL or CAD activity for effectiveness, suggesting, in turn, that *ml-o* resistance is independent of the phenyl propenoid pathway. Clearly, the resistances due to *Ml-a* and *ml-o* differ in underlying physiological mechanisms.

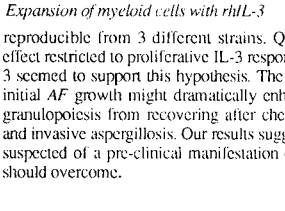
B4-103 *IN VITRO* INHIBITION OF HUMAN NORMAL GRANULO-MONOCYTIC PATHWAY BY *ASPERGILLUS FUMIGATUS*.

P Darodes de Tailly, JL Vignot, C Riché and MCLéglise .Depart. Pharmacology, University Hospital and Military Hospital, BREST, FRANCE. *Grants from INSERM-CRAM-Bretagne n°ANCO1-91.*

Our aim was the analysis of the interactions between *Aspergillus Fumigatus* (*AF*) and human normal granulopoiesis, because AF infection is a major problem among nosocomial infections.The occurence of invasive aspergillosis during long term aplasia (>1 month) is a frequent (>50 %) and life threatening (>80% mortality) complication in neutropenic (chemotherapy) or immunodepressed (AIDS) patients, and caused by *AF* in 90% of cases.

We used two complementary models of *in vitro* culture of granulo-monocytic (GM) progenitor cells from human umbilical cord blood (HCB): the colony Forming Units (CFU)-GM assay in semi-solid agar and a suspension culture for detailed analysis of the proliferative state of the cells, both stimulated by Interleukine-3 (IL-3) and GM-Colony Stimulating Factor. These models were exposed to increasing dilutions (0,01% to 5% v/v) of Aspergillus Conditioned Media (ACM) obtained from standardized filtrates of cultures of Aspergillus species in RPMI medium: *AF* (3 different strains), *A. Flavus*, *A Niger*, *A Penicilloides* .

On 25 different HCB samples, we observed an important inhibitory effect of *AF* conditioned media on the proliferation of GM progenitor cells, with a 50% inhibitory concentration of 0,12% (v/v), specific to the fumigatus species and



Expansion of myeloid cells with rhIL-3

ACM 1%, v/v	% control growth ± s.e.m.
A. fumigatus	8,5± 2.3
A. niger	94,5±10 *
A. penicill	117,5± 0.5*
A. flavus	93±24*

* Student's T test: p< 0,05

ACM from different species on CFU-GM

reproducible from 3 different strains. Qualitative studies suggested a cytostatic effect restricted to proliferative IL-3 responding cells. *In vitro* rescue assays with IL-3 seemed to support this hypothesis. The toxinogenesis contemporary with *in vivo* initial *AF* growth might dramatically enhance neutropenia duration by preventing granulopoiesis from recovering after chemotherapy, with unrestricted AF growth and invasive aspergillosis. Our results suggest that every long term aplasia should be suspected of a pre-clinical manifestation of AF infection which a specific therapy should overcome.

B4-104 FUNCTIONAL ANALYSIS OF THE *IPIO* GENES OF THE LATE BLIGHT PATHOGEN *PHYTOPHTHORA INFESTANS*, Anke de Jong, Pieter van West, Bart Thomma, Grardy v.d. Berg-Velthuis and Francine Govers, Department of Phytopathology, Wageningen Agricultural University, Binnenhaven 9, 6709 PD Wageningen, The Netherlands

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, *ipi*O1 and *ipi*O2, 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 *ipi*O mRNA level is strongly decreased. In *in vitro* grown mycelium *ipi*O gene expression is induced by nutrient deprivation (Pieterse *et al.*, MGG 244: 269-277). The two *ipi*O 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 the function of IPI-O, biochemical analysis and immunocytological localization were performed. In addition, *P. infestans* transformants producing anti-sense *ipi*O mRNA were obtained and these were tested for their ability to cause disease on potato leaves. Results of these experiments will be presented and the possible role of IPI-O during pathogenesis of *P. infestans* on potato will be discussed.

B4-105 HOST RESPONSES OF AMERICAN ELM TO DUTCH ELM DISEASE FUNGUS, Steven M. Eshita, Jennifer L. Koch, Joseph C. Kamalay, and *Lawrence R. Schreiber, USDA Forest Service and *USDA Agricultural Research Service - National Arboretum, 359 Main Road, Delaware, OH 43015

We have examined the xylem from mature American elms to determine if changes in expressed proteins or RNAs were correlated with *Ophiostoma ulmi* inoculation and the extent of Dutch elm disease (DED) development. Ramets of an American elm were inoculated with *O. ulmi* on June 9 or July 15 of 1993. Disease severity was monitored as the percentage of canopy exhibiting symptoms. Branches were harvested on July 2 and August 13, 1993. Although most of the inoculated trees did not survive beyond 1993, the few survivors were monitored in 1994 for DED and sampled until they also succumbed. Xylem was removed from branches and processed for protein and RNA extraction. Analysis of proteins by two-dimensional gel electrophoresis showed increased levels of low molecular weight proteins for all inoculated samples relative to uninoculated controls in 1993. The amounts of these proteins in inoculated samples did not correlate, however, with the percentages of canopy wilt. In 1994, changes in protein expression were not observed until branches exhibited disease symptoms. The inoculation-induced low molecular weight proteins were not detected in leaf tissue, indicating a xylem-specific response. Amino terminal sequences of two inoculation-induced 16 kDa proteins were distinct from each other and were not homologous to other proteins in sequence databases. The sequence information from the proteins and the RNAs will be used to isolate genes expressed in elm in response to fungal inoculation.

B4-106 CHARACTERIZATION OF A BINDING SITE FOR A FUNGAL GLYCOPEPTIDE ELICITOR SOLUBILIZED FROM TOMATO MICROSOMAL MEMBRANES, Angelika Fath and Thomas Boller. Friedrich Miescher-Institute, P.O.Box 2543, CH-4002 Basel, Switzerland.

Purified glycopeptides derived from yeast invertase act as highly potent elicitors in suspension-cultured tomato cells, inducing ethylene biosynthesis and phenylalanine ammonia-lyase half-maximally at concentrations of 1-5 nM. We previously demonstrated the presence of a high affinity binding site that specifically recognizes this glycopeptide in cells and microsomal membranes of tomato (Basse C., Fath A., and Boller, T., J. Biol. Chem. 268, 14724-14731, 1993). We now found that this binding site had a high affinity for N-linked glycans with nine mannosyl residues from fungal glycoproteins whereas it did not recognize the typical mammalian glycans with nine mannosyl residues. The elicitor binding site could be solubilized in an active form from the microsomal membranes with the neutral detergents dodecylmaltoside, dodecylsucrose, NP40, and with the zwitterionic detergent ZW 3-14. Ligand saturation studies and competition experiments with unlabeled glycopeptides and glycans demonstrated that the detergent-solubilized elicitor binding site had similar binding properties as the membrane-bound binding site (K_d=1.5 nM). Currently, an affinity-chromatography technique is being established for the purification of the glycopeptide binding site.

B4-107 MOLECULAR AND CELLULAR DETERMINANTS FOR THE RECOGNITION, ATTACHMENT AND PENETRATION OF EPITHELIA BY *CANDIDA ALBICANS*, Neil A.R Gow, Alistair J.P. Brown, Ed T. Buurman, Alex Häusler, Bernhard Hube, Graham W. Gooday, Thanuja H.S. Perera, and Caroline Westwater, Department of Molecular Cell Biology, Marischal College, University of Aberdeen, Aberdeen AB9 1AS, Scotland, UK.

During the establishment of infections by *Candida albicans*, the breaching of the human epithelium may be aided by tropic movements, molecular recognition, adherence to and enzymatic breakdown of the tissue. We have shown that hyphae of *C. albicans* exhibit a range of behavioral and tropic movements (thigmotropism) when grown on smooth or contoured surfaces *in vitro*. These may facilitate penetration *in vivo* at sites on epithelia which have a weakened integrity. The glycosylated component of *C. albicans* mannoproteins is thought to be important in the attachment and recognition of the surface, but the relative roles of O-linked and N-linked mannan and of specific glycosyl residues in the host-fungus interaction is not known. To address these questions we have isolated two *C. albicans* homologues of the *Saccharomyces cerevisiae MNT1* gene and have characterised the mannosyl transferase activities and the expression of these two genes in the yeast and hyphal forms. We intend to use gene disruption to determine the effect of specific alterations in the mannan component on the interaction with the host. Penetration of the epithelium is also thought to be facilitated by the production of secretory aspartyl proteinases (sap). We have characterized the expression of seven *C. albicans SAP* genes and shown that *SAP2* is the dominant transcript in yeast cells and *SAP4,5 & 6* are expressed in hyphae. *SAP2* is induced by peptides products of sap2 hydrolysis of protein. Gene disruption of individual and multiple *SAP* loci is now underway to establish the contribution of these gene products to pathogenesis.

B4-108 MOLECULAR BASIS OF THE *ALTERNARIA ALTERNATA* F. SP. *LYCOPERSICI* - TOMATO INTERACTION

Jacques Hille, Bert Overduin, Erik van der Biezen, Frank Takken, Laurent Mesbah, Tarcies Kneppers, John Nijkamp, and Mark van Haaren, Department of Genetics, Institute for Molecular Biological Sciences, BioCentrum Amsterdam, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

The tomato disease Alternaria stem canker, characterised by dark brown cankers on stems and leaf necrosis, is caused by the fungal pathogen *Alternaria alternata* f. sp. *lycopersici*. The main chemical determinants for this plant disease are AAL toxins excreted by the fungus. In tomato there is a single locus, *Asc*, that confers resistance to fungal infection and insensitivity to AAL toxins. It is our aim to understand the molecular basis of plant susceptibility and plant resistance to this fungal infection. As a first step, the *Asc* locus will be isolated and characterised. This disease resistance locus from tomato has been mapped on chromosome 3. Both transposon tagging and map based approaches are followed to clone *Asc*. Using genetically linked transposon insertions, *Asc* targeted transposon tagging experiments were carried out. Screening of 20,000 plants resulted in the identification of 1 plant susceptible to fungal infection and harbouring a number of transposon insertions. Progenies of this plant are being tested for association of the transposon with the mutated *Asc* locus. The *Asc* locus has been mapped in a 2 cM interval flanked by molecular markers. These markers have been used to select YAC clones from a tomato genomic library. Current research focuses on getting the *Asc* locus on a contig of YAC clones in order to construct a physical map of this region and to delimit the position of the *Asc* locus.

B4-109 A PLANT PROMOTER TURNED ON BY A SUSCEPTIBLE FUNGAL INFECTION, James K.Roberts and Anthony J. Pryor Division of Plant Industry, CSIRO, GPO 1600, Canberra, ACT 2601 Australia

Obligate biotrophic fungal pathogens have evolved a complex and finely tuned molecular interaction with their plant hosts as a prerequisite for a susceptible infection. In this interaction, basidiomycete rust fungi develop elaborate host-pathogen interfaces (haustoria) and initiate a number of changes in subcellular structure and biochemistry of the host plant cell. Using the flax (*Linum usitatissimum*) and flax rust (*Melampsora lini*) system we have isolated a cDNA from an mRNA that shows a 10-fold increase in abundance during the susceptible reaction but does not change in the resistant situation. This pattern of expression and the inferred translation product of this gene is unlike any stress or Pathogenesis Related (PR) protein. The inferred gene product does show some features common to aldehyde dehydrogenases. The 5' promoter region of this gene has been isolated, fused to the GUS reporter gene and tested in both transient and stable transformation assays. The transient assay system using a ballistic particle delivery into leaf cells has demonstrated that 2 kbp of the 5' region contains sufficient information to express the GUS reporter gene in leaves infected with rust. This system is currently being used to ascertain the minimal size of this 5' promoter required for the specific expression in infected leaves. The 2 kbp-GUS fusion has been used to transform both resistant and susceptible genotypes of flax via agrobacterium mediated transformation. In both genotypes there is a low constitutive level of GUS expression, an observation consistent with the observed expression pattern of the native gene. In rust infected leaves there is no change in expression in the resistant genotype while in the susceptible interaction GUS activity is highly expressed in those cells in the immediate vicinity of the rust infection. Detailed histology of this induction pattern is under current investigation.

B4-110 CONTROL OF CELL DIVISION AND DEVELOPMENT IN *CANDIDA ALBICANS*.

John Rosamond, Jia-Ching Shieh and Gavin Sherlock, School of Biological Sciences, 2.205 Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, U.K.

START is the major regulatory event of the mitotic cell cycle in budding yeast and occurs late in G1 phase. Completion of START requires the activation of an evolutionarily conserved, 34kDa protein kinase by interaction with G1 cyclins, and commits a cell to a round of mitotic division as opposed to alternative developmental fates.

We are investigating the events at and dependent on START in *Candida albicans* in relation to bud and germ tube formation. We have isolated genes encoding p34 (*CaCDC28*) and two G1 cyclins (*CaCLN1* and *CaCLN2*) from libraries of *Candida albicans* genomic DNA by functional complementation of appropriate conditional mutations in *Saccharomyces cerevisiae*. *CaCDC28* encodes a 317 residue protein kinase that is 78% identical to Cdc28 of *Saccharomyces cerevisiae* and which contains all of the characteristic conserved amino-acid motifs found in this family of proteins. Interestingly, both *Candida* G1 cyclins have most similarity to *Saccharomyces cerevisiae CLN3* which acts as an upstream regulator of other G1 cyclins in regulating both bud emergence and DNA synthesis. In addition, we have shown that the pathway leading to DNA synthesis is also conserved, and that *Candida albicans* contains functional homologues of *Saccharomyces cerevisiae CDC4* and *CDC7*, the only known homologues of these genes. The structure and expression patterns of each of these genes will be described.

B4-111 A FINE STRUCTURE AND CYTOCHEMICAL STUDY OF THE INTERACTION BETWEEN *Fonsecaea pedrosoi* AND RAT NEUTROPHIL, Sonia Rozental*, Wanderley de Souza**, Luiz H. M. Leal** and Celuta S. Alviano#, *Instituto de Biofisica and #Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil; **Universidade Estadual do Norte Fluminense, Campos, RJ, Brasil.

Fonsecaea pedrosoi is one of the etiologic agents of chromoblastomycosis, a subcutaneous disease which shows no major tendence to disseminate to deeper organs. The induction of a granulomatous reaction is frequently observed in subcutaneous mycosis. Our previous studies demonstrated that *Fonsecaea pedrosoi* is able to survive and proliferate in resident macrophages and that activated macrophages are fungistatic but not fungicidal. By contrast our recent studies revealed that neutrophils are able to kill *F. pedrosoi* cells in periods shorter than 10 minutes. Various phases of the interaction process were analysed by light, transmission (TEM) and scanning (SEM) electron microscopy. The kinetc analysis of the interaction process demonstrated no significant difference during the first hour. Electron microscopy and videomicroscopy images showed that neutrophils readly associated with and killed extracellular fungi, however few fungi were ingested. During this process the activation of respiratory burst takes place as evaluated by light and electron microscopy. It seems to be the more important antifungal neutrophil mechanism in addition to peroxidase activity. Acid and alkaline phosphatase production were also detected during the host cell-parasite interaction by using cytochemical analyses.

This work has been suported by FINEP and CNPq.

B4-112 HOST FLAVONOIDS REGULATE VIRULENCE GENE TRANSCRIPTION AND STIMULATE SPORE GERMINATION IN A PHYTOPATHOGENIC FUNGUS, Yijun Ruan, Jie He and David C. Straney, Department of Botany, University of Maryland, College Park, MD 20742

In legume plants some flavonoids have fungistatic properties and are identified as phytoalexins. Pisatin is the isoflavonoid phytoalexin produced by garden pea in response to microbial challenge and other environmental stresses. The *PDA*1 gene carried by *Nectria haematococca* MPVI (anamorph: *Fusarium solani*), a pea pathogen, encodes pisatin demethylase which detoxifies pisatin. The expression of this gene is induced by the presence of pisatin and repressed by glucose and amino acids. In order to study this fungal regulatory system, we recently developed an *in vitro* transcription system and used it to dissect the *PDA*1 promoter. Analysis of cis-acting elements utilized promoter deletions, DNA elements competition and fusion of DNA fragments to a heterologous promoter. Results identified *i*) pisatin-responsive element, *ii*) a Sp1-like general activating element and *iii*) a negative-acting element between the above two elements. Activity of the pisatin-responsive element is closely linked to its ability to bind a 35 kDa protein which is present only in pisatin-treated mycelia. Development of the in vitro transcription system has allowed rapid promoter analysis independent of fungal transformation.

Pisatin also regulates another trait in this fungus likely involved in pathogenesis. Pisatin stimulates germination of macroconidia. Non-toxic flavonoids which activate *nod* gene expression in pea rhizobia also produce this response. Inhibitors of cAMP-dependent protein kinase (PKA) prevent the plant flavonoid-stimulated germination, but not nutrient-activated germination, suggesting that these different stimulatory signals may act through separate signal pathways. The well characterized exudation of flavonoids from legume roots, and the inhibition of spore germination in root exudate we observe with PKA inhibitor, together suggest that plant flavonoids are a stimulatory signal in rhizosphere. The flavonoid response is significant in suggesting that both bacteria and fungi evolved an ablility to recognize specific flavonoids released by their leguminous host.

B4-113 CLONING OF A FUNGAL HYDROPHOBIN EXPRESSED EARLY IN EUCALYPT MYCORRHIZA DEVELOPMENT, Denis Tagu and Francis Martin., Microbiologie Forestière, INRA, 54280 Seichamps, France.

Ectomycorrhizal symbiosis between trees and soil-borne ectomycorrhizal fungi yields an intimate relationship between the plant and its symbiotic partner. The development of ectomycorrhiza involves the differentiation of structurally specialised fungal tissues and interfaces between the symbionts and a highly co-ordinated metabolic interplay. Morphogenesis of ectomycorrhiza results from the expression of plant and fungal developmental programmes and a complex set of signals triggers morphogenetic and physiological changes.

In order to characterized genes involved in the differenciation of the fungal mycorrhizal tissues, a differential screening was performed on a cDNA library from eucalypt ectomycorrhiza. Fungal mRNA populations were characterized and about 50 % of these populations were affected by the formation of the symbiotic organ. One of the clone shared identities with hydrophobins; these are small fungal polypetides involved in multilayer hyphae differentiation and in pathogenicity. The amino acid sequence is characterized by the presence of conserved 8 and by a high hydrophobicity. Our cDNA clone encoded a protein which harboured these characteristics. In ectomycorrhiza, we demonstrated that over accumulation of hydrophobin transcripts took place very early in the colonization process. This could serve as a molecular marker of mycorrhiza differentation and could be a tool for analysing putative root signals triggering symbiotic development.

The role of these hydrophobic proteins on the mycorrhiza formation will be discuss.

B4-114 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 crops including a number of tropical and temperate fruits. The maceration of fruit tissue is caused by the action of an array of pectinolytic and other hydrolytic enzymes secretion by the pathogen. We are using gene disruption as a tool for determining the importance of these enzymes in pathogenicity. Pectin lyase (E.C. 4.2.2.10) degrades pectin by β-(*trans*)-elimination. 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. We have used this locus to optimise gene disruption in *G. cingulata*. Transformants of *G. cingulata* appear at a low frequency but integration can occur with a high rate of homologous recombination. 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, and this method will be used to perform gene-disruptions in *G. cingulata* routinely. 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 and cell surface proteins will also be presented.

B4-115 ISOLATION AND NUCLEOTIDE SEQUENCE OF THE TRICHODIENE SYNTHASE GENE FROM THE MACROCYCLIC TRICHOTHECENE-PRODUCING FUNGUS *MYROTHECIUM RORIDIN* , Susan C. Trapp, and Bruce B. Jarvis (Department of Chemistry and Biochemistry, University of Maryland, College Park, MD USA 20742); Thomas M. Hohn (Mycotoxin Research Unit, USDA/ ARS, Peoria, IL USA 61604)

Trichodiene synthase (TS) catalyzes the cyclization of farnesyl pyrophosphate to trichodiene in the sesquiterpene biosynthetic pathway of simple and macrocyclic trichothecene-producing fungi. Trichothecene mycotoxins are produced by at least 8 genera of fungi and uniquely by 2 plant species of the genus *Baccharis*. The trichothecene mycotoxins have been associated with incidents of mycotoxicoses, feed refusal in grains, and plant pathogenicity. The trichodiene synthase gene (*Tri5*) has been isolated and nucleotide (nt) sequence determined from the macrocyclic trichothecene-producing fungus *Myrothecium rordin* (MR). Two *Tri5* genes have been sequenced and characterized previously in simple trichothecene producers, *Fusarium sporotrichiodes* and *Gibberella pulcaris*. A MR cosmid library was prepared using pSuperCOS-P1 vector, previously described by Hohn et al. A probe was prepared from the *Tri5* sequence of *Fusarium sporotrichiodes* and used to screen the MR cosmid library. Fifteen cosmids were isolated containing a portion of *Tri5* gene. Upon restriction analysis, MRcos14 and MRcos13 were found to be similar and were used to identify and deduced the sequence of the *Tri5* gene by subcloning two 1.7 kb *Hind* III fragments into pBluescript II KS vector. The gene consists of a 1211-nt open reading frame which contains a 58-nt intron. The predicted amino acid sequence (contains 385 residues) and has approximately a 70 % homology with the corresponding *Tri5* genes from *Fusarium sporotrichiodes* and *Gibberella pulcaris*.

B4-116 RECOGNIZING SYSTEM DURING APPRESSORIUM FORMATION IN *Magnaporthe grisea*, Jin-zhong Xiao, Takashi Kamakura, Tadakazu Watanabe and Isamu Yamaguchi, Microbial Toxicology Laboratory , The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01, Japan

Pathogenesis by *Magnaporthe grisea*, the causal fungus of rice blast disease, involves formation of a special infection structure termed an appressorium, which is a specific cellular differentiation from the germ tube. Results obtained were that *M. grisea* germ tubes were potentially anti-hydrotactic although water was essentially required for initializing conidial germination. After germination, germ tubes penetrated soft surfaces directly, but differentiated to form appressoria on solid materials and then penetrated the hard surfaces by penetration hyphae elaborated from the appressoria. We show that increase in frequency of appressorium formation was well correlated with the substratum hardness. Several proteolytic and glycolytic enzymes were found to inhibit germling adhesion and appressorium formation, but not conidial germination. Furthermore, we observed that concanavalin A specifically suppressed appressorium formation but did not significantly affect conidial germination and germling adhesion. These results infer that a) the anti-hydrotaxis of germ tubes relates with the aggressiveness of this pathogen; b) the hardness of substratum triggers appressorium formation; and c) glycoprotein(s) is involved in this signalling system. Approaches toward isolation of genes specifically expressed during the recognizing stage of appressorium formation were in progress by several differentail screening strategies.

Fungal Morphogenesis and Pathogenesis; Emerging Antifungal Targets and Resistance Mechanisms

B4-200 **GENE REGULATION DURING HYPHAL DEVELOPMENT IN *CANDIDA ALBICANS***

Sheila Black, David A Bailey, Gwyneth Bertram, Pascale A F Feldmann, Bernard Hube, David A Schofield, Rachel Smith, Rolf K Swoboda, Graham W Gooday, Neil A R Gow, and Alistair J P Brown, Department of Molecular and Cell Biology, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, UK.

We are interested in the control of hyphal development in *Candida albicans* and its role in the virulence of this pathogenic fungus. As a result we have studied the expression of numerous genes during the yeast-to-hyphal transition. The transition was induced using either serum addition combined with a temperature increase (25°C to 37°C), or a pH shift (pH 4.5 to 6.5) combined with a temperature increase (25°C to 37°C). Quantitative northern analysis revealed that the levels of relatively few mRNAs were unaffected by these changes. These mRNAs encoded a MAP kinase kinase (*HST7*) and glucosamine-fructose-6-phosphate (*GFA1*), and depending upon the conditions used to induce the transition, a chitin synthase (*CHS1*). Some glycolytic mRNAs (*PYK1, PGK1, GMP1* and *ADH1*) decreased transiently during the transition. In contrast, the levels of many other mRNAs increased transiently. These included the actin (*ACT1*), cytochrome-c (*CYC1*), plasma membrane ATPase (*PMA1*), the fungal-specific essential translation elongation factor 3 (*TEF3*), a ribosomal protein (*RP10*), chitin synthases (*CHS2, CHS3*), heat-shock proteins (*HSP90, HSP60*) and ubiquitin (*UBI1*) mRNAs. In all of these cases the changes in mRNA level were associated with the conditions used to induce dimorphism rather than a specific response to the transition *per se*. In constrast a few mRNAs appeared to be controlled specifically in response to the transition. These included a secretory aspartyl proteinase mRNA (*SAP2* which was down-regulated) and an mRNA of unknown function (*HYR1*, for HYphally Regulated, which was up-regulated). Although some of these changes may be important for the transition, it is likely that none of the genes we have studied so far play a role in the regulation of hyphal development. Therefore we have embarked on new screening strategies which should identify genes that exert a positive influence on hyphal development.

B4-201 DIFFERENTIATION OF CLINICAL BLASTOMYCES DERMATITIDIS ISOLATES BY PROTEIN PROFILE AND RESTRICTION FRAGMENT LENGTH POLYMORPHISMS. Stanley W. Chapman, Donna C. Sullivan, and Brenda Chapman. Department of Medicine, University of Mississippi Medical Center, Jackson, MS 39216

The differentiation of medically important fungi for epidemiological purposes has lagged far behind that of other medically important infectious agents. Recently, we have distinguished clinical isolates of Blastomyces dermatitidis (BD) using two methods: protein profiles and restriction fragment length polymorphisms (RFLP). The former employed SDS-PAGE analysis of eleven BD isolates. The number, molecular weight, and distribution of polypeptides into specific fractions (cytoplasmic or cell wall) were used to compute isolate similarity by considering the protein profiles as unweighted parameters. All isolates had similar numbers of polypeptides and specific fraction distributions, with only minor differences in protein molecular weights and total number of bands. A similarity matrix was calculated for all 11 isolates and the isolates were divided into three groups based on percent similarity. Group I, comprised of three isolates, shared 92% of total polypeptides. Group II, comprised of seven isolates, shared 90% within the group and 87% with group one. Group III was composed of a single isolate (isolate 3) and shared only 80% similarity to the either of the other two groups. In order to extend these studies, RFLP analysis was initiated. Genomic BD DNA from isolate 4, a member of group II, was digested with the restriction enzyme HindIII and cloned into the plasmid vector pBluescript. Forty insert containing clones were screened by Southern blot hybridization to HindIII digested DNA from isolates 3 (group III) and 4 (group II). Six clones detected RFLPs. These could be divided into two types: those clones which identified major differences in the molecular weight (>5 kb) of the hybridizing bands and those with only minor differences (<1kb). An additional seven clones also detected seven distinct regions of DNA heterogeneity in that a single BD DNA insert detected multiple hybridizing bands. One clone, pBC1C5, detected both RFLPs and heterogeneity. Further blotting studies employing all isolates as well as ATCC isolates of African origin are currently underway.

B4-202 Virulence mechanisms in the pathogenic yeast *Candida albicans*

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We are interested in the mechanisms by which *Candida albicans* is able to colonize and persist in systemic infections. In this regard, the ability to undergo a morphological conversion from yeast to hyphal phase, while not absolutely essential to virulence, is likely to be extremely important.

To better understand the molecular basis of the yeast/hyphal transition, we have set out to identify genes expressed specifically in the yeast or hyphal phase, using the PCR based RNA display method of Liang and Pardee (1992). In an ongoing study, we have so far identified six differentially regulated genes, among which are a previously identified pH-regulated cell surface glycoprotein, and a novel hyphal-induced superoxide dismutase.

We have also identified a *C. albicans* homolog of the mammalian enzyme leukotriene A4 hydrolase (LTA4 hydrolase) which converts LTA4 to LTB4. LTB4 is chemotactic for, and stimulates lysosomal enzyme release from, neutrophils, the critical immune cell in host defense against systemic candidiasis. Since *C. albicans* lacks arachidonic acid, the precursor of all leukotrienes, the LTA4 hydrolase homolog could be relevant in the interaction of the yeast with its mammalian host. We have expressed the gene in *E. coli*, and purified the enzyme as a fusion protein; it is active on LTA4, catalyzing its conversion to a compound different than LTB4. Conceivably, this enzyme is made by *C. albicans* to compete with host LTA4 hydrolase and reduce synthesis of LTB4, thereby reducing inflammation. We are currently constructing a *C. albicans* strain deleted for the LTA4 hydrolase homolog to directly assess its role in virulence in a mouse model for systemic infection.

B4-203 A PUTATIVE TRANSCRIPTION FACTOR OF THE DIMORPHIC FUNGAL PATHOGEN CANDIDA ALBICANS STIMULATES PSEUDOHYPHAL MORPHOGENESIS IN SACCHAROMYCES CEREVISIAE, Joachim F. Ernst, Anja Sonneborn and Volker Stoldt, Institut für Mikrobiologie, Universität Düsseldorf, D-40224 Düsseldorf, FRG

We have identified and sequenced a gene of the fungal pathogen *Candida albicans*, designated *EFG1*, whose overexpression stimulates pseudohyphal morphogenesis in the yeast *Saccharomyces cerevisiae*. The deduced Efg1 protein shows features of a transcription factor, including stretches of glutamine residues and a high proportion of alanine, serine, threonine and proline residues; its predicted molecular weight is 59.9 kDa. A central block of Efg1p contains 65% identical and 80% similar residues compared to the StuA protein required for conidiophore morphogenesis of *Aspergillus nidulans* and to the Ykl256/Phd1-protein of *S. cerevisiae*, whose function is unknown; this result suggests that Efg1p belongs to a conserved class of fungal proteins. During hyphal formation of *C. albicans EFG1* transcript levels decline rapidly indicating that high levels of *EFG1* expression depend on the yeast growth form. Experiments designed to disrupt both *EFG1* alleles or to overexpress Efg1p in *C. albicans* are in progress and will be discussed.

B4-204 *TEC*1, A NEW ROLE IN PSEUDOHYPHAL GROWTH. AN *ASPERGILLUS NIDULANS abaA* HOMOLOGUE? V.Gavrias and W.E. Timberlake, Myco Pharmaceuticals, One Kendall Square, Cambridge, MA 02139

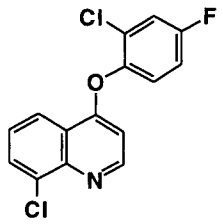
A typical feature of many pathogenic fungi is their ability to interconvert between a yeast and filamentous growth pattern. A characteristic of such dimorphic nature is the production of morphologically distinct cell types through alterations in the polarities and patterns of cell divisions. *Aspergillus nidulans* and *Saccharomyces cerevisiae* are providing insights into the mechanisms controlling such morphogenesis. They offer 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, suggesting the existence of a similar developmental pathway in yeast as well as the presence of an AbaA homologous protein. The *S.cerevisiae TEC*1 gene product, involved in Ty element transcriptional control, has been suggested to share a DNA binding domain with AbaA, and 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.

B4-205 MOLECULAR ANALYSIS OF BENZIMIDAZOLE RESISTANCE IN *RHYNCHOSPORIUM SECALIS*, Marie Claire Grosjean-Cournoyer, Ian Wheeler, Sheila Kendall and Derek Hollomon, Department of Agricultural Sciences, University of Bristol, Long Ashton, BS18 9AF, UK.

Benzimidazole fungicides inhibit the function of ß-tubulin. Resistance is associated with reduced binding to ß-tubulin, and in many pathogens is linked with increased sensitivity to phenylcarbamate fungicides. This negative cross-resistance has been exploited using mixtures as an anti-resistance strategy. We have examined the molecular basis of this negative cross-resistance in the plant pathogen *Rhynchosporium secalis*, and have identified several single DNA base changes in the ß-tubulin gene that confer resistance. Only changes at amino acid codons 198 and 200 have so far been identified with benzimidazole resistance in field strains (Table), although in laboratory generated mutants changes at 10 other sites within the ß-tubulin protein can confer resistance. Replacement of glutamic acid 198 with the smaller amino acid glycine, confers negative cross-resistance, whereas other changes at codons 198 and 200 generate resistance to both fungicide groups. Some of these changes are accompanied by poor pathogenicity indicating that the function of ß-tubulin is impaired. This work not only provides information on the structure of the benzimidazole binding site, but should lead to rapid and precise methods for identification of the various resistance alleles, and better management of benzimidazole/phenylcarbamate mixtures as an anti-resistance strategy.

B4-206 DIHYDROORTATE DEHYDROGENASE: A NEW ANTIFUNGAL TARGET IDENTIFIED BY MODE OF ACTION STUDIES ON THE 8-CHLOROPHENOXYQUINOLINE FUNGICIDE LY214352, Matthew J. Henry, Amy J. Smith, Gary D. Gustafson, Clive Waldron and George E. Davis, DowElanco, Discovery Research, 9330 Zionsville Rd., Indianapolis IN 46268-1054

A new antifungal target has been identified by characterizing the biochemical mechanism of LY214352.



The enzyme dihydroorotate dehydrogenase (DHO-DH) has been shown biochemically and genetically to be the target site. This study combined a traditional biochemical approach of mode of action studies with a molecular biological approach of cloning and identifying a fungicide resistance gene. Differential growth inhibition of *Aspergillus nidulans* sensitive and resistant strains correlates with sensitivity of the isolated enzyme. The DHO-DH gene from *A. nidulans* has been cloned and has significant homology to *E. coli* DHO-DH. Resistance is caused by a single nucleotide change in the gene encoding the target enzyme. Unlike most reported inhibitors of DHO-DH, LY214352 is competitive with the cofactor ubiquinone, and is uncompetitive with the substrate dihydroorotate.

B4-207 CLONING OF THE STE20 PROTEIN KINASE GENE FROM *CANDIDA ALBICANS*, Ekkehard Leberer, Karen L. Clark, Daniel Dignard and David Y. Thomas, NRC Biotechnology Research Institute, 6100 Royalmount Avenue, Montreal, Quebec H4P 2R2, Canada

In haploid*S. cerevisiae* cells, the Ste20p protein kinase is required for transmission of the mating pheromone signal from the β and γ-subunits of the mating response G-protein to a downstream MAP kinase cascade, assembled by the Ste11p, Ste7p and Fus3p/Kss1p protein kinases (Leberer *et al.*: EMBO J. 11, 4815-4824, 1992). This cascade carries the signal to a transcription factor encoded by the *STE12* gene. In diploid cells, the Ste20p, Ste11p and Ste7p protein kinases and the Ste12p transcription factor are also involved in the regulation of the morphological switch from the yeast to the filamentous form in response to nitrogen starvation (Liu *et al.*: Science 262, 1741-1744, 1993). It is conceivable that similar regulatory components are involved in the morphological switching of *C. albicans*, which is a dimorphic fungus and an opportunistic human pathogen.

We have cloned the *STE20* gene homologue from *C. albicans* by functional complementation in *S. cerevisiae*. The deduced protein shares, in the kinase domain, more than 90% sequence identity with the *S. cerevisae* protein. The *C. albicans* gene compensates both the mating and filamentous growth defects of *ste20* -deleted *S. cerevisiae* cells. This high degree of structural and functional similarity suggests that the Ste20p protein kinase might also play a role in the morphological switching of *C. albicans*.

B4-208 EVIDENCE FOR GROWTH INHIBITORS OF CANDIDA ALBICANS IN HUMAN VAGINAL FLUID, Shawn Lockhart[a], Rudolph Galask[b], Philip Wertz[c], and David R. Soll[a], [a]Department of Biological Sciences, [b]Department of Obstetrics and Gynecology, [c]Dowes Institute for Dental Research, The University of Iowa, Iowa City IA 52242

Approximately 40% of healthy women carry Candida as a commensal in the vulva and vaginal regions without any symptoms of vulvovaginitis. More interestingly, approximately 60% of women do not carry detectable levels of Candida in the vaginal canal. The difference between the vaginal fluid of women with no carriage and women with high carriage or an infection may be the key to understanding the basis of vaginal candidiasis. Vaginal canals of women with no, low, and high carriage (or infection) were washed with a small volume of sterile saline, and the lavage was centrifuged to remove vaginal mucosal cells and filtered to remove bacteria and endogenous yeast. The sterile fluid was then inoculated with a laboratory strain of Candida albicans and growth was assessed. Of eight women with no yeast carriage, the vaginal fluid of five supported growth with short generation time and high final concentrations, that of two supported growth at a reduced level and one did not support growth. Of four women with low to medium carriage, the vaginal fluid of three supported high levels of growth and that of one did not support growth. Finally, of three women with very high carriage (or infection), the vaginal fluid of one supported a high level of growth while that of two did not support growth. An analysis of fluid for total protein, total glucose, total charrable carbon and lactate demonstrated no significant differences between fluid supporting and not supporting growth. Addition of zinc, a growth limiting component of laboratory medium, did not activate growth. These results suggest that vaginal fluid can contain labile or stable inhibitors of Candida growth and these natural components may represent the natural defense mechanisms against yeast infections in vaginal fluid. We are presently trying to identify these components.

B4-209 MYRISTOYLCOA:PROTEIN N-MYRISTOYLTRANSFERASE IS AN ESSENTIAL ENZYME IN *C. NEOFORMANS* AND *C. ALBICANS*. Jennifer K. Lodge, Robin Weinberg*, Emily Jackson-Machelski, Steve Lee*, Dena Toffaletti+, John Perfect+, and Jeffrey I. Gordon, Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO,*GD Searle Co., St. Louis, MO, and the +Department of Medicine, Duke University, Durham, NC.

MyristoylCoA:protein N-myristoyltransferase (Nmt) is a monomeric enzyme that catalyzes the cotranslational transfer of myristate from myristoylCoA to the N-terminal glycine of cellular proteins with diverse biological functions. Studies of *S. cerevisiae* strains have established that Nmt is essential for vegetative growth. *nmt1-181* encodes a mutant enzyme with a Gly451 to Asp substitution that produces a reduced affinity for myristoylCoA, global defects in protein N-myristoylation at ≥30°C, growth arrest at various stages of the cell cycle within 1 h after cells are shifted to ≥30°C and lethality within 8 h. The growth arrest phenotype and loss of viability can be correlated with the appearance of nonmyristoylated pools of two essential cellular N-myristoylproteins, ADP ribosylation factors 1 and 2 (Arf1p and Arf2p). *nmt1-181* strains can be rescued at 37°C by overexpressing nmt181p or by adding myristate to the media. Metabolic labeling studies revealed that *C. neoformans* and *C. albicans*, two of the leading causes of systemic fungal infections in immunocompromised humans, produce several N-myristoylproteins during vegetative growth, the most prominently labeled being Arf. To determine whether Nmt is an essential enzyme in these organisms, we introduced a Gly^{487} -> Asp mutation into *C. neoformans NMT* and a Gly^{446} -> Asp mutation in *C. albicans NMT* by homologous recombination. These mutations, analogous to the mutation in *nmt1-181*, produce a temperature dependent reduction in the activity of the fungal Nmts as judged by co-expression of the wild type or mutant Nmt together with their Arf substrates in *E. coli*. Strains with the mutant allele are myristic acid auxotrophs at 24°C. This phenotype is stable with a reversion frequency of $< 10^{-8}$. Withdrawal of myristate from the media produces lethality within 24-48 h for both organisms. This lethality is associated with undermyristoylation of cellular Arfs. An immunosuppressed rabbit model of cryptococcal meningitis was used to establish that the *nmt487D* strain is completely cleared from the cerebrospinal fluid within 12 d while an isogenic *NMT* strain produces continuous infection and death. Finally, *in vitro* studies of purified human and fungal Nmts revealed significant differences in their peptide substrate but not their acylCoA specificities. Together, these data indicate that Nmt is an attractive target for development of fungicidal drugs.

B4-210 CHARACTERISATION OF THE *USTILAGO MAYDIS* KILLER TOXIN P4 PRECURSOR

Susanne Logemann[1,2], Dietmar Wurm[1] and Jeff Schell[1], Max-Planck-Institut für Züchtungsforschung, Carl-von-Linne'-Weg 10, 50829 Koeln, Germany; [2]Institute of Plant Molecular Sciences, Clusius-Laboratory, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

Some strains of the corn smut fungus *Ustilago maydis* carry myco-viruses which code for so called 'killer' proteins. These proteins have the ability to specifically kill cells or arrest cellular growth of *Ustilaginales*. The genetic information of the viruses is located on a segmented double stranded RNA-genome, of which the M-2 segment codes for the killer toxin ([1]). In *Ustilago*, the killer protein is translated as a larger precursor protein, posttranslationally maturated and secreted by the fungal cells. The mature killer protein of strain UmP4 was designated "Ustilin". Major parts of the M2 segment were cloned and the sequences for Ustilin were fused to the proteinase inhibitorII signal sequence from potato and transformed to plants. Ustilin was proved to be biologically active and secreted into the intercellular space of transgenic tobacco leaves ([2]). In order to obtain the complete sequence of the Ustilin precursor, this proteine was isolated and characterised. Glycosylation of the precursor was demonstrated by ConA-blotting and the N-terminal protein sequence was determined.

1 Shelbourne, S.L.; Day, P.R. and Buck, K.W. J. of Gen. Virology (1988), 69, 975-982
2 Logemann, S.; Bollhoefer, C. and Schell, J. J. of Cellular Biochem., Suppl. 18A, p 88 (1994)

B4-211 CLONING OF A *CANDIDA ALBICANS* PEPTIDE TRANSPORT GENE: A MOLECULAR APPROACH TO DRUG DELIVERY, M.Lubkowitz[1], M. Basrai, F. Naider[2], and J. Becker[1], [1]Dept. of Microbiology, University of Tennessee, Knoxville, Tennessee, USA; [2]Dept. of Chemistry, City University of New York, Staten Island, NY, USA.

A *Candida albicans* peptide transport gene, *CaPTR2*, was cloned from a *C. albicans* genomic library by functional complementation of a peptide transport deficient mutant (strain *ptr2-2*) of *Saccharomyces cerevisiae*. *CaPTR2* restored peptide transport to transformants as determined by uptake of radiolabeled dileucine, growth on dipeptides as sources of required amino acids, and restoration of growth inhibition by toxic peptides. Plasmid curing experiments demonstrated that the peptide transport phenotype was plasmid borne. *CaPTR2* was localized to Chromosome R of *C. albicans* by CHEF gel chromosome blots. Deletion subclones and frame shift mutagenesis were used to narrow the peptide transport complementing region. DNA sequencing of the complementing region identified an open reading frame of 1869 bp containing an 84 nucleotide intron. The deduced amino acid sequence predicts a protein (CaPtr2p) of 70 kDa consisting of 623 amino acids with 12 hydrophobic segments. A high level of identity was found among CaPtr2p and peptide transport proteins of *S. cerevisiae* and *Arabidopsis thaliana*. Further sequence analyses has identified similarity among additional membrane proteins mediating the uptake of small peptides into various organisms. This growing group of proteins has been termed the **PTR** (**P**eptide **TR**ansport) family. This study represents the first steps in the genetic characterization of peptide transport in *C. albicans* and initiates a molecular approach for the study of delivery of drugs against antifungal targets within this pathogen.

B4-212 **MOLECULAR CLONING, CHARACTERIZATION AND DISRUPTION OF CHITINASES FROM *Candida albicans*,** *Kenneth J. McCreath, Charles A. Specht and Phillips W. Robbins, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA. 02139*

Candida albicans is a dimorphic fungus that can grow in a yeast or filamentous (hyphal) phase depending on environmental conditions. It's genome is diploid but a sexual cycle has still to be elucidated. *C. albicans* exists as a commensal of warm-blooded animals and humans, colonizing the mucosal surfaces of the oral cavity and vaginal tract. Pathogenicity of this organism is normally held in check by host-cell defences, but it is a major cause of nosocomial infection.

Chitin is a major component of *C. albicans* cell wall and enzymic chitinase activity can be detected throughout the growth phase, both in whole cells and culture supernatant. We have cloned three chitinase genes from this human pathogen (*CHT1*, *CHT2* and *CHT3*). *CHT2* and *CHT3* have been sequenced in full and both predicted proteins have a molecular weight of 60kDa. The two genes are about 38% similar to each other and 36% similar to the chitinase from *Saccharomyces cerevisiae*. Homology is greater around the proposed catalytic domain, which they share with chitinases from fungi, plants and bacteria. Transcription of both *CHT2* and *CHT3* is greatest when the organism is grown in the yeast phase, rather than hyphal. A transcript of *CHT1* could not be found in either growth condition. When *CHT2* and *CHT3* were transformed into a deletion strain of *S. cerevisiae* a low level of chitinase activity could be detected in washed cell preparations. *CHT2* and *CHT3* have been sequentially knocked-out using the ura-blaster strategy and phenotypic characterization of the null mutants will be presented.

B4-213 EXTRACELLULAR ANTIFUNGAL PROTEINS IN SUGAR BEET LEAVES, Klaus K. Nielsen, Karsten M. Kragh, Susan Madrid and Jørn D. Mikkelsen. Danisco Biotechnology, Langebrogade 1, DK-1001 Copenhagen K, Denmark.

The intercellular washing fluid from leaves of sugar beet contains a number of different proteins exhibiting *in vitro* antifungal activity against *Cercospora beticola*, the causal agent of leaf spot disease. Thirteen chitinases, belonging to 4 structural classes, and 9 β-1,3-glucanases have been purified and characterized, and genes encoding several of these enzymes have been isolated. One of the chitinases, an acidic class IV chitinase, contains posttranslational modifications in the hinge region, in that the proline residues are hydroxylated and glycosylated with xylose. Another family of antifungal proteins is identified as small, monomeric, basic proteins consisting of 46 amino acids, 8 of which are cysteines. Two proteins belonging to this family, being 54% identical, show strong antifungal activity against *C. beticola* and other filamentous fungal pathogens. Another two families of small (30 and 91 amino acid residues, respectively), basic, cysteine-rich proteins with antifungal potentials have been identified. Genes encoding some of the antifungal proteins have been cloned and constitutively expressed in transgenic potato and sugar beet plants.

B4-214 STABLE TRANSFORMATION OF *COLLETOTRICHUM TRIFOLII* BY ELECTROPORATION, Nichole R. O'Neill[1], Nancy Brooker[2], James A. Saunders[1], and John Lydon[2], [1]Soybean and Alfalfa Research Laboratory, [2]Weed Science Laboratory, USDA, Agricultural Research Service, Beltsville, MD 20705.

Electrotransformation was evaluated as a procedure for transforming *Colleotrichum trifolii,* the causal agent of alfalfa anthracnose. Whole fungal spores and spores generated from protoplasts were subjected to a range of electroporation conditions. Transformation frequency and fungal viability was optimized using an exponential pulse wave generator and varying the number of pulses, field strength of the pulse (kV/cm), and internal resistance to control pulse duration. Transformants were obtained using a fungal expression vector that contained a bacterial gene encoding hygromycin B phosphotransferase. Isolates transformed using either polyethylene glycol (PEG) or electroporation were evaluated for changes in virulence, race specificity, mitotic stability, and hyg B phosphotransferase gene integration. Restriction digests of the putative transformants provided evidence for single copy, tandem repeat integration, and multiple random integration events in the fungal genome. Transformation by both procedures resulted in isolation of mitotically stable transformants which varied in cultural characteristics and virulence but retained race specificity of the wild-type. Electroporation and PEG DNA uptake resulted in similar transformation frequencies. The introduction of stable, dominant, selectable, antibiotic resistance genes in this race will facilitate disease resistance studies by enabling rapid detection of the fungal race in host tissues expressing specific resistance interactions.

B4-215 IMMUNOSUPPRESSANTS CYCLOSPORIN AND FK506 AS NOVEL ANTIFUNGAL AGENTS AGAINST CRYPTOCOCCUS NEOFORMANS, Audrey Odom, Tamara Breuder, Eric Lim, John Perfect, and Joseph Heitman, Departments of Genetics, Pharmacology, and Medicine, Howard Hughes Medical Institute, Duke University Medial Center, Durham, NC 27710

The immunosuppressants cyclosporin A (CsA) and tacrolimus (FK506) are antifungal natural products whose mode of action is highly conserved from the yeast *Saccharomyces cerevisiae* to man. Both drugs exert their effects by binding immunophilins cyclophilin A or FKBP12, respectively. The resulting protein-drug complexes inhibit the Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin, known to regulate signal transduction and cation transport. We have defined conditions under which CsA and FK506 are toxic to the central nervous system pathogen, *Cryptococcus neoformans. C. neoformans* is a ubiquitous basidiomycetous yeast of increasing importance as an opportunistic pathogen in AIDS patients and other immunocompromised individuals. Therapeutic options for cryptococcosis are currently limited.

Previous studies on CsA toxicity in *C. neoformans* have been conflicting. We find that *C. neoformans* strain H99 is extremely sensitive to both CsA and FK506 at 37°C but not at 30°C *in vitro*. The vast majority of mutants isolated as resistant to CsA or to FK506 are cross-resistant to both drugs, implicating a common downstream target for drug action. Lastly, several mutants resistant to FK506 but not to CsA lack FK506 binding activity in an *in vitro* ^{3}H-ligand binding assay, implying that an FKBP-FK506 complex is toxic in *C. neoformans*. Our studies suggest that calcineurin, the only known common target of CsA and FK506, is required for *C. neoformans* growth at elevated temperature *in vitro* and, by extension, may therefore be necessary for virulence *in vivo*.

Despite potent antifungal activity *in vitro*, both CsA and FK506 exacerbate cryptococcal meningitis in a rabbit model system. This may reflect an inability to cross the blood brain barrier (CsA) or an inability of the fungicidal effects to overcome the potent immunosuppressive effect (FK506). Non-immunosuppressive analogs of both CsA and FK506 are available and might prove useful as novel antifungal agents.

B4-216 DETECTION OF A CYCLIC PEPTIDE SYNTHETASE GENE IN *LEPTOSPHAERIA MACULANS*, Eric A. Pedersen, M. Soledade C. Pedras[1] and Janet L. Taylor. National Research Council of Canada, Plant Biotechnology Institute, Saskatoon, SK, Canada, S7N 0W9. [1]Department of Chemistry, University of Saskatchewan, Saskatoon, SK, Canada, S7N 0W0.

Leptosphaeria maculans, causal organism of blackleg in crucifers, produces a complex array of nonspecific phytotoxins, epipolythiodioxopiperazines (EPTs), and a host specific phytotoxin, phomalide (a cyclic depsipeptide). The precursor of the EPTs is phomamide, a dipeptide. As with many peptide toxins, the biosynthesis of both phomamide and phomalide may be catalysed by cyclic peptide synthetases, large multifunctional enzymes employing a thiotemplate mechanism. We have used degenerate oligonucleotides based on highly conserved amino acid motifs present in all known cyclic peptide synthetases to obtain a PCR product from a highly aggressive isolate of *L. maculans*. The PCR product was used to screen a Lambda genomic library of the pathogen. Several clones have been isolated, restriction mapped and partially sequenced. Thus far, sequencing has revealed an open reading frame of 3250 bp, the derived amino acid (aa) sequence contains a 576 aa domain which has high homology to cyclic peptide synthetase enzymes.

B4-217 ISOLATION OF MDR-LIKE GENES FROM *ASPERGILLUS FUMIGATUS* AND *ASPERGILLUS FLAVUS*. ROBERT B. PEERY AND PAUL L. SKATRUD, INFECTIOUS DISEASE RESEARCH, LILLY RESEARCH LABORATORIES, LILLY CORPORATE CENTER, INDIANAPOLIS IN 46285

Systemic *Aspergillus* infections present a serious clinical problem with a high mortality rate. Good non-toxic anti-*Aspergillus* compounds are not available. Most anti-fungal compounds are developed to treat *Candida albicans* infections because they represent the vast majority of clinically observed systemic fungal infections. Unfortunately, these anti-*Candida* compounds are frequently ineffective against *Aspergillus* species. Part of the reason for their ineffectiveness in *Aspergillus* may be due to the presence of multiple drug resistance (*mdr*) genes similar to the *mdr1* gene found in drug-resistant human cancer cells.

Two members of the ATP-binding cassette (ABC) family from *Aspergillus fumigatus* and one from *Aspergillus flavus* were cloned by using degenerate PCR to generate a hybridization probe. Both *A. fumigatus* and *A. flavus* contain at least one 12 transmembrane/2 ATP-binding domain genes (*aAu-mdr1* and *Afl-mdr1*) possessing high homology to the leptomycin B resistance gene from *Schizosaccharomyces pombe*. In addition, another gene (*Afu-mdl1*)was found in *A. fumigatus* which encodes a 6 transmembrane/1 ATP binding domain ABC transporter exhibiting high homology to *mdl1* and *mdl2* of *Saccharomyces cerevisiae*.

A plate assay was devised to screen compounds for the ability to potentiate activity against *Aspergillus* in antifungals normally possessing anti-*Candida* activity only. R106I, a cyclic depsipeptide with excellent anti-*Candida* activity but very poor activity against *Aspergillus* species, was used as the antifungal in a limited screen. Compounds such as verapamil and trifluoroperazine which are known human mdr blockers, exhibited the capacity to potentiate the activity of R106I against *A. fumigatus*. These data suggest the possibility of developing new compounds or combinations of compounds, that circumvent efflux as a mechanism of resistance.

B4-218 A MITOCHONDRIAL dsRNA ELEMENT FROM *CRYPHONECTRIA PARASITICA*: TRANSMISSION AND EXPRESSION, James J.Polashock and Bradley I. Hillman, Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903

Although most viruses associated with hypovirulence of the chestnut blight fungus, *Cryphonectria parasitica,* contain large (> 10 kbp) double-stranded (ds) RNA genomes and are localized in the cytoplasm, we have recently described a small, 2.7 kbp dsrna element associated with mitochondria of hypovirulent strain NB631 of *C. parasitica*. Here we present evidence that the element is inherited at high frequency (at or near 100%) through asexual spores (conidia). Also, unlike other *C. parasitica* dsRNAs which are not transmitted through the sexual cycle, NB631 dsRNA is passed through sexual spores (ascospores), but only if the infected strain acts as the female parent. Like other *C. parasitica* virus-like dsRNAs, NB631 is transmitted efficiently via hyphal anastomosis. This process appears to involve movement of whole mitochondria across the anastomosis bridge as evidenced by subsequent recombination of mitochondrial DNA. Altered mitochondrial genotypes were stable upon subculture and through conidia, and have always accompanied dsRNA transmission by anastomosis. Movement and recombination of the mitochondria was shown to occur in both directions following anastomosis, and also occurred in the absence of virus infection. NB631 dsRNA contains one UAG and nine UGA terminators in the single ORF thought to be translated in mitochondria. To begin to examine expression of NB631 dsRNA, we have assembled a full-length cDNA clone and used site-directed mutagenesis to alter these codons for cytoplasmic translation. Expression of wild-type and mutant clones is currently being examined in vitro and in vivo.

B4-219 NOVEL CHITINASES FROM TOBACCO EXHIBIT ANTIFUNGAL ACTIVITY, Anne S. Ponstein, Marianne B. Sela-Buurlage, Marion Apotheker-de Groot, Sandra A. Bres-Vloemans, J.A.van der Knaap, Huub J.M. Linthorst, John F. Bol, Ben J.C. Cornelissen and Leo S. Melchers, MOGEN Int. Einsteinweg 97, 2333 CB Leiden, The Netherlands.

Tobacco plants respond to pathogen attack by the synthesis of a large number of pathogenesis-related (PR) proteins. These proteins are subdivided into five distinct groups based on enzymatic, structural and serological data. Within each group of tobacco PR proteins a division can be made between basic, vacuolarly targeted proteins and acidic, extracellularly targeted proteins, now generally referred to as class I and class II proteins, respectively. Class I proteins are potent antifungal proteins able to inhibit the growth of for instance *Trichoderma viride*, *Fusarium solani* and *Alternaria solani* (PR-2, PR-3) and *Phytophthora infestans* (PR-5).

Here we report on the purification of two novel chitinases from TMV infected tobacco leaves. One of the chitinases (CBP20) was characterized as a class I PR-4 chitinase. The protein appeared to inhibit *Fusarium solani* both alone and in synergy with the class I ß-1,3-glucanase (PR-2) and the class I chitinase (PR-3) purified from tobacco leaves. *Alternaria radicina* was not sensitive to CBP20 alone. However, combinations of class I chitinases or ß-1,3-glucanases and CBP20 were active.

The sequence of the second chitinase (*chi*-V) differs from all known chitinases and PR-proteins identified so far but it shows sequence identity with bacterial chitinases. In contrast to the bacterial chitinases the enzyme lacks detectable exo-chitinase activity. Thus, it seems to hydrolyze chitin in an endo-fashion, like the class I and the class II chitinases. *In vitro* assays show that Chi-V exhibits antifungal activity toward *Trichoderma viride* and *Alternaria radicina*. Chi-V interacts synergistically with a tobacco class I ß-1,3-glucanase in growth inhibition experiments with *Fusarium solani*.

The expression pattern and the *in vitro* activities of the novel chitinases suggest a role in the plants' general defense mechanism. The potential of these proteins for engineering fungal resistance in transgenic plants is under investigation.

B4-220 RECOMBINANT EXPRESSION, PURIFICATION AND FUNCTIONAL ANALYSIS OF THE WHITE PHASE-SPECIFIC GENE WH11 IN THE WHITE-OPAQUE PHASE TRANSITION OF C. ALBICANS STRAIN WO-1

Klaus Schröppel, Thyagarajan Srikantha and David R. Soll, Department of Biological Sciences, The University of Iowa, Iowa City IA 52242

Switching between a number of general phenotypes appears to provide Candida albicans with the variability necessary to rapidly adapt to new environmental challenges. In strain WO-1, cells switch spontaneously, reversibly and at high frequencies between a hemispherical white and a flat gray (opaque) colony morphology, which is accompanied by changes in most aspects of cellular morphology and physiology. Switching involves the activation of both white phase-specific and opaque phase-specific genes. The gene WH11 is transcribed selectively in the white budding phase. When white budding cells differentiate to the hyphal form or to the opaque phase, transcription of WH11 is deactivated. The gene is regulated by two activation sequences in the WH11 promoter, both of which form complexes specifically with white protein extracts but not opaque protein extracts. To examine the function of the Wh11 protein, we have generated antisera to a recombinant Wh11 protein. WH11 was subcloned into the expression vector pGEX-2T and rWh11 was overexpressed as a GST-fusion protein in E. coli. rWh11 was purified and repeatedly injected into a rabbit. Western blots with antiserum from the immunized rabbit specifically detected rWh11 as well as native Wh11 in white cell extract but not in opaque cell extract. Indirect immunoflourescence experiments are now being performed to localize the WH11 antigen in white budding cells.

B4-221 MULTIPLE-DRUG RESISTANCE GENES IN *CRYPTOCOCCUS NEOFORMANS* AND *AUREOBASIDIUM PULLULANS*. Paul L. Skatrud, Robert B. Peery and Susan J. Thornewell. Infectious Disease Research, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis IN 46285

Multiple-drug resistance proteins are members of a superfamily of transport proteins designated the ATP binding cassette (ABC) proteins. ABC family members share significant overall structural homology and are typically comprised of four membrane-associated domains: two transmembrane domains that each span the membrane several times and two ATP-binding domains.

An MDR-like gene (*Apu-mdr1*) was found in the polymorphic fungus *Aureobasidium pullulans* by DNA sequence analysis of cosmid DNA. The *Apu-mdr1* encoded protein was highly homologous with several members of the ABC family of proteins and consisted of 1,302 amino acid residues with a calculated molecular weight of 139.52 kD. Kyte-Doolittle analysis revealed twelve putative transmembrane regions and DNA data bank comparisons suggested the presence of two ATP-binding domains. Molecular karyotype analysis of *A. pullulans* and subsequent hybridization analysis located the *Apu-mdr1* gene on chromosome 3.

Two MDR-like genes have been identified in the human opportunistic fungal pathogen *Cryptococcus neoformans*. *cne-mdr1* was identified by PCR amplification of a conserved motif with degenerate primers. Sequence analysis of the resulting amplified DNA fragment revealed a high degree of similarity with members of the ABC family of transporter proteins. The PCR fragment was used to probe a genomic DNA library and a fragment was identified containing the entire gene. Sequence analysis suggested the presence of 13 introns. Two ATP-binding domains were identified by homology to motifs found in other ABC transporter proteins. *cne-mdr1* was localized to one of the larger of the 9 chromosomes found in *C. neoformans* by molecular karyotype analysis. The presence of a second MDR-like gene (*cne-mdr2*) was discovered during PCR amplification from *C. neoformans* cDNA with primers designed to amplify the 5' portion of *cne-mdr1*. The DNA sequence of a portion of *cne-mdr2* was determined and was found to be different than *cne-mdr1*. Analysis of the predicted protein sequence encoded by cne-mdr2 revealed a high degree of similarity to the *Schizosaccharomyces pombe* leptomycin B-resistance protein and other ABC transporter proteins.

B4-222 ONE OF THE PUTATIVE CONTROL GENES FOR HYPHAL FORMATION IN CANDIDA ALBICANS ENCODES A HOMOLOGUE OF A PI/PC-TRANSFER PROTEIN THAT IS ESSENTIAL FOR HYPHAL MORPHOGENESIS IN YARROWIA LIPOLYTICA, I. V. Slobodkin, C. A. Kumamoto, Dept. of Mol. Biology and Microbiology, Tufts University, Boston, MA 02111

Candida albicans is a dimorphic yeast that can grow in budding or hyphal form in response to changes in conditions. A number of researchers have shown that it is feasible to isolate mutants defective in hyphal formation. However, due to obstacles in molecular cloning in this organism, genes that control hyphal formation have been difficult to identify.

We have obtained a set of insertion mutants generated by random nonhomologous integrative transformation of *C.albicans*; the transforming DNA was a bacterial plasmid. A filtration-based enrichment procedure yielded a subset of strains defective in hyphal formation. Some of the mutants were completely unable to form hyphae, whereas some of them had partial defects. DNA flanking the insertions in these mutants was obtained for investigation by imprecise excision of the inserted plasmids.

One of the mutants carries a disruption in a gene that appears to be a *C.albicans* homologue of the *SEC14* gene described in other yeasts (*Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Yarrowia lipolytica*). This gene encodes a phosphatidylinositol/ phosphatidylcholine-transfer protein. *SEC14* is essential for hyphal formation in the dimorphic yeast *Y.lipolytica* (Lopez M.C., Nicaud J.-M., Skinner H.B., Vergnolle C., Kader J.C., Bankaitis V.A., Gaillardin C., The Journal of Cell Biology, **125**, no.1, 113-127). Its known function in *S.cerevisiae* is maintenance of the PC content of Golgi membranes (McGee T.P., Skinner H.B., Whitters E.A., Henry S.A., Bankaitis V.A, The Journal of Cell Biology, **124**, no.3, 273-287). *SEC14* is essential for growth in *S.cerevisiae*, but not in *Y.lipolytica*. The role of *SEC14* in hyphal formation in *C.albicans* is currently being investigated.

B4-223 **PATTERNS OF MEDICAL PATHOLOGY IN THE MYCOTIC PATIENT.** Jacob J. Steinberg, David Kardon, Stephen M. Factor, Autopsy Service, BMHC-MMC of the Albert Einstein College of Medicine, Bronx, NY 10461

We reviewed 155 autopsies of systemic fungal infections (excluding oral thrush & dermatomycoses). The mean age of death was 46y (vs mean autopsy population=57y). HIV+ cases were 57% vs 8% in the post 1981 general autopsy population. The M:F is 2:1 & 3:1 in HIV+. HIV-were mostly elderly; females were almost equally distributed; males predominated in early & late adulthood. Mean ages of infections per age group were (yrs): 1.3 (0-18); 31 (18-40); 51 (41-65); & 76 (>65). Hispanics & African-Americans tended to be younger (41y vs 55y). Candida (63%) was most common, followed by pneumocystis (pcp; 39%), cryptococcus (10%), aspergillus (6%), histoplasma (5%), & mucor (2%). Mean hospital stay was 34d, compared to 20d overall, & not dependent on HIV status. 122 had single infections, 29 had double, with candida & pcp as the most common combination, & four had triple. CNS (n=21) involvement from any fungus occurred in the younger age group (mean=30y), & usually included at least three other organ systems (septic, lung, GU). Males predominated, but HIV+ was equally divided. Heart (n=13) was 2:1 HIV-, with a slight female predominance. Mean age was 46y. GU (n=30) followed lung & blood involvement. Mean age was 53 y, M:F equally split, but HIV- predominated 3:1. Blood (n=27) closely paralleled GU, occurred in older patients (57y), 1:1 between M:F, & HIV-predominated by 4:1. Fungi in the lung (n=89) were mostly male (2.5:1), young (39 y), & HIV+ (3.5:1). The maximum organ systems involved was nine. Crypto (n=16) predominated in the lungs & meninges & was the most widely spread. The mean age was 45y, mostly males & HIV+. Females split between HIV+ & HIV-, & had longer hospitalizations than males (30d vs 24d). Rarer sites involved pituitary, thyroid, & face (skin). Asper (n=9) occurred in lung & GU, equally affected M:F, & led to briefer hospitalizations prior to demise (18d). Thyroid was an uncommon site, & CNS was absent. Mucor (n=3) remained a disease of the elderly (68y), with brief hospitalizations (15d), & HIV-. Histo (n=7) predominated in the lung, secondarily in spleen, lymph & liver, & occurred equally in mostly HIV+ M:F. Males had longer hospitalization (27d vs 15d). In candida, excluding thrush or esophagitis, only 7 of 52 (14%) were HIV+, suggesting that HIV+ does not predispose to systemic candidiasis. Of 48 cases of oral candidiasis, only one, a 3y HIV+ boy, had dissemination outside the GI tract. These data serve as an initial guide for evaluating patterns of fungal disease.

B4-224 ENGINEERING OF CONTROLLED CELL DEATH AT INFECTION SITES AS A DEFENCE STRATEGY OF POTATO AGAINST LATE BLIGHT DISEASE, Günter Strittmatter[1], Margot Egen[1], Jan Janssens[2], Cris Opsomer[2] and Johan Botterman[2]; [1]Max-Planck-Institut für Züchtungsforschung, Department of Biochemistry, D-50829 Köln, Germany; [2]Plant Genetic Systems, B-9000 Gent, Belgium

Race-specific resistance of potato cultivars against *Phytophthora infestans* (*Pi*) is characterized by a high frequency of hypersensitive cell death in the vicinity of infection sites, blocking the spread of fungal mycelia in the host tissue. In contrast, the defence response of susceptible cultivars is not sufficient to restrict the growth of the fungus, finally leading to late blight disaease. In order to improve their resistance, we try to engineer an artificial controlled cell death in susceptible potato cultivars by rapid and strictly localized synthesis of a cytotoxic compound in the vicinity of infection sites. This approach requires a promoter mediating transcriptional activation specifically at fungal infection sites. By expression studies with chimeric promoter/GUS gene constructs, we have identified a promoter region in the potato *prp1-1* gene with properties coming close to these requirements. Potato plants have been transformed with a construct combining the *prp1-1* promoter fragment with the bacterial barnase gene encoding a highly cytotoxic RNase. To minimize the detrimental effects of potential background barnase expression in non-infected tissue, the transgenic plants also expressed a specific inhibitor of barnase, barstar, under control of a constitutive promoter. In transgenic plants harboring the two-component system, the level of barnase expression is expected to exceed the level of barstar expression only in the vicinity of fungal infection sites, thereby causing strictly localized death of host cells. According to phytopathological evaluation, in 12 of 75 primary transformants the efficiency of fungal sporulation was reduced after infection with *Pi*. Cosegregation of reduced sporulation with the presence of the barnase gene in F_1 progeny of the most promising transgenic line has proven their causal relationship.

B4-225 INTEGRATIVE AND REPLICATIVE TRANSFORMATION OF *Aureobasidium pullulans* R106 TO HYGROMYCIN B RESISTANCE. Susan J. Thornewell, Robert B. Peery and Paul L. Skatrud, Infectious Disease Research, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis IN 46285

Transformation of *A. pullulans* R106 with hybrid hygromycin B phosphotransferase (*HPT*) genes containing heterologous promoters produced inconsistent results. The promoter from a highly expressed gene in *A. pullulans* was sought to alleviate this problem. The *A. pullulans* transcription elongation factor 1 gene (*TEF1*) was cloned and sequenced. Molecular karyotype analysis of *A. pullulans* by CHEF agarose gel electrophoresis revealed the presence of eight chromosomal bands. The *TEF1* gene was localized on chromosome VI. Three introns were present in the *TEF1* gene, the first of which interrupted the open reading frame after 5-bp. The deduced amino acid sequence exhibited high identity to *TEF1* encoded proteins of several other organisms. The *A. pullulans TEF1* promoter was fused in frame with the *HPT* gene. *A. pullulans* was transformed to hygromycin B-resistance (HmB^R). Analyses of 23 HmB^R transformants revealed integration of the transforming DNA in only eight of these transformants. In two transformants, integration into the largest chromosome (VIII) resulted in an alteration of the molecular karotype. In four other transformants integration occurred on chromosome VI (the chromosome containing *TEF1*) but only one was the result of homologous recombination with the genomic copy of the *TEF1* promoter. The remainder of the transformants contained replicative plasmids that could be visualized on an agarose gel by ethidium bromide staining. The plasmids were generally of 7-8 kb in size and one transformant appeared to contain four plasmids ranging in size from 4-8 kb, suggesting rearrangement of the transforming DNA. Only one plasmid (~4 kb) was rescued by transformation into *E. coli*. This plasmid was unable to transform *A. pullulans* to HmB^R. Linearized plasmid DNA consistently produced more transformants than circular plasmid DNA. A PCR-generated DNA fragment containing only the *TEF1* promoter plus the *HPT* open reading frame transformed *A. pullulans* at the same frequency as linearized plasmid DNA. Interestingly, a number of HmB^R transformants obtained with the PCR fragment contained plasmids suggesting ARS function within the *TEF1* promoter region.

B4-226 MOLECULAR CHARACTERIZATION OF RESISTANCE IN TOMATO TO *ALTERNARIA ALTERNATA F. SP. LYCOPERSICI* Erik van der Biezen, Frank Takken, Laurent Mesbah, Tarcies Kneppers, John Nijkamp and Jacques Hille, Institute for Molecular Biological Sciences, BioCentrum Amsterdam, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

The fungus *Alternaria alternata* f. sp. *lycopersici* produces host-selective AAL-toxins that are involved in the pathogenesis of the Alternaria stem canker disease in susceptible tomato cultivars. Sensitivity to AAL-toxins is controlled by the semi-dominant *Asc* locus which is expressed *in vitro* at all tested tomato tissues. It is hypothesized that *Asc* encodes a target for the AAL-toxins, and that differential affinity of this target to the AAL-toxins plays a role in the relative sensitivity to the toxins. Reduced affinity of this target results in reduced sensitivity to the toxins and, consequently, results in resistance to the pathogen. This model is supported by the observation that EMS-induced mutations at *Asc* result in insensitivity to AAL-toxins and resistance to the pathogen.

We started a transposon tagging experiment to inactivate and isolate the *Asc* gene encoding the putative target for the AAL-toxins. A closely linked *Ds* element has been transactivated by a stabilized *Ac* element, both in an *Alternaria* susceptible background. Selfed and oucrossed progenies are currently screened for insensitivity to AAL-toxins.

As an alternative strategy to isolate *Asc*, a map based cloning approach was initiated. The *Asc* locus mapped on chromosome 3 and two flanking RFLP markers were identified in a 2 cM region. The markers were used to isolate Yeast Artificial Chromosomes (YAC) containing tomato DNA inserts. Mapping of the YAC ends-probes allows construction of a contig harboring the *Asc* locus. The region containing *Asc* will be identified following complementation assays by genetic transformation.

B4-227 CHARACTERIZATION OF CHITIN SYNTHASE 2 OF *SACCHAROMYCES CEREVISIAE*: IMPLICATION OF TWO HIGHLY CONSERVED DOMAINS AS POSSIBLE CATALYTIC SITES, Hisafumi Yamada, Masayuki Sudoh, Shigehisa Nagahashi, Rumi Umeda, Naomi Ono, Toshiyuki Mio and Mikio Arisawa, Department of Mycology, Nippon Roche Research Center, Kamakura, Kanagawa 247, Japan

Chitin, a component of yeast cell wall, is synthesized by the enzyme called chitin synthase. Three chitin synthases are identified in both *Saccharomyces cerevisiae* and *Candida albicans*. In *Saccharomyces cerevisiae*, it is thought that chitin synthase 1, 2 and 3 are involved in repair of damaged chitin, primary septum formation and all other chitin syntheses, respectively. We have characterized chitin synthase 2 of *Saccharomyces cerevisiae* by means of site directed mutagenesis and subsequent expression of the mutant enzymes in yeast cells. Chitin synthase 2 shares a region whose sequence is highly conserved in all chitin synthases. This region is divided into three subdomains based on the frequencies of appearance of the conserved amino acids. In two out of three subdomains, any conserved amino acid could not be replaced by alanine to retain the enzyme activity. In the above two domains, we found unique sequences, E^{561}-D^{562}-R^{563} and Q^{601}-R^{602}-R^{603}-R^{604}-W^{605}, that were also conserved in all chitin synthases. Substitutions of E^{561}, R^{563}, R^{602} or R^{603} for the analogous amino acids restored the enzyme activity, whereas those of D^{562}, Q^{601}, R^{604} or W^{605} for the analogous amino acids drastically decreased the activity without affecting the Km values for the substrate significantly. Amounts of these mutant enzymes in the total membranes were more or less the same as that of the wild type enzyme. These results suggest that D^{562}, Q^{601}, R^{604} and W^{605} locate in the active site, and that they are functioning as catalytic sites of the enzyme.

Late Abstracts

THE DIMORPHIC INSECT MYCOPATHOGEN NOMURAEA RILEYI: IN VIVO DEVELOPMENT OF THE HYPHAL BODY-MYCELIA TRANSITION. Drion G. Boucias and J. Pendland, Department Entomology and Nematology, University of Florida, Gainesville, Florida 32611.

The dimorphic fungus, Nomuraea rileyi during its' in vivo development in host insects sequentially forms a yeast-like hyphal body and a elongate mycelial stage. Upon penetration of the host cuticle this pathogen replicates as budding hyphal bodies. These fungal cells lack the surface epitopes required for opsonin-mediated phagocytosis. With 4 days postchallenge, hyphal body concentration reaches a threshold at ~2.0 x 10^5 cells per µl hemolymph and are induced to synchronously produce germ tubes. The induction of this switch is due to a "morphogen" released during the late infection stage. An active fraction can be separated from infected hemolymph using Folchs extraction followed by a combination of normal and reverse phase chromatography. This active fraction is capable of inducing a transition of cell phenotype of selected vertebrate and invertebrate fungi. Plasma extracted from control hemolymph does not contain this morphogen. Germ tubes originating from hyphal bodies possess surface epitopes which are recognized by healthy hemocytes and which mediate the binding to the basal membranes of insect tissues (fat body, epithelia, etc.). Among the various epitopes present, a 76 kDa mannoprotein has been extracted which possesses laminin binding domains. Within 12-24h of the hyphal body-mycelial transition host larvae succumb to mycosis.

NUCLEAR-CYTOPLASMIC INTERACTIONS IN COMMON WHEAT RESISTANCE TO FUNGAL PATHOGENS, Parfenova T.A., Institute of Genetics and Cytology, Belarusian Academy of Sciences, Scorina St. 27, Minsk, 220734, Rebublic of Belarus

Cytoplasms of 19 species of Triticum and Aegilops were introduced into different common wheats. Genetic effects of individual cytoplasms on inspecific resistance to brown rust caused by Puccinia triticina and to head blight caused by Fusarium spp. were clarified using these alloplasmic lines. A significant effect of alien cytoplasms on wheat resistance to fungal pathogens was revealed. The share of its influence on grain quality of infected plants and on reproductivity of fungi was approximitely within the range of 2% - 12%. As dependant on its influence on the fungi spore forming ability, all alien cytoplasms can be divided in two main groups: cytoplasms increasing reproductive capacity of pathogens and those decreasing it. It was shown that some cytoplasms such as Ae.squarrosa interacting with most wheat genomes stimulate resistance mechanisms of plants. This conclusion is confirmed by the fact that peroxidase and acid phosphotase activity of fungi infected alloplasmic lines which have been determined to be more resistant was higher than that of the sensitive ones.

GENETIC ANALYSIS OF CULTIVAR SPECIFICITY AND RACE EVOLUTION IN THE SOYBEAN PATHOGEN, *PHYTOPHTHORA SOJAE*. Brett M. Tyler[1], Helga Förster[2] and Michael D. Coffey[2]. Depts of Plant Pathology, University of California, Davis, CA95616[1] and University of California, Riverside CA92521[2]

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. Avirulence against *Rps4* and *Rps6* cosegregated, suggesting either a single avirulence gene for both *Rps* genes, or two tightly linked avirulence genes. An RFLP linked to *Avr(Rps1b)* was identified. 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.

ROLE OF ATPase IN BIOTROPHIC NUTRITION OF THE RUST FUNGUS UROMYCES VICIAE-FABAE, Christine Struck, Matthias Hahn, Claudia Siebels, Hans-Jürgen Apell and Kurt Mendgen, Fakultät für Biologie, Universität Konstanz, D-78434 Konstanz, Germany

Biotrophic rust fungi are nutritionally highly specialized parasites. The haustoria seem to play the major role in nutrient uptake from the surrounding host cells, but the mechanism of transport is poorly understood. Active transport of nutrients across the host/haustorial interface has been suggested. The essential enzyme for an energy-forced transport process is the plasma membrane H^+-ATPase. Using plasma membrane-enriched vesicles of uredospores, germ tubes and isolated haustoria, we studied the properties of the H^+-ATPase. In uredospores and germ tubes only a low enzyme activity was detected. A drastic increase of ATPase activity could be observed with microsomal vesicles from haustoria which had been isolated from rust-infected leaves. In addition, the fluorescence probe oxonol VI has been used to measure the electrogenic proton pumping of the plasma membrane H^+-ATPase. Our results indicate that the enzyme is developmentally regulated. Several near-full length cDNA clones encoding the plasma membrane H^+-ATPase gene(s) have been isolated. First sequencing results of one of these clones have shown that it contains the highly conserved regions typical for both fungal and plant plasma membrane H^+-ATPase. Currently we are investigating the transcript levels of the gene during different developmental stages of the fungus in order to study the regulation of H^+-ATPase during pathogenesis.