Research into the major fungal pathogen, *Candida albicans* has firmly entered the post-genomics era. The current challenge is to apply these technologies to the analysis of *C. albicans* infections. Initial studies, which focused on the expression of specific virulence genes, have supported the view that secreted hydrolases and adhesins are expressed in a niche-specific fashion during infection. However, genome-wide expression profiling has revealed that most infection-related changes in *C. albicans* gene expression reflect environmental adaptation. Initial contacts with the host and disease progression are clearly associated with metabolic and stress adaptation. These studies, together with analyses of *C. albicans* mutants, indicate that physiological fitness plays a central role in the pathogenicity of this fungus, alongside virulence factors.

**Introduction**

*Candida albicans* is a major fungal pathogen of humans [1]. It exists as a commensal in many individuals, generating no obvious pathology, but can cause a range of infections in patients whose immune defenses have been compromised. *C. albicans* is an opportunistic pathogen whose invasion correlates with changes in environmental factors such as alterations to host immunity, competition from other saprophytes and physical perturbation of its niche, for example through surgery. Unlike other fungal pathogens, *C. albicans* has rarely been isolated from the environment. Many consider *C. albicans* to be obligately associated with mammalian hosts. Clearly a key to understanding the pathogenicity of this fungus lies in the regulatory processes that underpin its transition from commensal to pathogen. The immune status of the host is important for this transition [2]. However, the adaptation of *C. albicans* to new niches within the host also is critical for the transition to pathogenicity. The analysis of infection-associated gene expression is currently a major challenge in the *Candida* field. This is the topic of this review.

*C. albicans* can thrive in a range of niches within its human host. As a commensal it grows in the oral cavity and gastrointestinal tract [1]. As a pathogen it invades oral and vaginal epithelial surfaces, and in severely immunocompromised individuals it can be carried via the bloodstream to invade internal organs [1]. These niches are diverse in many respects. Their ambient pH can range from acidic to mildly alkaline. The nature and availability of nutrients varies significantly. In addition, *C. albicans* must (attempt to) counteract different types of immunological defense at these infection sites. Furthermore, as *C. albicans* colonizes a niche it probably alters that niche, for example by metabolizing available nutrients, possibly altering the ambient pH through its metabolic activity, and damaging host tissues. Therefore, patterns of *C. albicans* gene expression presumably vary both spatially and temporally depending upon the microenvironment (Figure 1). Ultimately, analyses of infection-associated gene expression must address this issue.

In this review we discuss the current status of this new field. We summarize the progress that has been made in investigating infection-associated gene expression in *C. albicans*, indicate key technical issues that are influencing this progress, highlight the main findings that have arisen from these studies, and suggest major questions that remain to be addressed.

**The focus on virulence factors**

Numerous studies have investigated the expression of subsets of *C. albicans* genes or proteins during infection or in infection models. Some of these genes are associated with major changes at the cellular level, such as yeast-hypha morphogenesis. These studies have focused on predicted virulence factors such as secreted aspartyl proteinases (SAPs), secreted lipases (LIPs), phospholipase B (PLBs), and adhesins such as the Als family and Hwp1. These studies have generally exploited PCR approaches to quantify specific and often low abundance transcripts within complex populations, or antisera directed against individual proteins.

The SAP gene family is the best characterized in terms of the expression patterns of individual gene members during *C. albicans* infections (reviewed in [3]). Nevertheless, there remain few reports of SAP gene expression...
patterns in clinical samples [4,5,6]. Expression of SAP1, SAP3, SAP4, SAP7 and SAP8 correlates with oral disease, whereas SAP1, SAP3 and SAP6-8 expression is associated with vaginal infection [6]. SAP7 expression was not detected in vitro, but was seen in clinical samples. Investigations of SAP gene expression ex vivo and in animal models of infection suggest that the various family members are expressed in a stage-specific and niche-specific fashion [4,6,7–10]. This differential regulation might relate to possible differences in Sap enzyme substrate specificities. In addition, the expression of some SAP genes might be regulated indirectly through the proteolytic activity of other family members [3,11].

The LIP gene family has at least ten members [12]. Expression of LIP5, LIP6, LIP8, and LIP9, but not the other members was detected in a mouse model of C. albicans peritonitis [12]. Phospholipase B expression has been detected immunologically in mucosal, gastrointestinal and systemic infection models [reviewed in 13]. Use of RT-PCR to distinguish between family members showed differential expression of PLB1 and PLB2 in samples from human oral and vaginal infections [3].

The agglutinin-like sequence (ALS) gene family encodes eight cell-surface GPI-anchored proteins which are presumed to promote adhesion of C. albicans cells to host tissues. More recently, however, it has been suggested that some Als proteins might be involved in growth-related functions (Lois Hoyer, personal communication). Immunohistochemical approaches have demonstrated expression of Als proteins by C. albicans cells infecting the kidneys, spleen, heart, liver and lungs of mice [15]. More recently, the expression of individual ALS genes has been detected during oral and vaginal infections by RT-PCR [16,17,18]. ALS expression patterns in clinical samples were similar to those observed in the corresponding animal models of oral, vaginal and systemic candidiasis, and in reconstituted human epithelial models [16,17,18,19]. This has reinforced the validity of these experimental models of C. albicans infection. ALS gene family members were differentially expressed in response to specific stimuli.
in vitro, for example during hyphal morphogenesis [14,20,21]. However, all ALS genes were expressed in the clinical samples and in the infection models. ALS6 and ALS7 were expressed at relatively low levels, and ALS4 appeared to be downregulated in vaginal samples, which was consistent with the idea that ALS genes might be differentially regulated in a niche-specific fashion.

HWP1 encodes a cell-surface adhesin that promotes strong interactions between C. albicans and host cells [22]. HWP1 is expressed during hyphal development [23,24]. Production of Hwp1 has been confirmed in the mouse model of systemic infection by immunohistochemistry [23]. More recently HWP1 expression has been observed in samples from human oral and vaginal infections by antibody and RT-PCR-based approaches [25,26]. These authors suggest that Hwp1 is important both for benign and invasive interactions of C. albicans with humans.

In general these studies support the view that secreted hydrolases (SAPs, LIPs and PLBs) contribute to the provision of nutrients and promote fungal penetration of host barriers [13], whilst the Als proteins and Hwp1 promote adhesion of fungal cells to host tissue [14,27]. These studies are starting to provide focused views on the contribution of specific virulence genes to different stages and types of C. albicans infection.

Technical challenges of going global

RT-PCR has been used for targeted analyses of select genes, but does not provide a global view of gene expression. Genome-wide studies are providing novel insights into the global responses of C. albicans to specific stimuli in vitro [28–33]. There is little doubt that the development of robust methods that allow genome-wide analyses of infection-associated gene expression in C. albicans represents an important objective. However, significant technical challenges must be overcome before this can be achieved. This explains why few genome-wide analyses of infection-associated expression have been published for C. albicans so far.

The first challenge is generation of sufficient fungal biomass for genome-wide analyses from infected tissues. This is not trivial. In some cases this problem has been partially solved by linear amplification of expressed sequences. This was sufficient to generate signals from mouse peritoneal and kidney infection models [34,35]. An alternative approach is to use ex vivo models that facilitate the generation of sufficient amounts of fungal biomass, such as cultured macrophage cell lines, reconstituted human epithelium, human blood fractions, or perfused pig livers [4,35,36,37,38,39]. Increasing inoculum size might facilitate the generation of fungal biomass, but might affect the experimental outcome, for example through effects of quorum sensing on virulence.

Secondly, during infections fungal cells are intimately associated with host tissue so that mammalian RNA inevitably 'contaminates' fungal RNA preparations. Hube and co-workers have found that mammalian sequences do not cross-react significantly with the C. albicans sequences on some microarrays. However, the hybridization signals on C. albicans microarrays were weak if the ratio of mammalian to fungal RNA exceeded 5:1 [35]. Some have attempted to enrich for fungal cells from mouse kidney homogenates by filtration and centrifugation, and then remove contaminating mammalian RNA by RNase treatment before preparing RNA from the fungal cell pellet [34]. Clearly controls are needed to test the impact of such enrichment procedures upon the transcriptome.

A third technical challenge relates to fungal population heterogeneity. It is becoming clear that individual fungal cells that occupy complex microenvironments within the host display significant population heterogeneity with respect to their molecular responses. Approaches such as transcript profiling, RT-PCR or proteomics average the behavior of whole populations, thereby masking this heterogeneity. One solution has been to use single cell profiling which exploits GFP-tagged strains to examine the molecular responses of individual C. albicans cells in specific microenvironments during infection [10,19,39,]. However, this approach is limited to examining single promoter-GFP fusions rather than the expression patterns of multiple genes.

A fourth challenge is that few parameters can be controlled in ex vivo and animal models of infection. This contrasts with experiments in vitro where many parameters such as nutrient availability can be specified. The changes observed in an infection transcriptome may therefore reflect C. albicans genes that are responding generically to the host microenvironment. This important philosophical issue raises key questions about experimental design. How best can one separate infection-specific changes from generic environmental responses? Lorenz et al. [38] addressed the issue by comparing the transcriptional response of phagocytosed C. albicans cells with cells exposed to carbon and nitrogen starvation in vitro. An alternative approach was taken by Hube’s group [35]. With a view to defining genes that are specifically associated with tissue invasion, they compared the behavior of invasive and non-invasive C. albicans strains and examined temporal changes in the transcriptome during the invasion process. Neither approach was entirely satisfactory: both yielded genes involved in generic environmental responses in addition to putative infection-associated genes.

A fifth challenge relates to host variability. This factor has an increasing impact upon experimental outcome as the complexity of infection models approaches that of the human host. For example, even individuals within an inbred mouse strain respond differentially to a C. albicans...
challenge. Clinical samples derived from a genetically and physiologically diverse human population show even greater variation, as indicated by recent work by Hube’s group [40**,]. Genome-wide studies of C. albicans expression patterns in clinical samples will have to address this. However, this microarray work from the Hube laboratory has validated the reconstituted human epithelium is a useful ex vivo model of mucosal infections [40**].

Global analyses reveal metabolic adaptation
Several studies have examined the global transcriptional response of C. albicans to the host using ex vivo and in vivo infection models. These include phagocytosis by neutrophils [41*], macrophages [38**], exposure to human blood and blood fractions [36*,37**], invasion of reconstituted human epithelium (RHE) and perfused pig liver [35**], and kidney and peritoneal infections in the mouse [34*,35**]. These studies have all highlighted the importance of micro-scale environmental adaptation in infection-associated gene expression. They have shown that C. albicans cells alter their metabolism to assimilate available nutrients and attempt to protect themselves from the immediate host defenses they encounter in each microenvironment within the host (Figure 1). This key point, which has now been reinforced by a recent microarray study of clinical samples from oral infections [40**], was not revealed by studies that focused on putative virulence factors.

C. albicans must assimilate nutrients to grow and develop an infection in vivo. Microarray studies suggest that the fungus programs significant changes in carbon metabolism following exposure to the host. Glycolysis is down-regulated, and fatty acid β-oxidation and the glyoxylate cycle are upregulated following phagocytosis by macrophages or neutrophils [37**]. By contrast, the majority of C. albicans cells in the plasma and in tissues appear to assimilate carbon via glycolysis and the TCA cycle [35**,37**]. These global observations are reinforced by studies of individual C. albicans gene sets and of mutants lacking individual genes [25,39,42–44]. Together, these studies re-emphasize the differences in transcriptional responses of C. albicans in different host microenvironments.

Changes in C. albicans nitrogen metabolism also occur following exposure to host immune defenses or growth in biofilms [37**,38**,41*,45]. Amino acid biosynthetic genes are induced following neutrophil attack and, as described above, SAP gene induction probably contributes to nitrogen availability. SAP genes are required for virulence [46,47]. However, amino acid biosynthetic genes do not appear to be upregulated during tissue invasion [35**]. This is consistent with the observation that the inactivation of the amino acid starvation response or specific amino acid biosynthetic pathways does not attenuate the virulence of C. albicans [48,49]. Therefore, tissues appear to be amino acid replete.

Genes involved in iron and phosphate assimilation are upregulated during the development of tissue infections [35**]. Also the expression of pH sensing functions changes during exposure to human blood and during tissue invasion [35**,36*,37**]. Again these transcript profiling data are consistent with mutant studies indicating that iron assimilation and pH sensing are required for the overall virulence of C. albicans [35**,50,51].

Stress adaptation also contributes to virulence
Genome-wide expression profiling has also reinforced the view that stress adaptation is essential for the virulence of C. albicans. Numerous stress genes are induced when C. albicans cells are exposed to macrophages, neutrophils, blood or epithelial cells, or during oral infections [36*,37**,38**,40**,41*] (Figure 1). These include functions involved in the detoxification of reactive oxygen and nitrogen species, such as catalase, superoxide dismutase and components of the glutathiodoxin and thioredoxin systems. In addition, protective functions such as heat shock proteins (chaperones) are induced. The data suggest that C. albicans counteracts host immune cells by inducing adaptive stress responses. This view is supported by the observation that the virulence of C. albicans is attenuated by inactivating catalase, superoxide dismutases, or a flavohemoglobin involved in NO detoxification [31,37**,52–54,], or by disrupting the stress-activated protein kinase, Hog1 [55], which is required for oxidative stress resistance [56,57]. Therefore, stress adaptation is essential for overall virulence.

Genomic studies in animal models of disseminated infections have indicated that some stress genes – mainly chaperones (heat shock proteins), rather than oxidative stress functions – are upregulated in infected kidneys and liver [34*,55**]. This might suggest that, having survived an initial onslaught from the host immune system to establish a tissue infection, fungal cells are infrequently exposed to an oxidative stress. This view is reinforced by a recent analysis showing minimal oxidative stress adaptation by C. albicans cells in the mouse kidney [58*]. Therefore, C. albicans appears to activate adaptive stress responses in a niche-specific fashion during disease establishment and progression.

Future perspectives
The recent application of genomics to the analysis of infection-related gene expression in C. albicans infection has broadened our outlook. An initial focus on putative virulence functions has been extended to include fitness attributes such as metabolic and stress adaptation. Clearly these play a central role in the infection process. But several new questions have been raised.

Genomic studies show that multifarious changes in C. albicans gene expression occur during infection. Genes
encoding key processes such as metabolic and stress adaptation are upregulated at certain stages of the disease process. Therefore it is not surprising that many C. albicans genes have been found to be essential for the overall virulence of the fungus in animal models [48,59]. However, genomic studies in *Saccharomyces cerevisiae* have revealed a poor correlation between the genes that are regulated in response to a stimulus and those that are essential for that response [60]. Functional redundancy and post-translational regulation account at least in part for this poor correlation. Therefore, by analogy, many C. albicans genes that are apparently regulated during infection processes may not truly be required for virulence.

Numerous C. albicans genes of unknown function are regulated following exposure to host factors [34*,35*,36*,37*,38*,41*]. Obviously, some of these genes might execute interesting functions relevant to infection that have not yet been revealed by *in vitro* studies, a view that has been reinforced by the analysis of an infection-associated gene [40**]. How do these genes contribute to infection?

‘Classical’ virulence attributes such as secreted hydrolases and adhesins are regulated during infection. However, attempts to identify infection-associated genes by expression profiling have tended to highlight functions involved in environmental adaptation. For example, recent attempts to isolate invasion-associated genes in *C. albicans* resulted in the identification of an environmental pH sensor [35**]. Having said that, functional analyses of a gene required for deep invasion of epithelial cells revealed that it is also required to maintain hyphal elongation [40**]. Clearly virulence factors are important, but is environmental adaptation the main key to pathogenicity?

These and other questions present major challenges for the future.

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**References and recommended reading**

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