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ARTICLE

Gene expression in fungi

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Abstract: This contribution is based on the four presentations made at the Special Interest Group (SIG) meeting titled Gene Expression in Fungi held during IMC9 in Edinburgh. This overview is independent from other articles published or that will be published by each speaker. In the SIG meeting, basic principles of *in vivo* animal models for virulence studies were discussed. Infection associated genes of *Candida albicans* and fungal adaptation to the host was summarized. Azole susceptibility was evaluated as a combined result of several changes in expression of pertinent genes. Gene transfer in fungi, resulting in fungal evolution and gene adaptation to environmental factors, was reported.

Key words:
animal models
Candida albicans
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fungal adaptation
gene transfer
virulence genes

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INTRODUCTION

Different studies have already described changes in gene expression levels during host-pathogen interactions. Monitoring gene expression by measuring mRNA levels in fungal DNA may identify candidate genes involved in fungal virulence. Fungal adaptation and host response should be evaluated in a reciprocal manner. Altered virulence and new phenotypes are a result of the environmental adaptation of fungi during evolutionary processes. Gene expression is the essential mechanism of fungal virulence, and therefore it should be evaluated carefully with regard to future projects to combat fungal disease.

CONTRIBUTIONS

The session began with the presentation of Aras Kadioglu, whose main topic was *in vivo* infection models. Animal models, particularly rodent models, are the primary steps for virulence studies. The immune status of the host, the course of the disease, and virulence genes of the pathogen should be examined. Thus, the model must be well-defined, and the characteristics of a good model should be detailed. Animal models have both strengths and weaknesses that are to be taken into consideration. Numerous genetically defined murine strains are already available (Clemons & Stevens 2006). His work on host-pathogen gene expression in respiratory infection models was presented, and focusses on innate and adaptive host immune responses to *Streptococcus* and *Pseudomonas* infections. He provided an overview of his own studies that concentrated on bacterial infection models in animals, but also introduced the basic principles of animal studies, which may be similar to those needed in mycological research. The presentation was an useful introduction to gene expression studies. *Streptococcus pneumoniae* is responsible for a huge burden of human disease and death, and the most common bacterial pathogen causing community-acquired pneumonia in the UK. Further, it is a major cause of infant mortality in developing countries, causing approximately 25 % of all preventable deaths in children under the age of five. *Pseudomonas aeruginosa* is an opportunistic pathogen that causes a range of infections, particularly chronic lung infections which are the major cause of morbidity and mortality amongst sufferers from the genetic disorder cystic fibrosis. Relatively little is known about the regulation of the host immune response and the interactions between innate and adaptive immunity during respiratory infections caused by these pathogens. He also discussed the various *in vivo* models established to replicate human upper respiratory tract carriage or acute or chronic infection of the lungs. These models had been used to study the role of both pneumococcal and pseudomonas virulence factors, and also to examine the host response to these bacteria in different disease conditions and locations. This research has shown that the contribution of each bacterial virulence factor varies, individually and collectively, according

to the *in vivo* location (niche) of the bacterium. In turn, the host responds accordingly, trying to challenge these factors in their locations. He discussed some of these bacterial and host factors and their role in disease using both microbiological and molecular techniques.

Duncan Wilson reported on on fungal adaptation to the host from the perspective of transcriptional profiling. His projects are "Infection-associated genes of *Candida albicans*" and "Endothelial flow model". He stressed that *C. albicans* was a versatile opportunistic fungal pathogen, capable of infecting virtually every organ of its human host. Such adaptation to different environmental conditions is mediated by rapid changes in gene expression. His group has established a range of infection models for studying the behaviour of *C. albicans* during different host-pathogen interactions and performed transcriptional profiling of *C. albicans* during different forms of infection. In his talk, he covered two forms of *C. albicans* infection, oral candidosis and deep seated liver infections, and discussed how gene expression analysis could give information as to the microenvironments faced by the fungus and the mechanisms employed to adapt to these environments. However, a conspicuous problem in interpreting *C. albicans* transcriptional data is the large number of regulated genes of unknown function evident during infection. They have begun to address this problem by systematically deleting *C. albicans* genes of unknown function which are up-regulated during infection, and analysing their role during host pathogen interactions. He gave two examples of previously unknown function genes and their role during different types of infection, illustrating how such analyses may lead to the identification of novel pathogenicity mechanisms.

Molecular aspects of pathogenesis in a fungal agent was the subject of Mette Jacobsen's presentation. She studied a clinical isolate of *Candida albicans* and three putative derivatives of this isolate obtained from the clinic by experimental exposure to antifungal agents *in vitro*. The parental isolate showed a previously undescribed phenotype of temperature-sensitive fluconazole resistance, while the isolates derived from the parent were resistant at 25 °C and 35 °C. Transcript profiling and qRT PCR analyses for expression of genes known to relate to azole resistance revealed no obvious single mechanism to explain the resistant phenotype. Ongoing analysis of whole genome DNA sequences from the isolates had so far revealed sequence differences between the parental isolate and its progeny in the gene *TAC1*, a transcriptional regulator of genes encoding antifungal efflux pumps. It was concluded that overall azole susceptibility phenotypes of these isolates *in vitro* was the combined result of several changes in expression of pertinent genes and of differences in the DNA sequence of their regulator.

GENE TRANSFER IN FUNGI

An overview of the field was presented by Ayse Kalkanci. The first report of transferring genetic information through a process known as transformation,was that of Frederick Griffith in 1928. In 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty suggested that DNA, rather than protein as widely believed at the time, might be the hereditary material of bacteria, and could be analogous to genes in more complex organisms. DNA-mediated transformation of a fungal species was first described by E. L. Tatum in 1973 (Fincham 1989). The terms horizontal, interspecific, and lateral gene transfer, have since come to be used to refer to the movement and fixation of DNA sequences between species. Horizontal gene transfer (HGT) is one of the main evolutionary driving forces in prokaryotes, but its relevance for eukaryotes remains controversial (Diggle 2010). Lateral gene transfer (LGT) events are well established between prokaryote species, and are also well established from prokaryotic sources to eukaryotic recipients.

For fungi, each of these terms refers to the transfer of a gene (a nucleic acid sequence) from one isolate to another by a processes other than the routine asexual cell division, sexual processes involving fusion of gametes, or parasexual exchanges between compatible isolates (Whitaker *et al*. 2009, Scannell *et al*. 2007).

Mating or recombination is fundamental to sex, but it is also a fundamental feature of Life, and may occur in non-sexual stages, as in some mitotic fungi. Mating in the hemiascomycete yeast is regulated by transcription factors encoded at the mating-type (*MAT*) loci. These are master regulators of cell identity, defining the programs of sexual differentiation in these species. Sexual reproduction was the major way of gene transfer in fungi, and fungal sex has become one of the most attractive topics of fungal research in the last few years (Zeyl 2009, Michod *et al*. 2008, Raudaskoski & Kothe 2010)

The sexual cycle of *Cryptococcus neoformans* can also generate recombinant progeny with altered virulence. In this basidiomycete, proteins are involved in regulating pheromone signaling by interacting with the pheromone receptor. Pheromone loss has been detected in all fungal phylogenetic lineages. The general role for pheromones is mate attraction, so this activity was not envisioned as occurring in fungi (Arnold *et al*. 2007, Casselton 2008). The emergence of cryptococcosis, due to an uncommon molecular type of *C. gattii* outside Australia, has emphasized the importance of understanding the pathobiology of an emerging pathogen. The predominant subtype in the outbreak is more virulent than the parental strain (Byrnes *et al*. 2009). This is explained by differences in the virulence of strains arising from evolutionary divergences in gene expression (Thompson & Regev 2009).

The discovery that the so-called white-opaque transition was an essential step in the mating process not only provided a mechanism for switching in the biology of *Candida albicans*, but also a means for elucidating the molecular basis of the switch event. Figs 1–2 show white and opaque colonies of *C. albicans*. White-opaque switching of *C. albicans* was found to be the result of sexual reproduction and found to be correlated with biofilm production. Opaque cells, through the release of pheromones, signaled white cells to form a biofilm that then

Fig. 1. White-bright colonies of *Candida albicans*.

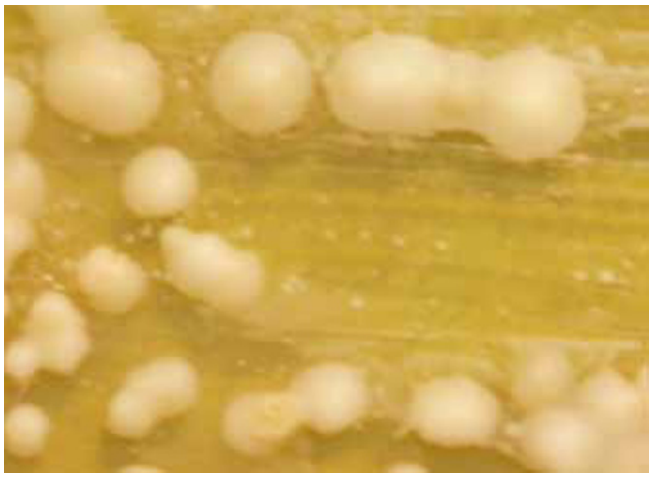

Fig. 2. Opaque colonies of *Candida albicans*.

facilitated mating; however, this process must still be verified *in vivo*. The link between biofilm formation and mating does not end with *C. albicans*, but is also found in bacteria (Soll 2009).

Gene transfer is a major force in microbial evolution, as evolution is driven by gene acqusition and loss, leading to increased fitness through homeostasis and altered virulence (Morschhauser *et al*. 2000). The acquisition of prokaryotic and eukaryotic genes by fungal genomes was been demonstrated. Type III and type IV secretion systems can be transfered from bacteria into eukaryotes. Fungal genomes were screened by phylogenomic comparison, and 713 acquired bacterial genes found in 88 % of the analyzed fungal genomes. Carbohydrate metabolism genes were the ones most commonly transfered from bacterial genomes during the evolutionary process (Fitzpatrick *et al*. 2008, Jackson *et al*. 2011, Moens *et al*. 1996, Hall & Dietrich 2007, Marcet-Houben & Gabaldon 2010, Khaldi *et al*. 2008)

The emergence of new pathogens, alterations of virulence, and resistance to antiofungal compounds are results of fungal evolution (Sapp 2007). Resistant isolates have frequently undergone several genomic changes that contribute to their reduced drug susceptibility. Future perspectives, and the clinical impact of gene transfer, will be major topics of molecular pathogenesis research, and can be expected also to provide insights into the study of the evolution of fungal organisms and eukaryotes as a whole.

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