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CELL SURFACE MANNAN AND THE CANDIDA-HOST INTERACTION

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The outer layer of the cell wall of Candida albicans is heavily enriched in glycosylated proteins that play critical roles in cell adherence, and act as major antigens and in the immunoregulation of the host. We are exploring the role of the O- and N-linked mannan in the host-fungus interaction via the analysis of strains in which a range of genes encoding mannosyl transferases have been disrupted. The C. albicans O-linked mannan consists of a tetrasaccharide in which Mnt1p and Mnt2p participate as partially functionally redundant enzymes in the assembly of the terminal two a-1,2-mannose residues. Deletion of either MNT1, MNT2 or both MNT1 and MNT2 resulted in strains with reduced adherence to epithelia and attenuation of virulence. Purified O-mannan oligosaccharides interfere with adhesin to buccal epithelial cells and oxidation of surface carbohydrates of C. albicans, but not C. glabrata blocked adhesion to epithelia. This suggests that O-mannan functions as a ligand in interactions with host surfaces. The C. albicans MNT1 family includes three other members (MNT3-5), which do not appear to participate in O-glycosylation. Recent advances in their roles in glycosylation and host-fungus interactions will be presented.
Mutants with deletions in the MNN4 gene are almost devoid in phosphomannan, a component of N-linked mannan which has been implicated in recognition of C. albicans by macrophages. However, mnn4 mutants were still able to be taken up by macrophages suggesting that this epitope is not a key mediator of macrophage recognition. Deletion of the OCH1 resulted in elimination of the outer N-mannan chains. The och1 mutant induced the cell wall salvage pathway and had elevated chitin levels. Microarray experiments showed that a range of genes encoding cell wall proteins were upregulated in the och1 mutant. Disruption of MNT1 and MNT2 had much less an effect on the cell transcriptome. Analysis of glycosylation mutants to date establishes that the carbohydrate epitopes of mannoproteins play key roles in aspects of C. albicans related to its pathogenesis including adherence, cell wall permeability, drug sensitivity, virulence, and yeast-hypha morphogenesis.

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Identification and validation of proteins that will assist in the development of an antifungal therapy for Aspergillus fumigatus.

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We at ACE BioSciences discover, validate and develop novel targets that will be central to the development of future therapies against pathogenic microorganisms. Studies are performed using an integration of proteomic, bioinformatic and molecular biology techniques. A pathogen of prime interest to us is the opportunistic fungus Aspergillus fumigatus. This organism is associated with an ever-increasing rate of infections in immunocompromised individuals and the lack of effective therapy is illustrated by mortality rates that can exceed 70%.

Presently, we are investigating the potential to prevent infections caused by A. fumigatus via a prophylactic antibody therapy. Such a treatment has the potential to prevent the function of fungal factors essential for growth and/or pathogenesis, and to assist in clearance of the pathogen by both innate and acquired arms of the immune system.

Proteomic studies have been used to identify novel cell surface exposed and secreted proteins. These proteins have been assessed, using functional, viability and bioinformatic assays, to determine their role in growth and/or pathogenesis. A number of interesting candidates have been revealed. One of particular interest, ACE5033, a protein previously considered to be solely cytosolic, is exposed on the surface of both AfC and AfM and appears to function as an adhesin. This surface localization is not unique to only the genome sequencing strain but is also relevant to other clinical isolates. Furthermore, antibodies targeted against this protein have the ability to reduce adhesion of AfC to lung epithelia in vitro.

Monoclonal antibodies will now be raised against this protein, in association with our collaborator Genmab, and favourable candidates will be tested for their ability to prevent A. fumigatus mediated infections in a suitable animal model. Thus, it is hoped that a new method of preventing aspergillosis will be developed.

CONTRIBUTION OF TWO GPI-ANCHORED PROTEINS TO THE FORMATION OF BIOFILMS BY CANDIDA ALBICANS

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Biofilms are three-dimensional associations of microorganisms that develop on various surfaces. The human pathogen Candida albicans colonizes the surfaces of catheters, prostheses and epithelia, developing biofilms that are extremely resistant to antifungal drugs and can act as a source of re-infection. To get a better understanding of the mechanisms responsible for biofilm formation by C. albicans, we have performed a broad analysis of biofilm-specific features by transcript profiling and observed that diverse C. albicans biofilms have homogeneous transcript profiles that differ significantly from those of planktonic cultures. Furthermore, we have identified a set of genes that are significantly overexpressed in mature biofilms. Among these, the PGA59 and PGA62 genes encode two related putative glycosylphosphatidylinositol (GPI)-anchored proteins that we confirmed to be located at the cell surface using GFP fusions. In order to investigate the contribution of PGA59 and PGA62 to biofilm formation, C. albicans strains with null mutations in either or both of these genes were constructed. We will present results that indicate that both protein play a role in the yeast-to-hypha transition and consequently biofilm formation.

SUSCEPTIBILITY TO ASPERGILLUS FUMIGATUS: ROLE OF HOST GENETIC BACKGROUND

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Aspergillus fumigatus is a ubiquitous and deadly pathogen that affects up to 20% of immunocompromised hosts. Known risk factors for invasive aspergillosis include neutropenia, exogenous immunosuppression and graft-versus-host disease. However, the host genetic determinants of susceptibility to A. fumigatus are not well characterized. Aspergillus infection of different inbred murine strains provides an ideal model for studying the genetic basis of host-pathogen interactions in invasive pulmonary aspergillosis (IPA). We hypothesized that different inbred murine strains will show varying susceptibility to invasive aspergillosis and highlight the genetic basis of host susceptibility to development of invasive pulmonary aspergillosis (IPA).

We used a novel inhalational method of A. fumigatus (AF 293) inoculation ($3x10^8$ conidia /ml) in a persistently neutropenic murine model. Susceptibility was measured by time to mortality and quantification of fungal burden with RT-PCR. Amongst exogenously immunosuppressed inbred strains (n= 10 per strain) of mice, we found varying susceptibility to IPA. Susceptible mice had 100% mortality by day 6 post infection while resistant mice had 0 % mortality by day 6, 50% mortality by day 9 and 70% mortality by day 14 post infection.
We have performed the first survey of susceptibility to aspergillosis amongst inbred murine strains. These inbred strains demonstrated a range of susceptibility to IPA that is undoubtedly due to genetic differences between these strains. These findings provide an ideal model to identify genes that regulate host defense in invasive aspergillosis. Further study of susceptible and resistant murine strains may contribute to understanding the genetic basis of susceptibility to A. fumigatus in humans. Ultimately, these results could allow for improved targeting of antifungal prophylaxis in immunosuppressed patients, as well as improvement in selection of donor-recipient pairs for organ transplantation.

Io-5

COMPARATIVE GENOMICS OF CRYPTOCOCCUS; GENOME AND TRANSCRIPTOME ANALYSIS OF THE CRYPTOCOCCUS SEROTYPE D GENOMES JEC21 AND B3501A AND COMPARISON WITH OTHER CRYPTOCOCCUS AND YEAST SPECIES.

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Cryptococcus neoformans is an encapsulated environmental yeast, a basidiomycete and an important human pathogen. Life-threatening infections caused by *C. neoformans* have been increasing steadily over the past 10 years because of the onset of AIDS and the expanded use of immunosuppressive drugs. Here we present the complete genome sequence of *C. neoformans* serotype D JEC21 and the almost complete genome sequence of the closely related strain B3501A. B-3501A and JEC21 are both Mating type (MAT) *alpha* strains of serotype D that were derived from the same genetic lineage. Though B-3501A and JEC21 are derived from the same genetic background, the two strains present significant differences in their biology. B-3501A is considerably more virulent in animal models and more thermotolerant at 40^{o}C than JEC21. Analysis of the genome data reveals very complex gene structures in *C. neoformans* with an average of > 6 exons per gene. The transcriptome also reveals many examples of apparently alternatively spliced genes, the presence of anti-sense transcripts and a couple of examples of polycistronic transcripts. The genome contains a high percentage of transposable elements with large apparent centromeric regions. We present a comparative analysis of these two strains in order to help account for the observed differences in virulence, thermo-tolerance and cellular differentiation. We also present comparative analysis of JEC21 with the other ongoing Cryptococcal genome projects and with the genome data from the previously published ascomycete yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*.

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GENOMIC DIVERSITY OF THE CRYPTOCOCCUS NEOFORMANS SPECIES COMPLEX

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Cryptococcus neoformans is a basidiomycetous yeast causing life-threatening infections mainly in immunocompromised hosts. The existence of the two varieties Cr. neoformans var. neoformans (serotype A, AD, D) and Cr. neoformans var. gattii (serotype B, C) has long been recognized. A third variety: Cr. neoformans var. grubii (serotype A) was described a few years ago. The observation of mating resulted in the description of a separate genus Filobasidiella.
Molecular studies on the intergenic spacer (IGS) and internal transcribed spacer (ITS) of the rDNA, mitochondrial large ribosomal subunit RNA (mtLrRNA), orotidine monophosphate pyrophosphorylase (URA5), diphenol oxidase (LAC) and phospholipase B (PLB1) genes showed that the three varieties belong to different phylogenetic lineages and may represent species. This result was supported by Amplified Fragment Length Polymorphism (AFLP) data and PCR fingerprinting. Recently var. gattii has been raised to the species level as Cr. gattii.
To gain a better insight into the phylogeny of the species complex a set of strains from different origin (clinical vs. environmental, different geographic regions etc.) are being studied for several regions of DNA.
To date we have studied three regions of the rDNA: ITS, large subunit rDNA (LSU) and small subunit rDNA (SSU). Besides these rDNA regions ATP6, elongation factor 1 alpha (EF1alpha), the largest and second largest subunit of the RNA polymerase II gene (RPB1 and RPB2), two mating type specific genes encoding protein kinases (STE12 and STE20), a gene associated with laccase production (LAC) and mtLrRNA are also being studied.
Results obtained so far show that six main groups can be distinguished and that there is a considerable amount of variation present within the Cr. neoformans species complex.
In addition we study the pathogenicity of the six main groups using the model organism Caenorhabditis elegans. The absence of an adaptive immune system in C. elegans allows the dissection of 'basic' cryptococcal virulence factors in this model system. Data obtained to date suggest the existence of additional virulence factors not previously described using other model organisms.