

***Aspergillus fumigatus* gene expression in experimental infections**
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The initiating events of human aspergillosis remain poorly characterized and current understanding of disease initiation is best approximated from infection modelling experiments. During murine neutropenia intranasally-inoculated *A. fumigatus* spores are observed as attached to the lung epithelium by 4 hours post-infection. After this they break dormancy, generate primary hyphae and penetrate the pulmonary epithelium (10-14 hours). Ultimately, an invasive mycelial lesion is established with concomitant degradation of host tissues, and varying degrees of inflammation dependent upon the immunosuppressive regimen adopted.

In humans such infection is hard to detect. This clinical challenge, coupled with the tissue damage associated with late stage infection, and suboptimal efficacy of antifungal therapy, contributes to observed high mortality rates.

The field has assembled a wealth of information on activities required by the fungus to successfully colonise the host. The spore is coated with hydrophobic proteins and can direct production of secondary metabolites and antioxidant enzymes during developmental growth. The cell wall has immunomodulatory properties, houses antigenic proteins and has been demonstrated to mediate adhesion to a subset of human tissue proteins. Cell wall molecules secreted from the growing hyphal tip have provided useful biomarkers of infection, and secreted proteases must be required for tissue degradation. Secondary metabolism remains relevant throughout the initiation of infection and a recently characterised methyltransferase appears to be a master-regulator of secondary metabolite loci. Many of these activities are regulated at the level of transcription, and are sensitive to environmental perturbation.

To probe the basis of fungal adaptation to the mammalian lung environment we have developed, and continue to optimise, methodologies capable of conveying genome-scale gene expression data, from minute samplings of invasive germlings, directly from the site of infection.

In developing our methodology we made a histological time course to identify a window of opportunity for germling rescue. We similarly profiled germinative growth in the laboratory reference culture to minimize the effects of morphological differences on our output. At 12-14 hours post-infection we performed bronchoalveolar lavage using sterile saline. Lung lavages (BALFs) were snap frozen and washed with ice cold water prior to RNA extraction to lyse contaminating host cells. We then prepared fungal RNA using standard laboratory RNA extraction methodology. To maximise the amount of total RNA obtained during this part of the process, we pooled bronchoalveolar lavage fluids from multiple mice, and then subjected the extracted RNA to two rounds of linear mRNA amplification. The amplification methodology worked well and we routinely obtained 250 µg of amplified RNA for labelling and array hybridisation.

A common reference design was adopted for the microarray experiment. We performed the infection experiment 5 times in total generating pooled (n=24) BALFs from five independent infections. These samples were co-hybridised with similarly amplified mRNA prepared from developmentally matched laboratory cultured

germlings. Fluorescent signal from 9075 out of a possible 9516 represented ORFs were detectable from these hybridisation analyses. Of 2178 genes (22.8 % of the whole genome) having a fold-change in log intensity ratio of 2 or greater, 1281 were up-regulated and 897 were down-regulated.

Viewing the entire transcriptional dataset as a function of chromosomal locus reveals several important trends, including biased induction of subtelomeric and lineage-specific genes and clusters of co-regulated neighbouring genes, including previously characterized secondary metabolite gene clusters.

93.6% of *A. fumigatus* genes having orthologues restricted to two very closely related, but differentially virulent, species *Neosartorya fischeri* and *Aspergillus clavatus* are more abundantly represented during the initiation of infection. Induced genes form a significantly increased proportion of differentially regulated functions in intermediate ($p < 0.001$) and subtelomeric ($p < 0.001$) regions of the chromosomes. While only 16% of the predicted *A. fumigatus* gene repertoire is housed within 300 kb of telomeres (classed as the subtelomeric gene repertoire in our analyses), 29% of transcripts having increased abundance, relative to laboratory culture, in the murine lung are located to such subtelomeric areas, compared to just 11% of down-regulated transcripts.

This initial laboratory/mouse comparison establishes a methodological platform upon which to delve still further into the molecular basis of the host-pathogen interaction. We can confidently identify differences *between* infectious isolates (based upon comparative studies of mutant strains) moreover we can study the *timecourse* of gene expression during initiation of murine infection to identify those gene functions most increased in activity during early stage disease. Such activities are candidates of rich diagnostic potential. Our continued study of gene expression during *Aspergillus*-host interactions will encompass perturbations of both host and fungal functions to further characterize landmarks of pathogenic and diagnostic importance. Currently we are tracking the protein products of many upregulated functions using epitope tags to parameterize the timescale, mechanics and final destination of fungal proteins which may ultimately be instrumental in distinguishing non-threatening carriage of fungal spores from life-threatening invasive fungal infection.

The studies presented result from a collaborative program of research in the Bignell and Nierman laboratories at Imperial College London and the J. Craig Venter Institute, respectively.