

X
MECHANISMS OF HUMAN
PATHOGENESIS

Chair:

Axel Brakhage & Laszlo Kredics



Xo-1

Expression profiles of *Aspergillus fumigatus* under human neutrophil attack

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The innate immune system has a central role in combating infections caused by *Aspergillus fumigatus*. Macrophages and neutrophils are two cell types of the innate immune system that are very important in preventing and eliminating *A. fumigatus* infections. Macrophages are the first line of defense, engulfing and killing inhaled conidia and possibly early germinating conidia before they can establish hyphal growth. Neutrophils attack and kill hyphae that invade tissue. The importance of neutrophils in combating and resolving infections caused by *A. fumigatus* is underscored by two observations. First, invasive aspergillosis disease is rare in people that have normal numbers of neutrophils, thus it is a disease primarily of a neutropenic host. Resolution of invasive aspergillosis disease strongly correlates with recovery of neutrophil numbers in patients that acquired disease while neutropenic. While these simple observations have been known for some time, we still know little about the interaction between *A. fumigatus*, and macrophages and neutrophils. We hypothesize that *A. fumigatus* responds to host cells of the innate immune system, macrophages and neutrophils, with specific transcriptional responses to defend against attack by these immune cell types, ultimately contributing to fungal virulence.

To begin to study the relationship of the interaction between neutrophils and *A. fumigatus* and virulence, we have undertaken a study of the global patterns of gene expression in *A. fumigatus* hyphae in the presence of human neutrophils in culture. We have identified the mRNAs with the most or least relative abundance when compared to hyphae in cell culture medium lacking neutrophils over a 60 minute time course. There are 532 *A. fumigatus* genes including 20 transcriptional regulators whose mRNAs are more abundant or less abundant by a factor of two fold or more, 428 more and 104 less. We have explored the expression pattern of these *A. fumigatus* genes under conditions variably related to virulence including oxidative stress, osmotic stress, anaerobic stress, glucose to sorbitol carbon source shift, carbon source starvation, and under secondary metabolite inducing conditions in an *A. fumigatus laeA* loss-of-function mutant strain. Our analysis of the pattern of expression of these genes will reveal information on the conditions imposed by the neutrophils on *A. fumigatus* hyphae and on the survival strategy of the fungus in responding to these killing conditions.

Phase-specific gene expression underlying morphological adaptations of the dimorphic human pathogenic fungus, *Coccidioides posadasii*

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Coccidioides posadasii is a dimorphic fungal pathogen that grows as a filamentous saprobe in the soil and as endospore-producing spherules within the host. To identify genes specific to the pathogenic phase of *C. posadasii*, we carried out a large-scale study of gene expression in two isolates of the species. From the sequenced *C. posadasii* genome, we chose 1000 open reading frames to construct a 70mer microarray. RNA was recovered from both isolates at three life-cycle phases: hyphae, presegmented spherules, and spherules releasing endospores. Comparative hybridizations were conducted in a circuit design, permitting comparison between both isolates at all three life-cycle phases, and among all life cycle phases for each isolate. By using this approach, we identified 92 genes that were differentially expressed between pathogenic and saprobic phases in both fungal isolates, and 43 genes with consistent differential expression between the two parasitic developmental phases. Genes with elevated expression in the pathogenic phases of both isolates included a number of genes that were involved in the response to environmental stress as well as in the metabolism of lipids. The latter observation is in agreement with previous studies demonstrating that spherules contain a higher proportion of lipids than saprobic phase tissue. Intriguingly, we discovered statistically significant and divergent levels of gene expression between the two isolates profiled for 64 genes. The results suggest that incorporating more than one isolate in the experimental design offers a means of categorizing the large collection of candidate genes that transcriptional profiling typically identifies into relevant strain-specific and species-wide differences.

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PHI-base: A database of experimentally verified pathogenicity, virulence and effector genes in fungal and Oomycete pathogens of animals and plants

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Fungal and Oomycete pathogens of plants and animals are a major global problem. In the last 15 years many pathogenicity and virulence genes required for disease ability have been determined for over fifty different species. In addition, other studies have characterised effector genes required to activate host responses. By studying these types of pathogen genes novel targets for control can be revealed. In this presentation, we will describe the creation of a Pathogen Host Interaction database (PHI-base), which systematically compiles the genes involved in pathogen-host interactions (Winnenburg *et al.*, 2006). Each PHI-base entry is curated by domain experts and supported by strong experimental evidence (gene disruption experiments) as well as literature references in which the experiments are described. Each gene in PHI-base is presented with its nucleotide and deduced amino acid sequence as well as a detailed description of predicted protein's function during the host infection process. To facilitate data interoperability, we have annotated genes using controlled vocabularies (Gene Ontology terms, EC Numbers, etc.), and provide links to other external data sources (for example, NCBI taxonomy and EMBL). We discuss the utilisation of PHI-base for the computational identification of pathogenicity genes through comparative genomics. In this context, the importance of standardising pathogenicity assays as well as integrating databases to aid comparative genomics is discussed. PHI-base can be found at <http://www4.rothamsted.bbsrc.ac.uk/phibase>.

References

Winnenburg, R., Baldwin, T.K., Urban, M., Rawlings, C., Kohler, J. and Hammond-Kosack, K.E. (2006) PHI-base: a new database for pathogen host interactions. *Nucleic Acids Research* 34, D459-D464.

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Role of myosin type II during cytokinesis in the dimorphic fungus *Penicillium marneffe*

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Penicillium marneffe is capable of alternating between a hyphal and a yeast growth forms, known as dimorphic switching, in response to a temperature signal. At 25 °C, *P. marneffe* grows in a filamentous form which resembles other saprophytic *Penicillium* species. Hyphal cells grow by apical extension and branching, they are separated by incomplete septa, and are usually multinucleated. A shift to 37 °C triggers the dimorphic switch. The process, known as arthroconidiation, involves the coupling of nuclear division with cell division to yield shorter, uninucleate cells which are separated by double septa. Subsequently, the cell wall material between the double septa is degraded and unicellular yeast cells released. At 37 °C, the growth form is an elongated unicellular and uninucleated yeast, which divides by fission. In addition to dimorphic switching, *P. marneffe* can also undergo asexual sporulation (conidiation) at 25 °C whereby conidia are produced by multicellular conidiophores. The different cell types produced during the two developmental programmes are strictly controlled. Myosins are motor proteins responsible for a number of cellular processes during vegetative growth and the different developmental programmes. There are three types of myosins in fungi: I, II and V. Type II myosins localize to the contractile ring and are involved in cytokinesis and cell division in yeasts. We have cloned the myosin type II homologue from *P. marneffe*, called myoB. Deletion of the myoB gene results in a very severe growth phenotype. On solid media, the deletion mutant is not able to undergo asexual development at 25 °C and does not grow at 37 °C. Microscopic examination of the deletion mutant revealed that the deletion mutant could not make septa and therefore was not capable of cell separation under any of the tested conditions (25 °C and 37 °C). Highly branched tips displaying a broom shape resembled wild-type conidiophores at 25 °C, and branched hyphae without septa appeared instead of arthroconidia at 37 °C. In addition, hyphal cells show a tendency to lyse, which is in agreement with the deletion mutant being more sensitive to calcofluor white than the wild-type strain. Nuclear distribution is unaffected in most hyphal cells except those tips showing aberrant morphologies.

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Analysis of the regulation, expression and localisation of the isocitrate lyase from *Aspergillus fumigatus*, a potential target for antifungal drug development

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Invasive aspergillosis, caused by *Aspergillus fumigatus*, is a severe systemic infection in immunocompromised patients. New drug targets are required, since therapeutic treatment often fails and is hampered by severe side effects of antifungals. Enzymes of the glyoxylate bypass are potential targets, since they are absent in humans, but required for growth of *Aspergillus* on C2-generating carbon sources like acetate, ethanol and fatty acids. The key enzyme isocitrate lyase (ICL) can be inhibited by 3-nitropropionate, both as a purified enzyme and within intact cells. However, *in vivo* inhibition is partially compensated by the up-regulation of ICL promoter activity. Promoter activity is most likely controlled by the transcriptional activator FacB, because putative binding sites have been detected in the promoter region. Additionally, a promoter::lacZ-fusion shows a similar expression pattern in *A. fumigatus* and *Aspergillus nidulans*. ICL was found in distinct subcellular structures within growing hyphae, but only under conditions requiring ICL activity. In contrast, ICL was constitutively found in conidia, suggesting a specific role during germination. Lipids, as potential substrates, were detected in conidia and macrophages. Additionally, germinating conidia within macrophages contain ICL, suggesting that the glyoxylate shunt might be a relevant target for development of antifungals. Deletion mutants of the coding region of isocitrate lyase have been constructed and will be checked for attenuation in virulence in a murine infection model.

Construction and characterization of a gliotoxin non-producing strain of *Aspergillus fumigatus*

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Gliotoxin is a secondary metabolite produced by *Aspergillus fumigatus* that has diverse array of biologic effects in the immune system including inhibition of macrophage and polymorphonuclear cell function and induction of apoptosis in T cells. In experimental models of aspergillosis, gliotoxin production in situ by invading *Aspergillus* hyphae have been determined to be high enough to result in immunosuppression of the host. Therefore, it has been speculated that gliotoxin production by *A. fumigatus* contributes to the pathobiology of invasive aspergillosis, but this has never been directly experimentally tested. To test if gliotoxin production is a virulence trait, we have constructed *gliP* deletion mutants in *A. fumigatus*. *gliP* is the gene that codes for a nonribosomal peptidyl synthase, the first enzymatic step in gliotoxin biosynthesis. Thus deletion of *gliP* would be expected to result in strains incapable of producing gliotoxin. We identified *gliP* deletion mutants by Southern blot analysis and tested them for production of gliotoxin. The parental strain and deletion mutants were grown for 72 hours in minimal medium with 3% (w/v) glucose as a carbon source and the gliotoxin produced extracted with chloroform and the residue dissolved in methanol. Gliotoxin was identified and measured by liquid chromatography - mass spectroscopy. None of the *gliP* deletion mutants produced detectable gliotoxin, which was readily detected in the parental strain. Gliotoxin production was restored in a deletion mutant that was transformed with a plasmid carrying a full-length copy of *gliP*. We are in the process of testing the virulence of the *gliP* deletion mutant, the restored strain and the parental strain has been tested in an immunosuppressed murine model for invasive pulmonary aspergillosis.