

Chapter 13

Transcriptome in Human Mycoses

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Abstract Mycoses are infectious diseases caused by fungi, which incidence has increased in recent decades due to the increasing number of immunocompromised patients and improved diagnostic tests. As eukaryotes, fungi share many similarities with human cells, making it difficult to design drugs without side effects. Commercially available drugs act on a limited number of targets, and has been reported fungal resistance to commonly used antifungal drugs. Therefore, elucidating the pathogenesis of fungal infections, the fungal strategies to overcome the hostile environment of the host, and the action of antifungal drugs is essential for developing new therapeutic approaches and diagnostic tests. Large-scale transcriptional analyses using microarrays and RNA sequencing (RNA-seq), combined with improvements in molecular biology techniques, have improved the study of fungal pathogenicity. Such techniques have provided insights into the infective process by identifying molecular strategies used by the host and pathogen during the course of human mycoses. In this chapter, the latest discoveries about the transcriptome of major human fungal pathogens will be discussed. Genes that are essential for host-pathogen interactions, immune response, invasion, infection, antifungal drug response and resistance will be highlighted. Finally, their importance to the discovery of new molecular targets for antifungal drugs will be discussed.

13.1 Introduction

Fungi are eukaryotic microorganisms widely distributed in nature, existing as yeasts, molds, and mushrooms. Fungi are important decomposers of biomass and are useful in baking and wine fermentation. However, fungi can also cause severe, life-threatening infections in humans, animals, and vegetables, which can result in enormous economic losses. Humans are constantly in contact with fungi by inhaling spores in the air and ingesting them as nutritional sources. Worldwide, human

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mycoses have increased in incidence due to the high prevalence of immunocompromised patients, becoming a major public-health concern. Although fungal infections are widespread, they are often overlooked, and in general, public-health agencies perform little surveillance of fungal infections (Brown et al. 2012). Fungi cause a wide spectrum of disease, ranging from asymptomatic infection to disseminated and fatal diseases. Nevertheless, fungal infections are not frequently diagnosed, which impairs the proper epidemiological surveillance of these diseases. Therefore, more data about the life cycle of pathogenic fungi and the pathogenesis of their infections will aid the development of therapeutic approaches and diagnostic tests. Although research funding for human mycoses remains lower than that for other areas of medical microbiology, the number of publications in the field of medical mycology has increased over the past several decades.

Fungi can infect several anatomical sites, resulting in different clinical symptoms. The most prevalent are cutaneous, mucosal, and pulmonary diseases. These infections can be acquired from trauma to the skin and mucosa, direct or indirect contact with infected humans and animals, contact with contaminated fomites, or inhalation. Airborne fungal infections typically result in pulmonary diseases. Skin and nail infections affect both healthy and immunocompromised individuals, decreasing quality of life by causing discomfort and pruritus. Cutaneous infections are most commonly caused by dermatophytes, a closely related group of keratinophilic molds that infect humans and animals. They are directly or indirectly transmitted between infected organisms and contaminated objects, such as towels and manicure appliances (Peres et al. 2010a). *Candida* species can also cause skin and nail infections, but they more commonly cause oropharyngeal (thrush) and vulvovaginal candidiasis. Many *Candida* species are harmless and are commensal microorganisms. However, immune-system impairment favors their pathogenicity, and they can cause opportunistic infections. *C. albicans* is part of the normal microbiota of mucous membranes of the respiratory, gastrointestinal, and female genital tracts. Changes in the host's immunological status enable its invasive behavior, leading to tissue damage and dissemination through the bloodstream to other organs (Mayer et al. 2013).

Deep fungal infections are mainly caused by *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, and *Blastomyces dermatitidis*. Some fungal diseases are endemic, such as blastomycosis (*B. dermatitidis*) and histoplasmosis (*H. capsulatum*), which are mainly found in the United States, and paracoccidioidomycosis (*P. brasiliensis*), which is primarily found in Latin America. Others are cosmopolitan and are encountered worldwide (Brown et al. 2012). Fungal spores are present in the environment and can be inhaled; upon reaching the lungs, they adhere to the parenchyma and initiate the infectious process. From the lungs, they can enter the bloodstream and disseminate to other organs, mainly the liver and spleen. Table 13.1 summarizes the major human fungal pathogens and their associated diseases. Overall, treatment of clinical mycoses can be a very long and expensive process that is often associated with uncomfortable side effects that lead to treatment interruption (Martinez-Rossi et al. 2008).

Table 13.1 Main fungal pathogens and their associated diseases in humans

Fungi	Main species	Disease
<i>Aspergillus</i>	<i>A. fumigatus</i>	Pulmonary infections (invasive aspergilosis and aspergilloma) Allergy
<i>Blastomyces</i>	<i>B. dermatitis</i>	Skin lesions Pulmonary infections
<i>Candida</i>	<i>C. albicans</i>	Cutaneous infections—skin and nails
	<i>C. glabrata</i>	Oropharyngeal candidiasis (thrush)
	<i>C. parapsilosis</i>	Vulvovaginal candidiasis
<i>Coccidioides</i>	<i>C. immitis</i>	Pulmonary infections
<i>Cryptococcus</i>	<i>C. neoformans</i>	Meningitis Pulmonary infections (pneumonia)
<i>Dermatophytes</i>	<i>Trichophyton rubrum</i>	Cutaneous infections—skin, nail, and hair (tinea or ringworms)
	<i>Trichophyton mentagrophytes</i>	
	<i>Microsporum canis</i>	
<i>Histoplasma</i>	<i>H. capsulatum</i>	Pulmonary infections
<i>Malassezia</i>	<i>M. furfur</i>	Cutaneous infections—skin (pityriasis versicolor)
<i>Paracoccidioides</i>	<i>P. brasiliensis</i>	Pulmonary and systemic infections
<i>Penicillium</i>	<i>P. marneffei</i>	Pulmonary infections
<i>Pneumocystis</i>	<i>P. carinii</i>	Pneumonia

Some fungal species are found as filamentous or yeast forms, while others are dimorphic (i.e., found in both forms). In dimorphic fungi, the yeast form represents the parasitic phase, and the hyphae form represents the saprophytic phase. The filament (or hyphae) is a tubular multi-cellular structure, and cells are divided into compartments by the formation of a septum. Yeasts are round, single cells that reproduce by budding and some species can form pseudohyphae, a chain of interconnected yeast cells. Fungi can undergo sexual or asexual reproduction, producing spores that can be inhaled or enter the body at sites of tissue damage. Once the spores reach their appropriate niche, they develop into hyphae that invade the tissue in search of nutrients.

The genomes of several fungal pathogens have been sequenced, enabling the design and analysis of microarrays, representing a large amount of transcriptomic data to be explored. Fungal transcriptomics have been used to analyze gene expression and regulation in response to antifungal exposure, environmental changes, and

interaction with the host during infection. The transcriptional profile may help elucidate several aspects of fungal biology, including signaling pathways that enable fungal survival, and help predict molecular targets for the development of novel antifungal drugs (Peres et al. 2010b; Cairns et al. 2010). Transcriptional and proteomic analyses have been used to identify connections among signaling and metabolic pathways that govern fungal development, morphogenesis, and pathogenicity as well as the host's immune response. Recent advances in molecular biology methods, especially RNA-seq by next-generation sequencing, have enabled the study of the whole transcriptome. These analyses provide insights into the functionality of the genome, revealing molecular components of cells and tissues involved in physiological and pathological processes. Transcriptome allows the analysis of all transcript species, including mRNAs, which encode proteins, as well as non-coding RNAs (ncRNAs) and small RNAs (sRNAs), which regulate gene expression and maintain cellular homeostasis. Furthermore, transcriptional profiling by RNA-seq is useful to determine gene structures at transcription initiation sites, 5'- and 3'-ends, and introns as well as splicing patterns (Wang et al. 2009).

This chapter will discuss recent advances in fungal transcriptomics arising from microarray and RNA-seq analyses. The contribution of these findings to the understanding of fungal biology and fungal diseases will be highlighted. The intrinsic relationship of the outcome of fungal infections and the immunological status of the host stresses the need to evaluate the host immune response to fungi. Furthermore, knowledge of the genes expressed in response to stressful environmental conditions and the gene networks that regulate the transcriptome during a fungal infection will help elucidate the pathogenesis of fungal infections and identify possible molecular targets for the development of novel therapeutic agents. Such information will aid both the treatment and prevention of fungal infections.

13.2 Host Immune Response to Fungal Infections

The host's immunological status is the primary determinant of the severity of fungal infections, which can range from asymptomatic to severe and disseminated. Immunocompromised patients often suffer from severe, disseminated, and fatal fungal infections. Host-pathogen interactions are complex and involve several molecules on the surface of both host and fungal cells. These surface molecules participate in pathogen recognition, fungal adhesion to host cells, downstream intracellular signaling, and metabolic pathways that mediate pathogen survival and host immune responses. An understanding of the infective process requires molecular knowledge of the pathogen strategies for infecting the tissue as well as the host responses aimed at eliminating the pathogen and maintaining cellular integrity. The development of experimental models has improved the study of infectious diseases, and most of these models utilize immunosuppressed mice, because most fungal species cause opportunistic infections. However, for some pathogens, such as anthropophilic dermatophytes, these models are not suitable and *ex vivo* and *in vitro* assays have being

performed, providing insights into the pathogenic process and immune response triggered by the fungus.

Fungal diseases can result from poor immune response or from exacerbated activation of the immune system, such as the inflammatory response. Therefore, the interplay of the innate and adaptive immune systems and their appropriate activation are crucial for successful pathogen clearance and cellular homeostasis. Recent studies have characterized different mechanisms underlying the host's immune response to fungi. The innate immune response is comprised of the epithelial barrier, mucosa, and phagocytes (i.e., neutrophils, macrophages, and dendritic cells [DCs]), which play essential roles in preventing the entry of pathogenic microorganisms and rapidly killing these pathogens, as well as activating the adaptive immune response. Complement and other molecules, such as antimicrobial peptides and mannose-binding lectin, are also important host defense mechanisms. Pattern-recognition receptors (PRRs) on the surface of host cells interact with pathogen-associated molecular patterns (PAMPs) on the surface of pathogens, such as mannoproteins and β -glucan in fungi. The molecular interaction between PRRs and PAMPs triggers intracellular signaling pathways that initiate early inflammatory and non-specific responses in the host and upregulates virulence factors in the pathogen that enhance survival. PRRs include the toll-like receptors (TLRs) TLR2, TLR4, and TLR9, complement receptor 3, mannose receptor, Fc γ receptor, and Dectin-1 (Romani 2011).

In general, a Th1 response is correlated with protective immunity against fungi. The Th1 response is characterized by the production of interferon gamma (IFN- γ) and leads to cell-mediated immunity. Antigen-presenting cells (APCs), such as macrophages and DCs, initiate the Th1 response once their PRRs engage with fungal PAMPs, which leads to cellular activation and elicits effector properties. Th1 cells are essential for optimal activation of phagocytes at the site of infection through producing signature cytokines, such as like IFN- γ . Moreover, Th17 cells support Th1 cellular responses in experimental mucosal candidiasis, indicating an important role for Th17 cells in promoting neutrophil recruitment and Th1 immune responses (Conti et al. 2009; Romani 2011; Bedoya et al. 2013). Whole-genome transcriptional analyses have identified specific transcriptional profiles of host cells in response to various fungal species. Different cell types respond to fungal stimuli by activating distinct intracellular signaling pathways downstream of different PRRs. This mechanism confers plasticity to immune cells, such as DCs and macrophages, which shapes T-cell responses during fungal infections. The distinct signaling pathways in phagocytes influence the balance between innate and adaptive immune responses and the balance between CD4⁺ T cells and regulatory T cells. Together, the balance between these processes establishes the outcome of the infection (Romani 2011).

H. capsulatum is a dimorphic fungus that causes respiratory infections and disseminated disease in immunocompromised hosts. The *H. capsulatum* hyphae produce spores (conidia) in the environment, which can be inhaled by humans. Inside the host, the conidia undergo morphological changes to form yeast cells. Once inhaled, the conidia are captured by phagocytic cells, such as macrophages, and trigger the host immune response. On the other hand, yeast cells use alveolar

macrophages as vehicles to spread to different organs, such as the liver, spleen, lymph nodes, and bone marrow (Deepe 2000). Transcriptomic analysis by microarrays has revealed that conidia and yeast cells induce different transcriptional responses in macrophages. In response to infection with *H. capsulatum* conidia, macrophages specifically upregulated type 1 IFN-induced genes, including IFN- β and a classic type 1 IFN secondary response signature, in addition to general inflammatory genes. This effect was dependent on interferon regulatory factor 3 (IRF3) and independent of the TLR signaling pathway. This expression profile suggested that type 1 IFN contributes to the outcome of infection with *H. capsulatum*. Indeed, although ELISA failed to detect IFN- β protein, macrophages lacking the type 1 IFN receptor IFNAR1 did not express IFN- β in response to conidia exposure. Furthermore, IFNAR1 knockout mice showed a decreased fungal burden in the lungs and spleen after intranasal infection with conidia and yeast cells as compared to wild type mice. Therefore, IFNAR1 signaling might contribute to the virulence and disease burden of *H. capsulatum* infection rather than conferring protection. The authors suggested that type 1 IFNs might modulate cytokine production, apoptosis of infected macrophages, or specific aspects of the adaptive immune response to *H. capsulatum* (Inglis et al. 2010).

However, in the pathogenic yeast *C. neoformans*, which causes severe meningoencephalitis in immunocompromised patients, type 1 IFN signaling directs cytokine responses toward a protective type 1 pattern during murine cryptococcosis. IFNAR1 and IFN- β knockout infected mice displayed higher fungal burdens in the lungs and brain as well as decreased survival as compared to wild type mice (Biondo et al. 2008). Likewise, *C. albicans* induced the expression of type 1 IFN genes and proteins in DCs but not in macrophages. This pathway relied on Myd88 signaling, which is a TLR adaptor molecule. IFNAR1 and IFN- β knockout mice also displayed a lower survival rate and increased fungal burden in the kidneys. Therefore, the type 1 IFN response was protective against *C. albicans* by stimulating the host's innate immune defense mechanisms (Biondo et al. 2011). These studies highlight the complexity of the immune response to fungal pathogens and demonstrate the various roles that type 1 IFN plays.

The major virulence factor of *C. neoformans* is its polysaccharide capsule, which interferes with recognition by immune cells. Microarray analysis showed profound differences in gene expression profiles of DCs during phagocytosis of encapsulated versus non-encapsulated isogenic strains of *C. neoformans* opsonized with mouse serum. In general, the non-encapsulated strain induced the expression of genes involved in DC maturation, chemokines, and cytokines, characterizing an immunostimulatory response. In contrast, the encapsulated strain caused a downregulation or no change in the expression of these genes, indicating that the capsule prevented the activation of immune response-related genes. Among the proteins encoded by the genes upregulated in response to the non-encapsulated *C. neoformans* strain were CD86, CD83, the transcription factor Relb, ICAM1, major histocompatibility complex class II (MHC-II)-related genes (H2-D1, H2-Q7, and H2-Q8), and the pro-inflammatory cytokines IL-12, TNF- α , and IL-1. These proteins are involved in DC activation, maturation, and migration through the endothelium as well as

induction of an inflammatory response. Several chemokines were also upregulated in DCs stimulated with the non-encapsulated strain, including CCL3, CCL4, CCL7, CCL12, CXCL10, CCL22, and the chemokine receptor CCR7, which contributes to the accumulation of inflammatory cells at the site of infection. Among the proteins encoded by the genes downregulated by the encapsulated strain were E74-like factor 1 (Elf1) and sequestosome 1 (Sqstm1), which regulate the expression of cytokines genes and the induction of NF- κ B signaling, respectively (Lupo et al. 2008).

P. brasiliensis causes pulmonary and systemic infections. Once in the lungs, the yeast cells interact with resident phagocytic cells, such as macrophages and DCs. Microarray analyses of murine macrophages and DCs after phagocytosis of *P. brasiliensis* identified differential expression of genes encoding inflammatory cytokines, chemokines, signal-transduction proteins, and apoptosis-related proteins (Silva et al. 2008). Among the genes upregulated in macrophages were the pro-inflammatory chemokines CCL21, CCL22, and CXCL1. CXCL1 and CCL22 recruit neutrophils and monocytes, respectively, while CCL21 mediates the homing of lymphocytes to secondary lymphoid organs. Upregulation of the gene encoding NF- κ B might account for the upregulation of pro-inflammatory chemokines and cytokines (e.g., TNF- α) that increase the cytotoxic activity of macrophages (Silva et al. 2008). Expression of the TNF- α gene and protein increased upon macrophage infection with *P. brasiliensis*. This was consistent with previous findings that p53KO mice, which are deficient in TNF- α , were unable to control *P. brasiliensis* infection, given the increased fungal burden and the absence of a well-formed granuloma (Silva et al. 2008; Souto et al. 2000). After exposure to *P. brasiliensis*, macrophages highly expressed apoptotic genes, including caspases 2, 3, and 8, which may represent a mechanism of eliminating the fungus without damaging host tissues. On the other hand, the fungus induced the expression of matrix metalloproteases genes, which may have facilitated fungal invasion, given their role in tissue remodeling (Silva et al. 2008).

Expression of the gene encoding IL-12 was downregulated in macrophages interacting with *P. brasiliensis*, which might represent a strategy of the fungi to evade the immune system. However, this gene was upregulated in DCs interacting with *P. brasiliensis* (Tavares et al. 2012) and *C. neoformans* (Lupo et al. 2008). IL-12 is associated with resistance to paracoccidioidomycosis and cryptococcosis by inducing IFN- γ production and Th1 protective responses. IL-12p140 knockout infected mice displayed decreased survival, higher fungal burden, and decreased production of IFN- γ (Decken et al. 1998; Livonesi et al. 2008). In addition to IL-12, DCs exposed to *P. brasiliensis* expressed genes encoding other pro-inflammatory cytokine and chemokine genes, such as TNF- α , CCL22, CCL27, CXCL10, and NF- κ B, concomitantly with the downregulation of the NF- κ B inhibitor N κ -RF encoding gene. Both macrophages and DCs expressed CCL22 in response to *P. brasiliensis*, which might have increased the microbicidal activity of macrophages by stimulating a respiratory burst and the release of lysosomal enzymes. The chemokines expressed by macrophages and DCs in response to *P. brasiliensis* mediate the accumulation of leukocytes at the site of infection in order to control fungal invasion (Silva et al. 2008; Tavares et al. 2012).

Innate immune cells represent the first line of defense against pathogenic microorganisms (Mullick et al. 2004; Kim et al. 2005; Fradin et al. 2007) and include polymorphonuclear cells (PMNs, neutrophils), eosinophils, basophils, and monocytes. Monocytes release various cytokines in response to infection in order to amplify and coordinate the immune response. The dynamics of the molecular response triggered by *C. albicans* was measured in human monocytes using a time-course assay. This analysis identified a pattern of gene expression that might account for the recruitment, activation, and viability of phagocytes as well as the enhancement of chemotaxis and inflammation (Kim et al. 2005). Increased expression of genes encoding the pro-inflammatory cytokines TNF- α , IL-6, and IL-1 α was correlated with neutrophil infiltration at the site of infection. In addition, there was an upregulation of genes encoding the chemokines CCL3, CCL4, CCL20, CCL18, CXCL1, CXCL3, and IL-8, which are involved in the activation and recruitment of phagocytes and lymphocytes, as well as genes encoding the chemokine receptors CCR1, CCR5, CCR7, and CXCR5. This expression pattern likely favored the recruitment of inflammatory cells during the yeast phase of *C. albicans* life cycle. Moreover, TNF- α is essential for mounting a Th1 immune response and successfully controlling the infection. On the other hand, the expression of T cell-related genes was unchanged during the time-course analysis, suggesting that T cell regulatory molecules were not important in the early response of monocytes to *C. albicans*. It was proposed that in the early stages of infection, monocytes overexpress genes encoding various pro-inflammatory cytokines, chemokines, and chemokine receptors as well as COX2, IL-23, which are important for inflammation, and heat-shock proteins, which are implicated in the induction of inflammatory cytokines and chemokines. Thus, these changes in gene expression allow cellular recruitment and activation. Along with the pro-inflammatory response, increased expression of genes encoding anti-apoptotic molecules (XIAP and BCL2A1) may have protected the monocytes from cellular damage and death. The gene encoding the transferrin receptor (CD71) was upregulated, suggesting that iron deprivation might be a defense mechanism against infection. Indeed, iron is essential to the virulence of several pathogens (Kim et al. 2005).

Neutrophils display a potent set of hydrolytic enzymes, antimicrobial peptides, and oxidative species within their intracellular granules. These cells have an immediate and pronounced effect on *C. albicans* (Fradin et al. 2005). Granulocyte-like cells phagocytose and kill *C. albicans* and prevent hyphal growth, and undergo apoptosis after pathogen exposure. During this process, granulocytes upregulate inflammatory genes and downregulate anti-*Candida*-response genes, depending on the size of the inoculum. Among the upregulated genes were inflammatory mediators, including IL-1 β , TNF- α , COX2, and the chemokine CCL3. On the other hand, genes encoding myeloperoxidase, which causes hyphal damage, and defensins, such as human neutrophil protein 1 (HPN1), were downregulated. These changes may represent mechanisms by which *C. albicans* survives the early stages of infection (Mullick et al. 2004).

In another study, a microarray of immune-related genes was used to evaluate the early response of PMN cells to *C. albicans* hyphal cells, UV-killed and live yeasts.

In PMNs, the transcriptional profiles induced by live yeasts and hyphae were more similar to one another than to that induced by dead yeasts. This suggested that fungal viability had a more significant effect on PMN gene expression than cellular morphotype. The presence of *C. albicans* did not affect the expression of genes encoding granule proteins. Nevertheless, *C. albicans* induced the upregulation of pro-inflammatory genes and cell-to-cell signaling (leukemia inhibitory factor [LIF]), signal transduction proteins, cell stimulatory factors, vascular endothelial growth factor, and PMN-recruitment chemokines (CCL3 and CXCL2). Importantly, these gene expression changes were irrespective of fungal cell type or viability. Furthermore, the few genes that were downregulated in response to *C. albicans* were involved in the regulation of cell signaling and growth (Fradin et al. 2007). In addition, exposure to viable *Candida* cells upregulated genes encoding stress-response proteins, including heat shock proteins (HSPA8, HSPCA, HSPCB, and HSPH1). This demonstrated a direct effect of live cells on PMN cells and monocytes (Kim et al. 2005). Interestingly, these genes also regulate CXC-type chemokines, indicating that this antimicrobial response amplifies the overall immune response by recruiting additional cells to the infection site. Overall, this transcriptional profile suggested that PMNs contribute to the immunological response to *C. albicans* by expressing genes involved in cellular communication, which may recruit more PMNs or other immune cells.

Systemic candidiasis is characterized by *C. albicans* entering the bloodstream, disseminating throughout the body, and causing microabscesses. In the blood vessels, the fungus must adhere to and invade endothelial cells (ECs); thus, the ECs have the potential to influence the host response to vascular invasion. Microarray transcriptional analysis of ECs in response to *C. albicans* identified the upregulation of genes with several functions, especially chemotaxis, angiogenesis, cell death, proliferation, intra- and intercellular signaling, immune response, and inflammation (Muller et al. 2007; Barker et al. 2008; Lim et al. 2011). These results support an important role for ECs in innate immunity. *C. albicans* induces several genes that are targets of the pro-inflammatory transcription factor NF- κ B, which plays a central role in EC transcriptional regulation during *C. albicans* infection. In addition, the expression of chemokines, including IL-8, CXCL1, CXCL2, CXCL3, CXCL5, and CXCL6, indicates that ECs help recruit neutrophils and monocytes to the infection site (Muller et al. 2007). The overexpression of genes involved in stress and wound healing, coding for pro-inflammatory mediators such as IL-1, calgranulin C, E-selectin, and prostaglandin-endoperoxide synthase 2, correlated with the endothelial damage caused by *C. albicans*. The ECs also upregulated anti-apoptotic genes, suggesting that ECs respond to *C. albicans* by undergoing cellular proliferation (Barker et al. 2008). However, another transcriptional profile revealed that apoptotic genes were upregulated in ECs infected with a high density of *C. albicans*; the difference in the number of fungal cells may have been responsible for this difference in expression profile. In general, human umbilical vein ECs infected with high densities of *C. albicans* displayed a stronger and broader transcriptional responses than cells infected with low densities. Again, this effect may have been related to the number of cells or even to secreted molecules involved in quorum

sensing. The authors hypothesized that in microenvironments with a high density of yeast cells, such as microabscesses, the fungus triggers apoptosis, which disrupts the endothelial barrier and permits fungal dissemination to different organs and tissues (Lim et al. 2011).

Given the complex and dynamic nature of host-pathogen interactions, techniques that measure both the host and pathogen responses are crucial for characterizing their interaction. Recent advances in molecular techniques have identified the molecular responses of the host and the pathogen during the infection. In dual transcriptomics, microarrays and RNA-seq have been used to identify molecular patterns of the pathogen and the host. These studies have provided insight into the dynamics of the infectious process (Westermann et al. 2012). Dual transcriptomics by RNA-seq was performed during DC phagocytosis of *C. albicans*, and gene interactions were predicted using a systems biology approach. Specifically, RNA-seq identified 545 *C. albicans* and 240 DCs genes that were differentially expressed. The genes were clustered by their expression kinetics over the duration of the interaction, and selected genes were used to infer gene interactions. After experimentally validating one of these gene interactions, the authors proposed a model in which PTX3, an opsonin secreted by DCs that facilitates phagocytosis through dectin-1, binds to the *C. albicans* cell wall, leading to its remodeling, which is mediated by the transcription factor Hap3 during invasion of innate immune cells. Remodeling of the fungal cell wall compromises the ability of immune cells to recognize fungi, thus attenuating the immune response (Tierney et al. 2012).

Microarray-based dual transcriptomics was performed on *A. fumigatus* interacting with bronchial epithelial cells. The resulting expression patterns indicated activation of the host's innate immune response (Oosthuizen et al. 2011). *A. fumigatus* is a major cause of pulmonary fungal infections, including invasive aspergillosis, aspergilloma, and allergy. During infection, environmental conidia enter the airways through inhalation. There, they germinate into hyphae and penetrate the lung parenchyma. Upon invasion, the fungus can disseminate to other organs and tissues. In response to *A. fumigatus* conidia, bronchial cells upregulated genes involved in innate immunity, chemokine activity, and inflammation. Among the overexpressed genes were those encoding the chemokines CCL3 and CCL5, which recruit leukocytes to the site of infection, matrix metalloproteinases (MMP1 and MMP3), and glutathione transferase (MGST1), which protects against oxidative damage. By comparing the expression profiles of two different cell lines, the authors identified only 17 genes in common. This demonstrates the variability in gene expression between a cell line and primary cells resulting from exposure to the same fungus (Gomez et al. 2011; Oosthuizen et al. 2011). The commonly expressed genes mainly encoded chemokines and regulators of the innate immune response. In particular, IL-6, a potent pro-inflammatory cytokine, was highly expressed in response to *A. fumigatus* conidia. This was consistent with earlier findings that IL-6-deficient mice were susceptible to invasive pulmonary aspergillosis and had impaired protective Th1 responses (Oosthuizen et al. 2011; Cenci et al. 2001). In addition, genes involved in nucleosome organization and chromatin assembly were overexpressed. Genes involved in mitosis and cell cycle progression were downregulated, suggest-

ing decreased proliferation and cell cycle arrest during infection with *A. fumigatus* (Gomez et al. 2011).

Moreover, in response to *A. fumigatus* human monocytes presented a coordinated expression of genes involved in fungal death and invasion (Cortez et al. 2006). Among the highly expressed genes were pro-inflammatory genes, such as IL-1 β , CCL3, CCL4, IL-8, PTX3, and SOD2, and regulators of inflammation, such as IL-10, COX2, and HSP40. Moreover, several anti-inflammatory genes were downregulated, such as CD14, which is involved in phagocytosis, and CCL5, which is a Th1 chemokine. This differential regulation of pro- and anti-inflammatory genes likely balanced the innate immune response. Furthermore, the coordinated expression of genes involved in oxidative response may have both eliminated the fungus and protected the cell. This was supported by the high expression of superoxide dismutase (SOD2) and dual phosphatase (DUSP1) and downregulation of catalase (CAT), glutathione peroxidase 3 (GPX3), and peroxiredoxin 5 (PRDX5) (Cortez et al. 2006).

Dermatophytes are highly specialized fungi that use keratin as a nutrient source, and thus infect keratinized structures, such as skin, hair, and nails. Upon infecting the skin, dermatophytes first encounter keratinocytes, which represents an important barrier against pathogens and help mediate the immune response (Peres et al. 2010a). The transcriptional profile of keratinocytes was measured in response to *Arthroderma benhamiae*, a zoophilic dermatophyte, and *Trichophyton tonsurans*, an anthropophilic species. During interaction, these fungi induced different cytokine expression profiles in keratinocytes, which were correlated with the inflammatory response. *Arthroderma benhamiae* induced the upregulation of 48 cytokine-related genes. In contrast, *T. tonsurans* induced the differential expression of only 12 cytokine-related genes; 4 were upregulated, and 8 were downregulated. In infected keratinocytes, the zoophilic species induced the upregulation of pro-inflammatory genes and the concomitant secretion of cytokines IL-1 β , IL-6, IL-6R, and IL-17 and chemokines IL-8 and CCL2. This may promote the infiltration of inflammatory cells in the skin during infection. Moreover, the upregulation of IL-6, IL-6R, and granulocyte-colony stimulating factor (G-CSF) may have promoted tissue remodeling and wound healing. On the other hand, the anthropophilic species induced limited cytokine expression and release, including exotoxin 2, IL-8, and IL-16. This was likely responsible for the poor inflammatory response observed in *T. tonsurans* skin infection (Shiraki et al. 2006). Moreover, mice infected with *Arthroderma benhamiae* displayed an infiltration of PMNs, macrophages, and DCs in the skin as well as increased levels of TGF- β , IL-1 β , IL-6, and IL-22 mRNA in skin biopsies. Because these cytokines are involved in the establishment of the Th17 response, this pathway might regulate immunity against dermatophytes (Cambier et al. 2014).

In summary, fungal pathogens induce several changes in the host's target cells and innate immune cells. Studying the transcriptome of fungal-host interactions has elucidated the molecular patterns associated with protection from or progression of fungal infections. In general, fungi induce the upregulation of genes encoding cytokines, chemokines, and other pro-inflammatory molecules in host cells, which recruit inflammatory cells to the site of infection. Host cells exhibit different ex-

pression profiles in response to different fungal pathogens, which may account for the differences in outcomes of these infections. Moreover, some studies have identified molecular strategies by which fungi evade the host's immune system as well as host defense mechanisms that favor fungal survival. Transcriptomic analyses have generated hypotheses that can be further validated by reverse genetic approaches in order to better characterize the immune components that contribute to the outcome of fungal infections.

13.3 Metabolic Adaptation of Fungi During Infection

Fungal pathogens adapt to the host's microenvironment during infection, a process that requires dynamic responses to constantly changing conditions. In particular, nutrient availability can be limited in host niches, especially inside phagocytes. Thus, fungi undergo metabolic adaptations in order to control, for example, glycolysis, gluconeogenesis, the glyoxylate cycle, and proteolysis. This allows them to utilize diverse substrates as nutrient sources, evade the toxic conditions triggered by the immune response, and maintain their virulence despite changes in the physiological ambient (Brock 2009). Phagocytes produce reactive oxygen and nitrogen species (ROS and RNS), which induce oxidative and nitrosative stress and kill pathogens (Brown et al. 2009). Reactive species can alter or inactivate proteins, lipid membranes, and DNA. Pathogens can survive this toxic environment by producing protective enzymes, such as flavohemoglobin and S-nitrosoglutathione (GSNO) reductase, which confer resistance to nitrosative stress (de Jesús-Berrios et al. 2003), and superoxide dismutases, catalases, and peroxidases, which counteract oxidative stress. Non-enzymatic defenses include metabolites, such as melanin, mannitol, and trehalose (Missall et al. 2004). The ability of pathogens to sense and appropriately respond to environmental pH is essential for their survival in different host niches. In pathogenic fungi, the PACC/RIM signaling pathway has been implicated in survival, growth, virulence, and dissemination in different host niches (Cornet and Gaillardin 2014). The pH affects enzymatic activities, and the alkaline pH of human tissues influences nutrient uptake, because the solubility of essential elements, such as iron, is pH-dependent (Davis 2009). Iron is an essential micronutrient for both the host and pathogen, as it is required for several metabolic processes, including respiration and DNA replication. Iron, in the form of heme and iron-sulfur compounds, is an essential cofactor in various cellular enzymes, oxygen carriers, and electron-transfer systems. Iron homeostasis plays a key role in host-pathogen interactions. For instance, host tissues can restrict the availability of free iron in order to prevent infection. Accordingly, fungal pathogens have adapted strategies for iron uptake, including the production of metalloreductases, ferroxidases, and siderophores (Silva et al. 2011), in order to survive in iron-deficient niches.

Several pathways are crucial for fungal pathogens to survive in various host microenvironments during infection. *In vivo*, *ex vivo*, and *in vitro* infection models have identified the transcriptional profiles of fungal pathogens during infection

and interaction with host cells. These studies have helped elucidate the pathogenesis of superficial, deep, and bloodstream fungal infections. An *in vitro* study used microarray to assess the transcriptional profile of *C. albicans* during interaction with human blood. There was an upregulation of genes involved in stress response, such as *SSA4* (a member of the *HSP70* gene family), and anti-oxidative response, such as those encoding Cu/Zn superoxide dismutase (*SOD1*), catalase (*CAT1*), and thioredoxin reductase (*TRR1*). There was a simultaneous upregulation of genes encoding the glycolytic enzymes phosphofructokinase (*PFK2*), phosphoglycerate kinase (*PGK1*), and enolase (*ENO1*) as well as those encoding the glyoxylate cycle enzymes isocitrate lyase (*ICL1*), malate synthase (*MLS1*), and acetyl-coenzyme-A-synthetase (*ACSI*). This suggested that both pathways were important for fungal dissemination. Genes involved in fermentation, such as those encoding alcohol dehydrogenases (*ADH1* and *ADH2*), were also upregulated. Importantly, *C. albicans* isolated from infected mice exhibited similar transcription profiles, thus validating some of the *in vitro* results. Moreover, these data suggested that *C. albicans* uses alternative carbon sources during blood infection and dissemination (Fradin et al. 2003).

A subsequent study investigated the utilization of the glyoxylate cycle and glycolysis by *C. albicans* interacting with different blood fractions, including erythrocytes, PMNs (mainly neutrophils), PMN-depleted blood (consisting of lymphocytes and monocytes), and plasma. *C. albicans* cells were physiologically active and displayed rapid hyphal growth while interacting with plasma, erythrocytes, and PMN-depleted blood. On the other hand, growth of *C. albicans* cells was arrested when interacting with PMNs, and only 40% of the cells interacting with whole blood produced hyphae. *C. albicans* upregulated glyoxylate cycle genes when interacting with PMNs, but not when interacted with plasma. During interaction with plasma and PMN-depleted blood, *C. albicans* upregulated genes related to glycolysis. Global cluster analysis was used to compare the transcriptional profile of *C. albicans* interacting with whole blood and blood fractions. During interaction with whole blood, the upregulation of genes related to glycolysis and the glyoxylate cycle resulted from mixed populations of fungal cells that were internalized by phagocytes, which triggers a nutrient limitation response, and not internalized (Fradin et al. 2005). Indeed, starvation inside the phagosome activated the glyoxylate cycle, which allowed nutrient uptake and survival. Accordingly, *C. albicans* deficient in the gene encoding isocitrate lyase was less virulent than the wild type strain in murine infection (Lorenz and Fink 2001).

While interacting with neutrophils, *C. albicans* also activated nitrogen- and carbohydrate-starvation responses, as indicated by the upregulation of genes encoding ammonium permeases (*MEP2* and *MEP3*), vacuolar proteases (*PRB1*, *PRB2*, and *APR1*), carboxypeptidases (*PRC1* and *PRC2*), glyoxylate cycle enzymes (*MLS1*, *ICL1*, and *ACSI*), aminoacid transporters, and proteins involved in aminoacid metabolism. Similarly, *C. albicans* activated the oxidative stress response, as indicated by the upregulation of genes encoding peroxidases and reductases, including superoxide dismutases (*SOD1* and *SOD5*) and catalase (*CAT1*) (Fradin et al. 2005). The upregulation of these genes may have contributed to fungal survival. Furthermore,

C. albicans internalized by murine macrophages *in vitro* displayed growth arrest and downregulation of genes associated with translation machinery and glycolysis. On the other hand, there was an upregulation of genes encoding enzymes involved in the gluconeogenesis pathway (phosphoenolpyruvate carboxykinase and fructose-1,6-bisphosphatase), glyoxylate cycle (isocitrate lyase and malate synthase), tricarboxylic acid cycle (aconitase, citrate synthase, and malate dehydrogenase), and β -oxidation of fatty acids, as well as several transporters. This suggested a metabolic adaptation toward the use of alternative carbon sources. Other upregulated genes included those encoding proteins important for detoxification of reactive species (flavo-hemoglobin, cytochrome *c* peroxidase, peroxidases, and reductases), stress response (heat shock protein HSP78), metal homeostasis, and DNA repair (Lorenz et al. 2004). Moreover, enzymes such as flavo-hemoglobins and superoxide dismutases, important to counteract ROS and RNS, were also implicated in *C. albicans* virulence (Missall et al. 2004).

Transcriptional profiling of *C. albicans* was performed using biopsies of infected oral mucosa from 11 HIV-positive patients. The results were compared to those obtained after *in vitro* interaction with reconstituted human epithelia (RHE). Genes related to gluconeogenesis (*PCK1*), the glyoxylate cycle (*MLS1* and *ICL1*), β -oxidation of fatty acids, and amino acid and phosphate transport (*PHO84*) were upregulated. In the *in vitro* model, genes involved in nitric-oxide detoxification, such as those encoding flavo-hemoglobin (*YHB5*) and a sulfite transporter (*SSU1*), alkaline pH-responsive genes (*PHR1* and *PRA1*), and alkaline pH-induced genes of the Rim101 pathway were also upregulated. *SSU1*, *YHB1*, *YHB5*, *PHR1*, and *ICL1* were upregulated during oral mucosa infection. These changes reflected fungal responses to nitrosative stress, innate defense of epithelial cells against microbes, adaptation to the neutral-alkaline pH of the oral mucosa, and the use of alternative carbon sources at the site of infection. Despite the heterogeneity of the biopsy samples, there was a group of genes with similar expression profiles from both the patient samples and the *in vitro* RHE model. Genes related to iron acquisition (*CFL2* and *FRE4*) were upregulated in oral candidiasis but not in the *in vitro* model. In another study, *Δicl1* was impaired to damage RHE, suggesting the importance of the glyoxylate cycle in oral candidiasis (Wachtler et al. 2011). Moreover, epithelial escape and dissemination (*EED1*), a unique species-specific *C. albicans* gene, was involved in hyphal elongation during infection (Zakikhany et al. 2007). A time-course microarray analysis of wild type and *Δeed1* strains interacting with RHE showed that transcriptional differences between the strains increased over time. The mutant did not upregulate any genes across the entire time course, but did downregulate seven genes throughout the course of infection, including the hyphae-associated genes *ECE1* and *HYR1*. Other downregulated genes in the *Δeed1* strain included those encoding proteins involved in polarized growth, such as CDC42, RD11, MYO2, CDC11, CYB2, MOB1, and MLC1. A comparison of the transcriptional profile of several mutant strains suggested that *EED1* was the primary component of a regulatory network controlling hyphal extension. *EED1* is positively regulated at the transcriptional level by EFG1, a member of the APSES family of transcription factors, and is repressed by NRG1, a sodium regulator. UME6, another

transcriptional regulator involved in hyphal extension, is transcriptionally regulated by EED1. Both regulators participate in a pathway that controls the extension of *C. albicans* germ tubes into hyphae and the hyphae-to-yeast transition, which allows fungal dissemination within epithelial tissues (Martin et al. 2011).

In order to investigate expression changes in *C. albicans* during liver infection, transcriptional profiling was performed *in vivo* on mice infected by intraperitoneal injection as well as pig livers inoculated *ex vivo*. The upregulated genes encoded enzymes involved in glycolysis, such as phosphofructokinase (*PFK2*) and pyruvate dehydrogenase subunits (*PDA1* and *PDX1*), as well as those involved in acetyl-CoA biosynthesis and the tricarboxylic acid cycle (*KGD1* and *KGD2*). This gene expression modulation reflected the availability of carbohydrates and the utilization of glycolysis and respiration for energy production. However, the upregulation of *PCK1*, which encodes phosphoenolpyruvate carboxykinase, a key enzyme in gluconeogenesis, suggested that alternative carbon sources were also used. Other upregulated genes included *SAP2*, *SAP4*, *SAP5*, and *SAP6*, which encode the hyphae-associated aspartic proteases. Indeed, Sap2 is the major protease that enables the utilization of proteins as nitrogen sources. The upregulation of alkaline pH responsive gene (*PHR1*) suggested adaptation to an alkaline environment. Similarly, upregulation of genes encoding stress-response proteins, including heat shock proteins and molecular chaperones (*HSP78*, *HSP90*, *DDR48*, *HSP104*, *HSP12*, and *SSA4*), suggested that the heat shock response was triggered during the course of infection. However, genes related to oxidative, osmotic, and nitrosative stresses were not upregulated. On the other hand, genes related to iron, copper, zinc, and phosphate transport (*FTR1*, *CTR1*, *ZRT1*, *PHO84*, *PHO89*) were upregulated during liver infection, suggesting limited iron and phosphate in this environment. A comparison of the transcriptional profile of an invasive *C. albicans* strain with that of a non-invasive strain identified genes associated with liver invasion. One of these genes was *DFG16*, which encodes a membrane sensor in the RIM101 pathway that is crucial for pH-dependent hyphal formation, pH sensing, invasion at physiological pH, and systemic infection (Thewes et al. 2007; Martinez-Rossi et al. 2012; Rossi et al. 2013). In another study of systemic infection, rabbits were infected through the marginal ear vein, and infected kidneys were subsequently collected. *C. albicans* exhibited an upregulation of genes related to alternative pathways of carbon assimilation, such as β -oxidation of fatty acids, the glyoxylate cycle (*MLS1* and *ACSI*), and the tricarboxylic acid cycle (*CIT1*, *ACO1*, and *SDH12*), suggesting limited carbohydrate supply in the kidneys (Walker et al. 2009). Although genes involved in β -oxidation of fatty acids are upregulated in several models of infection, fatty-acid degradation is not essential for virulence of *C. albicans*. Nevertheless, disruption of genes involved in the glyoxylate cycle or gluconeogenesis significantly attenuated its virulence in mice (Ramirez and Lorenz 2007; Barelle et al. 2006).

C. albicans colonizes medical devices, such as intravascular catheters, by forming biofilms. Biofilms are comprised of heterogeneous microbial communities and form on biotic or abiotic surfaces embedded in an extracellular polymeric matrix. Such biofilms are associated with persistent infections and resistance to antifungal drugs and mechanical treatments. *C. albicans* forms a biofilm in four steps. First,

yeast cells attach to and colonize a surface; second, yeast cells form a basal layer that anchors the biofilm; third, hyphae grow and produce pseudohyphae and extracellular matrix; finally, the yeast cells disperse. In order to characterize biofilm formation in *C. albicans*, the transcriptional regulatory network was analyzed in mutants that are unable to form biofilms. A combination of whole-genome chromatin immunoprecipitation microarray (ChIP-chip) and genome-wide transcriptional profiling identified six master regulators that control biofilm formation in *C. albicans*: BCR1, TEC1, EFG1, NDT80, ROB1, and BRG1. Each regulator controlled the other five, and most of the target genes were controlled by more than one master regulator. Moreover, the biofilm network targeted approximately 15% of the entire genome (Nobile et al. 2012).

Microarray analyses were used to profile *C. neoformans* transcription in response to murine macrophages. *C. neoformans* exhibited a downregulation of genes encoding translational machinery and an upregulation of genes associated with lipid degradation and fatty-acid catabolism (lipases and acetyl coenzyme A acetyltransferase), β -oxidation, transport of glucose and other carbohydrates, response to nitrogen starvation, the glyoxylate cycle (*ICL1*), and autophagy (*ATG3* and *ATG9*). This suggested nutritional starvation and metabolic adaptation toward the use of alternative carbon sources and nitrogen uptake. Moreover, the upregulation of several genes encoding oxidoreductases, peroxidases, and flavohemoglobin denitrosylase (FHB1), which are important for nitrosative response and virulence, indicated the presence of oxidative and nitrosative stress (de Jesús-Berrios et al. 2003). Also, there was an upregulation of genes related to endocytosis, exocytosis, and synthesis of extracellular polysaccharides and cell wall components. Genes located in the mating-type (MAT) locus and several genes associated with virulence were also upregulated. These included those encoding inositol-phosphorylceramide synthase (*IPC1*), laccases (*LAC1* and *LAC2*), genes involved in capsule formation (*CAP10*, *CAS31*, *CAS32*, *CAS1*, and *CAS2*), and *PKA*, a gene in the Gpa1-cAMP pathway, that is essential for virulence. In particular, the Gpa1-cAMP pathway regulates capsule formation and melanin production. Moreover, calcineurin gene (*CNA1*), which is critical for virulence, was upregulated (Fan et al. 2005).

Transcriptional analyses of *C. neoformans* isolated from cryptococcal pulmonary infection in mice revealed the upregulation of genes encoding malate synthase, phosphoenolpyruvate carboxykinase, aconitase and succinate dehydrogenase as well as those involved in β -oxidation of fatty acids. These findings suggested glucose depletion and the use of alternative carbon sources in the lungs. Genes encoding glyoxylate cycle enzymes were strongly upregulated as well as genes involved in glycolysis (e.g., fructose 1,6-biphosphate, aldolase, hexokinase, and phosphofructokinase). In addition, there was an upregulation of genes encoding transporters for monosaccharides, iron, copper, acetate, trehalose, and phosphate, enzymes involved in the production of acetyl-CoA (e.g., acetylCoA synthetase [*ACS1*]), pyruvate decarboxylase, and aldehyde dehydrogenase. The upregulation of several stress-response genes, including flavohemoglobin denitrosylase, superoxide dismutase, *HSP12*, *HSP90*, and other virulence factors, might have protected against the stressful conditions in the lungs. This profile of upregulated genes sug-

gested the importance of generating and utilizing acetyl-CoA by *C. neoformans* during infection (Hu et al. 2008). Indeed, deletion of the *acs1* gene resulted in attenuated virulence and impaired growth on media containing acetate as a carbon source. Moreover, *ACS1* is regulated by serine/threonine protein kinase 1 (SNF1), which mediates glucose sensing, utilization of alternative carbon sources, and stress response. Deletion of the *SNF1* gene also reduced growth on acetate medium, decreased melanin production and caused loss of virulence in murine model (Hu et al. 2008). Although *C. neoformans* upregulated glyoxylate cycle genes during infection, *ICL1* and *MLS1* were not essential for establishing infection (Rude et al. 2002; Idnurm et al. 2007). On the other hand, deficits in β -oxidation pathways compromised the virulence of *C. neoformans* (Kretschmer et al. 2012).

C. neoformans var. *grubii* molecular type VNI and VNII were isolated from the cerebrospinal fluid (CSF) of two AIDS patients with cryptococcal meningitis (Chen et al. 2014). RNA was isolated from *C. neoformans* and used for transcriptome analyses by RNA-Seq. The *in vivo* transcriptomes were compared with the transcriptomes of each strain after incubation in pooled human CSF *ex vivo* or growth in YPD broth *in vitro*. As compared with growth in YPD, growth *in vivo* and *ex vivo* in CSF resulted in the upregulation of genes associated with pathogenicity, such as *CFO1*, *ENA1*, and *RIM101*. *CFO1* encodes a ferroxidase required for the utilization of transferrin, an important source of iron during brain infection and subsequent dissemination in infected mice (Jung et al. 2009). *ENA1* is a sodium transporter that is important for CSF infection, intracellular survival in macrophages, and survival during meningitis in rabbits (Idnurm et al. 2009). *RIM101* is a conserved pH-responsive transcription factor that regulates iron and metal homeostasis, capsule production, and cell wall formation in *C. neoformans* (O'Meara et al. 2010). Other genes, such as *SIT1* and *SRX1*, were upregulated only *in vivo*. *SIT1* is a siderophore transporter that is important for growth under iron-limiting conditions, suggesting a role for iron in CSF infection. *SRX1* is a sulfiredoxin that confers resistance to oxidative stress, which might improve survival in the presence of phagocytic cells in the CSF. Indeed, *Rim101* controls the expression of several genes, including *SIT1* and *ENA1* (O'Meara et al. 2010; O'Meara et al. 2013). *ICL1* was another gene upregulated in *C. neoformans* infecting human CSF. The *ICL1* gene was also upregulated in a rabbit model of cryptococcal meningitis, although isocitrate lyase was not essential for infection (Rude et al. 2002). However, there was an upregulation of trehalose-6-phosphate synthase (TPS1) encoding gene (Steen et al. 2003), which was essential for infection (Petzold et al. 2006). Thus, in human cryptococcal meningitis, a set of genes was similarly modulated between *C. neoformans* strains isolated from infected patients. Among the differentially modulated genes, novel genes with unknown functions were also identified (Chen et al. 2014).

Recently, another transcriptional profiling study using nanoString demonstrated high concordance between the downstream targets of PKA and *RIM101* in *C. neoformans*. A pairwise transcriptional analysis of the Δ *pka* and Δ *rim101* mutant strains revealed a strong correlation between the majority of *RIM101*-dependent and *PKA1*-dependent genes. This suggested that *PKA1* and *RIM101* interact or that they are in the same signaling pathway. In a murine model of lung infection, tran-

scriptional and ChIPseq analyses of wild type and $\Delta rim101$ strains revealed genes regulated by RIM101, including *cdal* and *kre6*. CDA1 regulates levels of chitosan in the cell, and KRE6 participates in β -glucan synthesis, consistent with a role for RIM101 in regulating cell wall remodeling (O'Meara et al. 2013).

In a murine model of pulmonary aspergillosis, *A. fumigatus* exhibited a down-regulation of genes related to ribosomal biogenesis and protein biosynthesis and an upregulation of genes related to siderophore biosynthesis and transport, including ferric-chelate reductases, aminoacid permeases, GABA and proline permeases, maltose permeases and transporters, and extracellular proteases. Elastinolytic metalloprotease, an aorsin-like serine protease, and dipeptidylpeptidases are antigenic virulence factors that are important for nitrogen uptake. Several genes encoding antioxidant enzymes were also upregulated, including a Mn-superoxide dismutase and the bifunctional catalase-peroxidase CAT2. The initiation of infection was likely associated with aminoacid catabolism, as indicated by the induction of the enzyme methylcitrate synthase, which detoxifies propionyl-CoA intermediates (McDonagh et al. 2008). During invasive aspergillosis, propionyl-CoA is a toxic product generated from the degradation of valine, methionine, and isoleucine derived from host proteins. The gene encoding methylcitrate synthase was upregulated, suggesting that aminoacids are used as nutrients during invasive aspergillosis. Moreover, an *A. fumigatus* strain deficient in methylcitrate synthase displayed attenuated virulence (Ibrahim-Granet et al. 2008). During invasive aspergillosis, many of the preferentially expressed genes were located in the sub-telomeric regions of chromosomes. The coordinated expression of clustered genes included those responsible for the biosynthesis of siderophores and the secondary metabolites pseurotin and gliotoxin, which are toxins (McDonagh et al. 2008).

While interacting with human neutrophils, *A. fumigatus* conidia upregulated genes encoding proteins involved in peroxisome biogenesis, β -oxidation of fatty acids (acyl-CoA dehydrogenase and enoyl-CoA hydratase), acetate metabolism (acetyl-coenzyme A synthetase), the tricarboxylic acid cycle (aconitate, succinate dehydrogenase, and malate dehydrogenase), and the glyoxylate cycle (isocitrate lyase), suggesting a state of carbohydrate starvation (Sugui et al. 2008). There was a strong upregulation of the gene encoding formate dehydrogenase, which detoxifies formate, an indirect product of the glyoxylate cycle (Prigneau et al. 2003). Neutrophils normally produce cytotoxic ROS; however, neutrophils from patients with chronic granulomatous disease (CGD) are unable to damage *A. fumigatus* hyphae. Indeed, hyphae exposed to normal neutrophils expressed higher levels of the genes encoding glutathione peroxidase and thioredoxin reductase than those exposed to CGD neutrophils (Sugui et al. 2008). Despite the involvement of ROS released by phagocytes in killing *A. fumigatus*, a triple *SOD1/SOD2/SOD3* mutant and the parental strain were similarly virulent in experimental murine aspergillosis in immunocompromised animals (Lambou et al. 2010).

Upon contact with bronchial epithelial cells, *A. fumigatus* upregulated approximately 150 genes, most of which were related to vacuolar acidification, siderophore biosynthesis, metallopeptidase activity, and formate dehydrogenase activity (Oosthuizen et al. 2011; Kane 2007). Formate dehydrogenase (*fdh*) was also significantly

upregulated upon incubation with neutrophils (Sugui et al. 2008) and biofilm formation (Bruns et al. 2010). Moreover, incubation with *A. fumigatus* induced cell death in monocyte-derived immature dendritic cells (iDC) (Morton et al. 2011). This effect was coincident with growth of the fungal germ tube and fungal upregulation of genes encoding enzymes related to oxidative stress response, ROS detoxification (e.g., superoxide dismutases), and pyomelanin biosynthesis. Additionally, *A. fumigatus* upregulated *ASPF1*, which encodes a ribotoxin that inhibits protein synthesis and induces apoptosis in iDCs *in vitro* (Ok et al. 2009).

Transcriptional profiling was performed on the dermatophyte *Arthroderma benhamiae* during an *in vivo* skin infection in guinea pigs. During acute infection, *Arthroderma benhamiae* upregulated genes encoding key enzymes of the glyoxylate cycle (MLS and ICL), formate dehydrogenase, monosaccharide transporter, oxidoreductase, opsin-related protein, and several proteases (Staib et al. 2010). The most highly upregulated gene was *SUB6* that encodes subtilisin 6, a protease previously characterized as the major allergen in another dermatophyte, *T. rubrum*. Sub6 has been shown to bind human IgE antibodies (Woodfolk and Platts-Mills 1998). The second most highly upregulated gene was that encoding an opsin-related protein with an unknown function. The same gene was previously shown to be upregulated during the parasitic phase of *Coccidioides immitis*, but its function is still unknown (Viriyakosol et al. 2013). Genes encoding proteases, such as subtilisins SUB1, SUB2, SUB6, and SUB7, the neutral protease NpII-1, and serine carboxypeptidase ScpC were also upregulated during infection (Staib et al. 2010). Proteases are the most commonly studied virulence factors of dermatophytes, and some possess keratinolytic activity, which allow them to infect the skin and nails (Monod 2008). Genes encoding SUB3, SUB5, and metalloprotease 4 (MEP4) were also upregulated in *T. rubrum* grown in keratin as the sole carbon source (Maranhão et al. 2007). Moreover, a *PACC/RIM101*-mutant strain of *T. rubrum* displayed decreased keratinolytic activity and impaired growth on the human nail *in vitro*, suggesting a role for RIM101 in the pathogenicity of *T. rubrum* (Ferreira-Nozawa et al. 2006; Silveira et al. 2010; Martinez-Rossi et al. 2012).

While interacting with human keratinocytes, *Arthroderma benhamiae* upregulated the *HYP A* gene, which encodes a hydrophobin (Burmester et al. 2011) that influences the organism's recognition by the immune system (Heddergott et al. 2012). Deletion of *HYP A* gene increased the susceptibility of *Arthroderma benhamiae* to human neutrophils and DCs. Compared to wild type, the $\Delta hypA$ mutant strain activated cellular immune defenses and increased the release of IL-6, IL-8, IL-10, and TNF- α to a higher degree. Moreover, conidia of the mutant strain were more easily killed by neutrophils (Heddergott et al. 2012). Indeed, surface expression of hydrophobin was shown to prevent immune recognition of *A. fumigatus* (Aimanianda et al. 2009).

Transcriptome data from various human fungal pathogens have identified global responses and survival strategies during interaction with host cells and infection of host niches. Accordingly, some pathways have been implicated in mycotic diseases, and fungi are able to proliferate and survive within the host by employing sophisticated mechanisms to quickly modulate gene expression and adapt to changes in

the environment. Genes that are upregulated during the infective process or during interaction with host cells are potentially important for virulence (Table 13.2), which has been evaluated by the functional characterization of mutant strains. Thus, genome-wide transcriptional analyses combined with genetic approaches have provided important insight into fungal responses, adaptive processes, virulence, and pathogenesis.

13.4 Transcriptome of Drug Response and Resistance

Microorganisms respond to sub-lethal doses of chemical and physical agents by synthesizing a variety of specific proteins and low molecular weight compounds that act to promote defenses or tolerance (Fachin et al. 2001). Fungi use numerous signal transduction pathways to sense environmental stress and respond appropriately by differentially expressing cell-stress genes (Martinez-Rossi et al. 2008). Thus, analyses of transcriptional changes in response to cytotoxic drugs have elucidated the mechanisms by which fungi adapt to physiological stress as well as the mechanisms of drug action.

Although there are several commercially available antifungal drugs, the number of cellular targets is limited. Some antifungal drugs target ergosterol, a sterol analogous to cholesterol that is the main component of the fungal cell membrane and has diverse functions, including maintaining membrane stability, integrity, and permeability. Other antifungal drugs target proteins involved in the biosynthesis of ergosterol. The ergosterol biosynthetic pathway has been well described in *Saccharomyces cerevisiae* and involves approximately 20 genes, including those that convert mevalonate to squalene (Fig. 13.1), which are the primary targets of antifungal drugs. The polyenes are a class of antifungal drugs that include amphotericin B (AMB) and nystatin. They bind to ergosterol and form pores in the membrane, which causes the leakage of intracellular contents and fungal cell death. AMB also induces oxidative damage to cellular membranes through the generation of ROS.

Azoles are the most commonly used class of antifungal drugs in clinical treatment and include ketoconazole, itraconazole, fluconazole, and voriconazole. They inhibit the activity of the enzyme cytochrome P450 lanosterol 14- α demethylase (ERG11), which is responsible for the oxidative removal of the 14 α -methyl group of lanosterol, an essential step in ergosterol biosynthesis. Azoles are first-line agents for the treatment of candidiasis, but their frequent use can result in resistance due to their fungistatic mechanism of action. Terbinafine (TRB) is another antifungal drug that belongs to the allylamine class and is most effective against dermatophytes. It inhibits ergosterol biosynthesis by inhibiting the enzyme squalene epoxidase (ERG1), which is responsible for converting squalene to lanosterol. Inhibition of ERG1 decreases the production of ergosterol and increases the accumulation of squalene to toxic levels.

Other antifungal drugs target DNA/RNA metabolism. Flucytosine is a cytosine analogue that was first used as an antitumor agent. It showed poor efficacy in the

Table 13.2 Putative fungal proteins associated with host interaction and pathogenesis

Protein description	Gene expression modulation and functional analysis	References
Isocitrate lyase (glyoxylate cycle enzyme)	Upregulated in <i>C. albicans</i> , <i>C. neoformans</i> , <i>A. fumigatus</i> and <i>A. benhamiae</i> . Gene inactivation attenuates virulence in <i>C. albicans</i> but not in <i>C. neoformans</i> and <i>A. fumigatus</i>	Fradin et al. (2003, 2005); Lorenz et al. (2004); Zakikhany et al. (2007); Fan et al. (2005); Chen et al. (2014); Sugui et al. (2008); Staib et al. (2010); Rude et al. (2002); Schöbel et al. (2007); Lorenz and Fink (2001); Wachtler et al. (2011)
Malate synthase (glyoxylate cycle enzyme)	Upregulated in <i>C. albicans</i> , <i>C. neoformans</i> , <i>A. fumigatus</i> and <i>A. benhamiae</i> . Gene inactivation does not attenuate virulence in <i>C. neoformans</i>	Fradin et al. (2003, 2005); Lorenz et al. (2004); Zakikhany et al. (2007); Walker et al. (2009); Hu et al. (2008); McDonagh et al. (2008); Staib et al. (2010); Idnurm et al. 2007; Cairns et al. 2010)
Acetyl-coenzyme-A-synthetase (glyoxylate cycle enzyme)	Upregulated in <i>C. albicans</i> , <i>C. neoformans</i> and <i>A. fumigatus</i> . Gene inactivation attenuates virulence in <i>C. neoformans</i>	Fradin et al. (2003, 2005); Walker et al. (2009); Sugui et al. (2008); McDonagh et al. (2008); Cairns et al. (2010); Hu et al. (2008); Thewes et al. 2007; Lorenz et al. 2004)
Aconitase (tricarboxylic acid cycle enzyme)	Upregulated in <i>C. albicans</i> , <i>C. neoformans</i> and <i>A. fumigatus</i>	Lorenz et al. (2004); Walker et al. (2009); Hu et al. (2008); Sugui et al. (2008)
Malate dehydrogenase (tricarboxylic acid cycle enzyme)	Upregulated in <i>C. albicans</i> , <i>C. neoformans</i> and <i>A. fumigatus</i>	Lorenz et al. (2004); Hu et al. (2008); Cairns et al. (2010); Sugui et al. (2008)
Phosphofruktokinase (glycolysis enzyme)	Upregulated in <i>C. albicans</i> and <i>C. neoformans</i>	(Fradin et al. 2003; Thewes et al. 2007; Hu et al. 2008)
Enolase (glycolysis enzyme)	Upregulated in <i>C. albicans</i> and <i>C. neoformans</i>	Fradin et al. (2003); Thewes et al. (2007); Hu et al. (2008)
Phosphoenolpyruvate carboxykinase (gluconeogenesis enzyme)	Upregulated in <i>C. albicans</i> and <i>C. neoformans</i> . Gene inactivation attenuates virulence in <i>C. albicans</i>	Zakikhany et al. (2007); Lorenz et al. (2004); Thewes et al. (2007); Hu et al. (2008); Barelle et al. (2006)

Table 13.2 (continued)

Protein description	Gene expression modulation and functional analysis	References
Flavo-hemoglobin denitrosylases (RNS detoxification)	Upregulated in <i>C. albicans</i> and <i>C. neoformans</i> . Gene inactivation attenuates virulence in <i>C. albicans</i> and <i>C. neoformans</i>	Hu et al. (2008); Lorenz et al. (2004); Zakikhany et al. (2007); Fan et al. (2005); de Jesus-Berrios et al. (2003); Missall et al. (2004); Brown et al. (2009)
Superoxide dismutases (ROS detoxification)	Upregulated in <i>C. albicans</i> , <i>C. neoformans</i> and <i>A. fumigatus</i> . Gene inactivation attenuates virulence in <i>C. albicans</i> and <i>C. neoformans</i> but not in <i>A. fumigatus</i>	Hu et al. (2008); McDonagh et al. (2008); Morton et al. (2011); Fradin et al. (2003); Lorenz et al. (2004); Fradin et al. (2005); Lambou et al. (2010); Missall et al. (2004); Brown et al. (2009)
Hydrophobin (cell surface protein)	Upregulated in <i>A. fumigatus</i> and <i>A. benhamiae</i> . Gene inactivation in <i>A. fumigatus</i> and <i>A. benhamiae</i> increases the susceptibility to the host immune response	Cairns et al. (2010); Burmester et al. (2011); Hedergott et al. (2012); Aimaniananda et al. (2009)

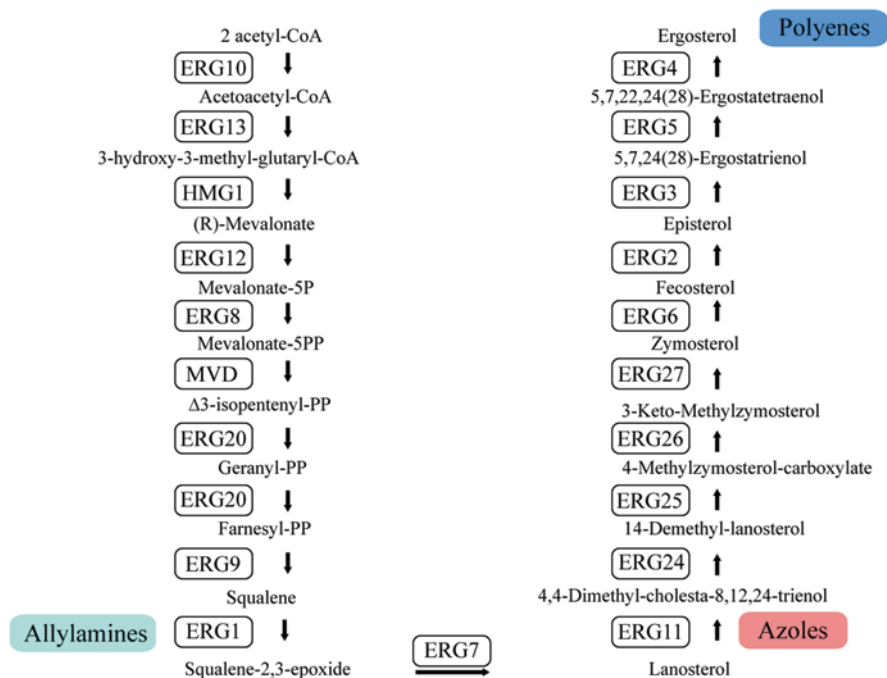


Fig. 13.1 Schematic representation of the ergosterol biosynthetic pathway

treatment of tumors but was shown to have antifungal properties. Flucytosine is transported to the cytoplasm of fungal cells through cytosine permease; in the cytoplasm, cytosine deaminase converts it to 5-fluorouracil, which blocks protein and DNA synthesis. When phosphorylated, 5-fluorouracil is incorporated into RNA, leading to miscoding and inhibition of protein synthesis. Furthermore, phosphorylated 5-fluorouracil can be converted into the deoxynucleoside form by uridine monophosphate pyrophosphorylase; thereafter, it inhibits the enzyme thymidylate synthetase and consequently disrupts DNA synthesis (Vermes et al. 2000).

More recently, the fungal cell wall has become a specific target of antifungal drugs, since it is absent from mammalian cells. Caspofungin was the first compound to target the fungal cell wall and was approved for clinical use in 2001. It is a member of the echinocandin class, which inhibits the enzyme (1,3)- β -D-glucan synthase (FKS1 and FKS2), thus preventing the synthesis of (1,3)- β -D-glucan and disrupting cell wall biosynthesis. In addition to caspofungin, two other echinocandins, micafungin and anidulafungin, are commercially available. These drugs are only available as intravenous infusions and are indicated to treat invasive aspergillosis and candidiasis. They have fungicidal activity against most *Candida* species and fungistatic activity against *Aspergillus* species. Although most fungal species encode orthologs of FKS1 and FKS2, echinocandins are not effective against Zygomycetes *C. neoformans*, or *Fusarium* spp. (Denning 2003).

Transcriptome analyses have been used to evaluate the responses of pathogenic fungi, such as *C. albicans*, *A. fumigatus*, and *T. rubrum*, to several antifungal drugs, including azoles, polyenes, terbinafine, and echinocandins (Liu et al. 2005; da Silva Ferreira et al. 2006; Cervellati et al. 2006; Yu et al. 2007b; Paião et al. 2007; Gautam et al. 2008; Diao et al. 2009; Zhang et al. 2009; Peres et al. 2010b). These studies revealed that the modulation of genes in the ergosterol biosynthetic pathway varies significantly among species and drugs (Table 13.3). For instance, in response to itraconazole, *C. albicans* upregulated the following genes related to ergosterol biosynthesis: *erg1*, *erg2*, *erg3*, *erg4*, *erg5*, *erg6*, *erg9*, *erg10*, *erg11*, and *erg25* (De Backer et al. 2001). In contrast, *T. rubrum* only upregulated *erg11*, *erg24*, and *erg25* (Diao et al. 2009). Similarly, in response to voriconazole *A. fumigatus* only upregulated *erg3*, *erg24*, and *erg25* (da Silva Ferreira et al. 2006). Although caspofungin and flucytosine do not primarily target the ergosterol biosynthetic pathway, they elicited the upregulation of some ergosterol biosynthetic genes in *C. albicans* (Liu et al. 2005) (Table 13.3).

In response to ketoconazole, *C. albicans* upregulated genes involved in the biosynthesis of ergosterol, lipids, and fatty acids. Ketoconazole also induced the expression of the major transporter genes *cdr1* and *cdr2* (Liu et al. 2005). Similarly, in response to ketoconazole, *T. rubrum* upregulated genes involved in the metabolism of lipids, fatty acids, and sterols, including *erg3*, *erg4*, *erg6*, *erg11*, *erg24*, *erg25*, and *erg26* as well as the multidrug-resistance gene encoding ABC1, which is a homolog of *C. albicans* CDR1 (Yu et al. 2007a).

In response to AMB, *C. albicans* downregulated genes related to ergosterol biosynthesis and upregulated genes related to cell stress, including those encoding nitric oxide oxidoreductase (YHB1), catalase 1 (CTA1), aldehyde oxidase 1 (AOX1), and superoxide dismutase 2 (SOD2) (Liu et al. 2005). *A. fumigatus* exposed to AMB upregulated *erg11* and downregulated *erg6*. In addition, it modulated genes involved in cell stress, transport, oxidative phosphorylation, nucleotide metabolism, cell cycle control, and protein metabolism. Moreover, in response to the oxidative damage caused by AMB exposure, *A. fumigatus* overexpressed several genes encoding antioxidant enzymes, such as Mn-SOD, catalase, the thiol-specific antioxidant protein LsfA, glutathione S-transferase (GST), and thioredoxin. *A. fumigatus* downregulated ergosterol biosynthetic genes in response to AMB, possibly in attempt to use alternate sterols or sterol intermediates in the cell membrane (Gautam et al. 2008).

C. albicans exposed to caspofungin induced the expression of genes encoding cell wall maintenance proteins, including a target of caspofungin (the β -1,3-glucan synthase subunit homolog to FKS3), a pH-regulated glucan-remodeling enzyme (PHR1), extracellular matrix proteins (ECM21 and ECM33), and a putative fatty acid elongation enzyme (FEN12). Interestingly, *fen12* was upregulated in response to caspofungin and downregulated in response to AMB. In response to flucytosine, *C. albicans* upregulated the *cdc21* gene, which encodes thymidylate synthetase. This enzyme is the target of flucytosine and is associated with DNA synthesis; therefore, its upregulation may prevent fungal death. Other genes that were upregulated

Table 13.3 Modulation of genes related to ergosterol biosynthesis in response to drug exposure

Gene	<i>C. albicans</i>						<i>T. rubrum</i>						<i>A. fumigatus</i>		
	ITRA	AMB	KTC	CPF	5-FC	ITRA	AMB	KTC	TRB	AMB	VRCZ				
<i>erg1</i>	13.2	-	-	-	-	-	-	-	-	-	-	-	-	-	0.34
<i>erg2</i>	3.9	1.35	2.06	1.26	0.70	-	-	-	-1.64	-	-	-	-	-	-
<i>erg3</i>	12.4	0.26	2.10	0.27	1.45	-	-	3.6	-2.1	-	-	-	-	-	2.34
<i>erg4</i>	11.6	0.73	1.57	0.61	1.00	-	-4.7	6.5	-	-	-	-	-	-	-1.38
<i>erg5</i>	12	1.05	1.40	3.13	1.39	-	-	-	-	-	-	-	-	-	-
<i>erg6</i>	50.3	0.68	1.92	0.80	0.98	2.27	-	37.5	-	-2.6	-	-	-	-	-
<i>erg7</i>	-	0.77	1.45	0.58	1.18	2.35	-	-	-	-	-	-	-	-	0.21
<i>erg9</i>	4.9	0.54	1.50	0.51	1.16	-	-	-	-	-	-	-	-	-	-
<i>erg10</i>	10.9	0.70	3.14	0.65	1.58	-	-	-	1.61	-	-	-	-	-	0.1
<i>erg11</i>	12.5	0.56	2.43	0.64	1.41	13.55	-	3.6	-	2.8	-	-	-	-	0.34
<i>erg13</i>	-	0.41	1.50	0.44	2.95	-	-	-	1.64	-	-	-	-	-	-
<i>erg20</i>	-	0.96	1.02	0.70	1.53	-	-	-	-	-	-	-	-	-	-
<i>erg24</i>	-	-	-	-	-	6.76	-	8.6	-2.01	-	-	-	-	-	2.44
<i>erg25</i>	4.2	0.43	2.52	0.53	2.73	7.92	-	11.9	-2.24	-	-	-	-	-	1.65
<i>erg26</i>	4.8	0.59	1.27	0.49	1.12	2.26	-	11.7	-	-	-	-	-	-	-
<i>erg251</i>	-	0.37	2.74	0.22	2.50	-	-	-	-	-	-	-	-	-	-

ITRA itraconazole, AMB amphotericin B, KTC ketoconazole, CPF caspofungin, 5-FC flucytosine, TRB terbinafine and VRCZ voriconazole

a nucleoside diphosphate kinase, and *FUR1* an uracil phosphoribosyltransferase, as well as genes associated with protein synthesis (Liu et al. 2005).

Terbinafine is commonly used to treat dermatophytosis. Exposure of *T. rubrum* to TRB decreased the expression of genes related to ergosterol biosynthesis, such as *erg2*, *erg4*, *erg24*, and *erg25*, and increased the expression of genes involved in lipid metabolism, such as *erg10*, *erg13*, and *ino1*. Although TRB primary target is squalene epoxidase (*ERG1*), *T. rubrum* did not differentially express *erg1* after exposure to TRB. It did, however, upregulate multidrug-resistance (*MDR*) genes, including *mdr1* and *mdr2* (Zhang et al. 2009). Indeed, *MDR2* is associated with drug susceptibility. Overexpression of *mdr2* likely causes the efflux of TRB, since deletion of *mdr2* increased *T. rubrum* susceptibility to TRB (Fachin et al. 2006).

The emergence of resistant strains is an important obstacle to effective antifungal therapy. Azoles are the first-line treatment for many fungal infections; however, their use may select for azole-resistant mutants. Several mechanisms contribute to drug resistance, including alteration of the drug target, increased drug efflux, and increased cellular stress responses. Both mutations in and overexpression of the ergosterol biosynthesis gene *erg11/cyp51* confer resistance to azoles in *C. albicans* and *A. fumigatus*. For instance, one mutation causes the synthesis of an alternative protein that is insensitive to azoles and diminishes drug efficacy. At least 12 different point mutations in *erg11* have been identified in azole-resistant clinical isolates of *C. albicans* (Shapiro et al. 2011).

Overexpression of efflux pumps are associated with antifungal resistance in *C. albicans*. *CDR1* and *CDR2* confer resistance to multiple azoles, while *MDR1* confers resistance to fluconazole (White et al. 2002). Similarly, azole-resistant clinical isolates of *C. glabrata* have been shown to overexpress genes encoding *CDR1* and *CDR2* as well as *SNQ2*, another ATP-binding cassette ABC transporter (Sanguinetti et al. 2005). In response to azoles and other structurally distinct drugs, *T. rubrum* overexpressed *TruMDR1* and *TruMDR2*, which encode ABC transporters. Thus, these genes may participate in drug efflux (Cervellati et al. 2006; Fachin et al. 2006).

In order to identify genes associated with fluconazole resistance, a laboratory strain of *C. albicans* susceptible to fluconazole was subjected to successive passages in media containing fluconazole to induce resistance, and the transcriptional profile was subsequently analyzed. Some genes were modulated in coordination with the upregulation of *CDR1* and *CDR2*. For instance, there was an upregulation of genes coding the glutathione peroxidase 1 (*GPX1*) and *RTA3*, a protein involved in 7-aminosterol resistance, and a downregulation of NADPH oxidoreductase (*EBP1*). Genes that were modulated in coordination with the overexpression of *MDR1* included the upregulation of genes encoding aldo-keto reductase family proteins (*IFD1*, *IFD4*, *IFD5*, and *IFD7*), methylglyoxal reductase (*GRP2*), pyrophosphate phosphatase (*DPP1*), and inositol-1-phosphate synthase (*INO1*) and the downregulation of multi-copper ferroxidase (*FET34*), phosphatidylethanolamine N-methyltransferase (*OPI3*), and Cu and Zn-containing superoxide dismutase (*IPF1222*) (Rogers and Barker 2002, 2003).

Genes encoding the ABC transporters CDR1 and CDR2 in azole-resistant *C. albicans* strains are regulated by the zinc cluster transcription factor TAC1. This transcription factor was initially identified in *C. albicans* in a search for genes containing the *cis*-acting drug-responsive element (DRE) Zn(2)-Cys(6) finger, which is present in the promoter region of the *cdr1* and *cdr2* genes. Further characterization showed that deletion of *tac1* gene prevented the upregulation of *cdr* genes. Moreover, introduction of a *tac1* allele recovered from an azole-resistant strain into an azole-susceptible strain induced overexpression of CDR1 and CDR2 (Coste et al. 2004). In addition, the TAC1 regulon contains 31 upregulated and 12 downregulated genes, including those encoding IFU5, HSP12, phospholipid flippase (RTA3), glutathione peroxidase (GPX1), histidine kinase (CHK1), sphingosine kinase (LCB4), NADH dehydrogenase (NDH2), and sorbose dehydrogenase (SOU1) as well as TAC1, CDR1, and CDR2. Among the downregulated genes in the TAC1 regulon are an iron transporter (FTR1), a putative glycosyl phosphatidyl inositol-anchored protein (IHD1), and an oligopeptide transporter (OPT6), all of them encoding integral membrane proteins, and the superoxide dismutase SOD5, which is a cell wall protein. Furthermore, ChIP-chip experiments demonstrated that TAC1 directly binds to the promoter region of eight of these genes, including CDR1, CDR2, GPX1, LCB4, RTA3, a putative lipase, and two genes with unknown functions (Liu et al. 2007).

A genome-wide expression analysis of resistant clinical isolates of *C. albicans* identified a transcription factor that was upregulated in coordination with MDR1. This gene encodes the multidrug resistance regulator MRR1, which is a zinc cluster transcription factor and the main regulator of MDR1 expression. Gain-of-function mutations in MRR1 are responsible for overexpression of MDR1 and are associated with fluconazole resistance in *C. albicans* (Morschhauser et al. 2007). In addition to regulating MDR1 expression, MRR1 regulated at least 14 other genes that may also contribute to fluconazole resistance. These genes encoded mainly oxidoreductases. Notably, MRR1 does not target CDR1 or CDR2. Overall, large-scale transcriptional analyses have identified several genes associated with drug response and resistance in pathogenic fungi (Table 13.4).

Transcriptional analyses have been performed in order to identify additional genes associated with azole resistance. Recently, RNA-seq analyses were performed on two isogenic *C. albicans* strains that differed only in fluconazole resistance. These studies identified novel genes associated with azole resistance, including the transcription factor CZF1, which is involved in the hyphal transition and the white/opaque switch. Inactivation of CZF1 increased the susceptibility to fluconazole as well as unrelated antifungal drugs, such as TRB and anisomycin. Furthermore, the CZF1 mutant strain displayed increased resistance to the cell-wall-disrupting agent Congo red. The mutant also overexpressed the gene encoding β 1,3-glucan synthase (GLS1), suggesting that CZF1 represses β -glucan synthesis and regulates cell wall integrity (Dharmgaye et al. 2012).

Table 13.4 Putative proteins associated with drug response and resistance in fungal pathogens

Protein description	Gene expression modulation and functional analysis	References
ATP-binding cassette (ABC) multidrug transporter (CDR1)	Up-regulated in <i>C. albicans</i> FLU resistant strains	Rogers and Barker (2002, 2003)
ATP-binding cassette (ABC) multidrug transporter (CDR2)	Up-regulated in <i>C. albicans</i> FLU resistant strains	Rogers and Barker (2002, 2003)
Transcriptional activator of CDR genes (TAC1)	Gain of function mutations lead to up-regulation of the CDRs genes and FLU resistance in <i>C. albicans</i>	Liu et al. (2007)
Lanosterol 14-alpha-demethylase Ergosterol biosynthesis (ERG11)	Mutations or overexpression lead to resistance to FLU in <i>C. albicans</i> and <i>A. fumigatus</i>	De Backer et al. (2001); Liu et al. (2005); Diao et al. (2009); Gibbons et al. (2012)
	Up-regulated in response to azoles in <i>C. albicans</i> and <i>T. rubrum</i>	
	Up-regulated in <i>A. fumigatus</i> biofilm cells	
Zn2-Cys6 transcription factor (UPC2)	Gain of function mutations lead to up-regulation of the ERG11 and FLU resistance in <i>C. albicans</i>	Silver et al. (2004)
MDR/MFS multidrug efflux pump (MDR1)	Up-regulated in <i>C. albicans</i> FLU resistant strains	Morschhauser et al. (2007); Zaugg et al. (2009)
	Up-regulated in response to TRB in <i>T. rubrum</i>	
Multidrug resistance regulator (MRR1)	Gain of function mutations lead to up-regulation of the MDR1 and FLU resistance in <i>C. albicans</i>	Morschhauser et al. (2007)
MDR/MFS multidrug efflux pump (MDR2)	Up-regulated in response to terbinafine in <i>T. rubrum</i>	Zaugg et al. (2009); Fachin et al. (2006); Gibbons et al. (2012)
	Up-regulated in <i>A. fumigatus</i> biofilm cells	
MDR/MFS multidrug efflux pump (MDR4)	Up-regulated in <i>A. fumigatus</i> biofilm cells	Gibbons et al. (2012)
Transcription factor (CZF1)	Up-regulated in <i>C. albicans</i> FLU resistant strains	Dhamgaye et al. (2012)

Table 13.4 (continued)

Protein description	Gene expression modulation and functional analysis	References
Thymidylate synthetase associated with DNA synthesis (CDC21)	Up-regulated in response to flucytocine	Liu et al. (2005)
(1,3)- β -D-glucan synthase (FKS3)	Up-regulated in <i>C. albicans</i> in response to caspofungin	Liu et al. (2005)
Heat shock protein 90 (HSP90)	Inhibition can improve azole and echinocandins effectiveness against <i>C. albicans</i> and <i>A. fumigatus</i>	Cowen (2009)
Catalase (CTA1)	Up-regulated in response to AMB in <i>C. albicans</i> and in <i>A. fumigatus</i>	Liu et al. (2005); Gautam et al. (2008)
Superoxide dismutase 2 (SOD2)	Up-regulated in response to AMB in <i>C. albicans</i>	Liu et al. (2005)

In pathogenic fungi, mitochondrial dysfunction has been associated with altered susceptibility to antifungal drugs. In *C. albicans*, inhibition or mutation of the mitochondrial complex I (CI) increased susceptibility to fluconazole even in resistant clinical isolates. Transcriptional analysis was performed on the Δ *goal* and Δ *ndh51* mutant strains, which are associated with CI-induced susceptibility to fluconazole. *GOA1* is required for function of the electron transport chain, and the Δ *goal* mutant accumulates ROS, undergoes apoptosis, and is avirulent. *Ndh51* encodes a 51-kDa subunit of the NADH dehydrogenase of the electron transport chain, and the Δ *ndh51* mutant exhibits defects in morphogenesis. RNA-seq analyses of these strains demonstrated downregulation of transporters, including the CDR1/CDR2 efflux pumps but not MDR1. Genes related to ergosterol biosynthesis were downregulated in the Δ *ndh51* mutant. In contrast, genes associated with peroxisomes, gluconeogenesis, β -oxidation, and mitochondria were downregulated in the Δ *goal* mutant (Sun et al. 2013). NDH51 is conserved among eukaryotes, including mammals; nevertheless, *GOA1* is only conserved in some *Candida* species. Therefore, fungi-specific mitochondrial genes may be targets for the development of novel antifungal drugs. Indeed, acriflavine, an acridine derivative that has antibacterial, antifungal, antiviral, and antiparasitic properties, induces the overexpression of genes involved in the mitochondrial electron transport chain of *T. rubrum* (Segato et al. 2008).

Interestingly, chemical inhibition of fungal HSP90 improved the activity of azoles and echinocandins against *C. albicans* and echinocandins against *A. fumigatus* (Cowen 2009). Inhibition of HSP90 prevents the stress-response cascade mediated by calcineurin, which is normally activated in response to antifungal drugs. Blunting of the stress-response cascade enhances the fungicidal effects of drugs, leading to cell death (Singh et al. 2009). It will be a challenge to develop an inhibitor selective for fungal HSP90 and inactive against human HSP90. Nevertheless, HSP90 may be a promising target for treatment of resistant fungal diseases and may combat the emergence of drug resistance (Cowen 2009).

In addition to the emergence of drug-resistant strains, another major clinical problem is the formation of microbial biofilms. Biofilms possess specific traits as compared to planktonic cells, such as intrinsic resistance to drugs. In immunocompromised individuals, both *C. albicans* and *A. fumigatus* can form biofilms on implanted medical devices, such as catheters, and cause persistent infections. In particular, biofilms have decreased susceptibility to antifungal drugs, such as polyenes and azoles. In order to understand their mechanisms of resistance, mature biofilms cells were exposed to fluconazole, AMB, and caspofungin. Fluconazole exposure did not significantly alter gene expression, and AMB exposure resulted in only minor alterations in gene expression. On the other hand, biofilms exposed to caspofungin underwent more pronounced alteration in gene expression, including the upregulation of several genes associated with biofilm formation, such as *ALS3*, a cell-wall adhesin, the transcription factor *TEC1*, and genes associated with cell-wall remodeling (Vediyappan et al. 2010). Furthermore, AMB and fluconazole bind to the extracellular matrix of the biofilm, which is comprised of β -glucans; such binding inhibits effective drug action (Vediyappan et al. 2010; Nett et al. 2007). An RNA-seq analysis

compared the transcriptional profile of an *A. fumigatus* biofilm to that of planktonic cells. Thousands of genes were differentially expressed between the biofilm and planktonic cells. Specifically, the biofilm exhibited an upregulation of secondary metabolism genes, cell-wall-related genes, sterol biosynthetic genes (e.g., *erg11*), transporters associated with antifungal resistance (MDR1, MDR2, and MDR4), and hydrophobins, which are associated with structural organization of biofilms (Gibbons et al. 2012). The complex gene network involved in biofilm formation (Nobile et al. 2008) is consistent with the fact that *C. albicans* can form biofilms in different niches, such as the bloodstream and oral cavity. This highlights the challenge inherent to treating these infections as well as the importance of searching for new antifungal targets.

In conclusion, analyses of the transcriptional changes that occur in response to cytotoxic drugs have identified genes with known mechanisms of action. Moreover, these studies have suggested novel effects of antifungal drugs. In addition, responses shared across multiple classes of antifungal agents were identified in *C. albicans* (Liu et al. 2005) and *T. rubrum* (Fachin et al. 2006; Paião et al. 2007; Peres et al. 2010b). Therefore, there may be non-specific responses to a drug challenge that allow fungi to adapt to stress.

13.5 Concluding Remarks

The pathogenesis of fungal infections involves changes in gene expression and metabolic pathways, which enable fungal invasion and survival. At the same time, fungi elicit host responses aimed at eliminating the pathogen. Genome-wide transcriptional profiling has identified the molecular responses of both host and pathogen during interaction and has provided insight into the adaptive responses that occur during the establishment of infection. The combination of large-scale transcriptomic analysis and systems biology approaches has enabled the development of regulatory molecular models that can assess the dynamic behavior of host-pathogen interactions and elucidate the pathogenesis of human mycoses. These regulatory models have been validated through reverse-genetic approaches by evaluating the physiological behavior of knockout strains *in vitro*, *ex vivo*, and *in vivo*. Furthermore, transcriptomics is a valuable source of data on gene expression regulation, gene structure and function, also providing information regarding the mechanisms of fungal responses and resistance to drugs. These insights will further support the development of novel therapeutic approaches to prevent and control fungal infections.

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References

- Aimanianda V, Bayry J, Bozza S et al (2009) Surface hydrophobin prevents immune recognition of airborne fungal spores. *Nature* 460(7259):1117–1121
- Barelle CJ, Priest CL, MacCallum DM et al (2006) Niche-specific regulation of central metabolic pathways in a fungal pathogen. *Cell Microbiol* 8(6):961–971
- Barker KS, Park H, Phan QT et al (2008) Transcriptome profile of the vascular endothelial cell response to *Candida albicans*. *J Infect Dis* 198(2):193–202
- Bedoya SK, Lam B, Lau K et al (2013) Th17 cells in immunity and autoimmunity. *Clin Dev Immunol* 2013:986789
- Biondo C, Midiri A, Gambuzza M et al (2008) IFN-alpha/beta signaling is required for polarization of cytokine responses toward a protective type 1 pattern during experimental cryptococcosis. *J Immunol* 181(1):566–573
- Biondo C, Signorino G, Costa A et al (2011) Recognition of yeast nucleic acids triggers a host-protective type I interferon response. *Eur J Immunol* 41(7):1969–1979
- Brock M (2009) Fungal metabolism in host niches. *Curr Opin Microbiol* 12(4):371–376
- Brown AJ, Haynes K, Quinn J (2009) Nitrosative and oxidative stress responses in fungal pathogenicity. *Curr Opin Microbiol* 12(4):384–391
- Brown GD, Denning DW, Gow NA et al (2012) Hidden killers: human fungal infections. *Sci Transl Med* 4(165):165rv113
- Bruns S, Seidler M, Albrecht D et al (2010) Functional genomic profiling of *Aspergillus fumigatus* biofilm reveals enhanced production of the mycotoxin gliotoxin. *Proteomics* 10(17):3097–3107
- Burmester A, Shelest E, Glockner G et al (2011) Comparative and functional genomics provide insights into the pathogenicity of dermatophytic fungi. *Genome Biol* 12(1):R7
- Cairns T, Minuzzi F, Bignell E (2010) The host-infecting fungal transcriptome. *FEMS Microbiol Lett* 307(1):1–11
- Cambier L, Weatherspoon A, Defaweux V et al (2014) Assessment of the cutaneous immune response during *Arthroderma benhamiae* and *A. vanbreuseghemii* infection using an experimental mouse model. *Br J Dermatol* 170(3):625–633
- Cenci E, Mencacci A, Casagrande A et al (2001) Impaired antifungal effector activity but not inflammatory cell recruitment in interleukin-6-deficient mice with invasive pulmonary aspergillosis. *J Infect Dis* 184(5):610–617
- Cervellati EP, Fachin AL, Ferreira-Nozawa MS et al (2006) Molecular cloning and characterization of a novel ABC transporter gene in the human pathogen *Trichophyton rubrum*. *Med Mycol* 44(2):141–147
- Chen Y, Toffaletti DL, Tenor JL et al (2014) The *Cryptococcus neoformans* transcriptome at the site of human meningitis. *MBio* 5(1):e01087–e01013
- Conti HR, Shen F, Nayyar N et al (2009) Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. *J Exp Med* 206(2):299–311
- Cornet M, Gaillardin C (2014) pH signaling in human fungal pathogens: a new target for antifungal strategies. *Eukaryot Cell* 13(3):342–352
- Cortez KJ, Lyman CA, Kottlilil S et al (2006) Functional genomics of innate host defense molecules in normal human monocytes in response to *Aspergillus fumigatus*. *Infect Immun* 74(4):2353–2365
- Coste AT, Karababa M, Ischer F et al (2004) TAC1, transcriptional activator of CDR genes, is a new transcription factor involved in the regulation of *Candida albicans* ABC transporters CDR1 and CDR2. *Eukaryot Cell* 3(6):1639–1652
- Cowen LE (2009) Hsp90 orchestrates stress response signaling governing fungal drug resistance. *PLoS Pathog* 5(8):e1000471
- da Silva Ferreira ME, Malavazi I, Savoldi M et al (2006) Transcriptome analysis of *Aspergillus fumigatus* exposed to voriconazole. *Curr Genet* 50(1):32–44

- Davis DA (2009) How human pathogenic fungi sense and adapt to pH: the link to virulence. *Curr Opin Microbiol* 12(4):365–370
- De Backer MD, Ilyina T, Ma XJ et al (2001) Genomic profiling of the response of *Candida albicans* to itraconazole treatment using a DNA microarray. *Antimicrob Agents Chemother* 45(6):1660–1670
- de Jesus-Berrios M, Liu L, Nussbaum JC et al (2003) Enzymes that counteract nitrosative stress promote fungal virulence. *Curr Biol* 13(22):1963–1968
- Decken K, Kohler G, Palmer-Lehmann K et al (1998) Interleukin-12 is essential for a protective Th1 response in mice infected with *Cryptococcus neoformans*. *Infect Immun* 66(10):4994–5000
- Deepe GS Jr (2000) Immune response to early and late *Histoplasma capsulatum* infections. *Curr Opin Microbiol* 3(4):359–362
- Denning DW (2003) Echinocandin antifungal drugs. *Lancet* 362(9390):1142–1151
- Dhamgaye S, Bernard M, Lelandais G et al (2012) RNA sequencing revealed novel actors of the acquisition of drug resistance in *Candida albicans*. *BMC Genomics* 13:396
- Diao YJ, Zhao R, Deng XM et al (2009) Transcriptional profiles of *Trichophyton rubrum* in response to itraconazole. *Med Mycol* 47(3):237–247
- Fachin AL, Contel EPB, Martinez-Rossi NM (2001) Effect of sub-MICs of antimycotics on expression of intracellular esterase of *Trichophyton rubrum*. *Med Mycol* 39(1):129–133
- Fachin AL, Ferreira-Nozawa MS, Maccheroni W et al (2006) Role of the ABC transporter TruMDR2 in terbinafine, 4-nitroquinoline N-oxide and ethidium bromide susceptibility in *Trichophyton rubrum*. *J Med Microbiol* 55(8):1093–1099
- Fan W, Kraus PR, Boily MJ et al (2005) *Cryptococcus neoformans* gene expression during murine macrophage infection. *Eukaryot Cell* 4(8):1420–1433
- Ferreira-Nozawa MS, Silveira HCS, Ono CJ et al. (2006) The pH signaling transcription factor PacC mediates the growth of *Trichophyton rubrum* on human nail *in vitro*. *Med Mycol* 44(7):641–645
- Fradin C, Kretschmar M, Nichterlein T et al (2003) Stage-specific gene expression of *Candida albicans* in human blood. *Mol Microbiol* 47(6):1523–1543
- Fradin C, De Groot P, MacCallum D et al (2005) Granulocytes govern the transcriptional response, morphology and proliferation of *Candida albicans* in human blood. *Mol Microbiol* 56(2):397–415
- Fradin C, Mavor AL, Weindl G et al (2007) The early transcriptional response of human granulocytes to infection with *Candida albicans* is not essential for killing but reflects cellular communications. *Infect Immun* 75(3):1493–1501
- Gautam P, Shankar J, Madan T et al (2008) Proteomic and transcriptomic analysis of *Aspergillus fumigatus* on exposure to amphotericin B. *Antimicrob Agents Chemother* 52(12):4220–4227
- Gibbons JG, Beauvais A, Beau R et al (2012) Global transcriptome changes underlying colony growth in the opportunistic human pathogen *Aspergillus fumigatus*. *Eukaryot Cell* 11(1):68–78
- Gomez P, Hackett TL, Moore MM et al (2011) Functional genomics of human bronchial epithelial cells directly interacting with conidia of *Aspergillus fumigatus*. *BMC Genomics* 11:358
- Heddergott C, Bruns S, Nietzsche S et al (2012) The *Arthroderma benhamiae* hydrophobin HypA mediates hydrophobicity and influences recognition by human immune effector cells. *Eukaryot Cell* 11(5):673–682
- Hu G, Cheng PY, Sham A et al (2008) Metabolic adaptation in *Cryptococcus neoformans* during early murine pulmonary infection. *Mol Microbiol* 69(6):1456–1475
- Ibrahim-Granet O, Dubourdeau M, Latge JP et al (2008) Methylcitrate synthase from *Aspergillus fumigatus* is essential for manifestation of invasive aspergillosis. *Cell Microbiol* 10(1):134–148
- Idnurm A, Giles SS, Perfect JR et al (2007) Peroxisome function regulates growth on glucose in the basidiomycete fungus *Cryptococcus neoformans*. *Eukaryot Cell* 6(1):60–72
- Idnurm A, Walton FJ, Floyd A et al (2009) Identification of ENA1 as a virulence gene of the human pathogenic fungus *Cryptococcus neoformans* through signature-tagged insertional mutagenesis. *Eukaryot Cell* 8(3):315–326

- Inglis DO, Berkes CA, Hocking Murray DR et al (2010) Conidia but not yeast cells of the fungal pathogen *Histoplasma capsulatum* trigger a type I interferon innate immune response in murine macrophages. *Infect Immun* 78(9):3871–3882
- Jung WH, Hu G, Kuo W et al. (2009) Role of ferroxidases in iron uptake and virulence of *Cryptococcus neoformans*. *Eukaryot Cell* 8(10):1511–1520
- Kane PM (2007) The long physiological reach of the yeast vacuolar H⁺-ATPase. *J Bioenerg Biomembr* 39(5–6):415–421
- Kim HS, Choi EH, Khan J et al (2005) Expression of genes encoding innate host defense molecules in normal human monocytes in response to *Candida albicans*. *Infect Immun* 73(6):3714–3724
- Kretschmer M, Wang J, Kronstad JW (2012) Peroxisomal and mitochondrial beta-oxidation pathways influence the virulence of the pathogenic fungus *Cryptococcus neoformans*. *Eukaryot Cell* 11(8):1042–1054
- Lambou K, Lamarre C, Beau R et al (2010) Functional analysis of the superoxide dismutase family in *Aspergillus fumigatus*. *Mol Microbiol* 75(4):910–923
- Lim CS, Rosli R, Seow HF et al (2011) Transcriptome profiling of endothelial cells during infections with high and low densities of *C. albicans* cells. *Int J Med Microbiol* 301(6):536–546
- Liu TT, Lee RE, Barker KS et al (2005) Genome-wide expression profiling of the response to azole, polyene, echinocandin, and pyrimidine antifungal agents in *Candida albicans*. *Antimicrob Agents Chemother* 49(6):2226–2236
- Liu TT, Znaidi S, Barker KS et al (2007) Genome-wide expression and location analyses of the *Candida albicans* Tac1p regulon. *Eukaryot Cell* 6(11):2122–2138
- Livonesi MC, Souto JT, Campanelli AP et al (2008) Deficiency of IL–12p40 subunit determines severe paracoccidioidomycosis in mice. *Med Mycol* 46(7):637–646
- Lorenz MC, Fink GR (2001) The glyoxylate cycle is required for fungal virulence. *Nature* 412(6842):83–86
- Lorenz MC, Bender JA, Fink GR (2004) Transcriptional response of *Candida albicans* upon internalization by macrophages. *Eukaryot Cell* 3(5):1076–1087
- Lupo P, Chang YC, Kelsall BL et al (2008) The presence of capsule in *Cryptococcus neoformans* influences the gene expression profile in dendritic cells during interaction with the fungus. *Infect Immun* 76(4):1581–1589
- Maranhão FCA, Paião FG, Martinez-Rossi NM (2007) Isolation of transcripts over-expressed in human pathogen *Trichophyton rubrum* during growth in keratin. *Microbial Pathog* 43(4):166–172
- Martin R, Moran GP, Jacobsen ID et al (2011) The *Candida albicans*-specific gene EED1 encodes a key regulator of hyphal extension. *PLoS One* 6(4):e18394
- Martinez-Rossi NM, Peres NTA, Rossi A (2008) Antifungal resistance mechanisms in dermatophytes. *Mycopathologia* 166(5–6):369–383
- Martinez-Rossi NM, Persinoti GF, Peres NTA et al (2012) Role of pH in the pathogenesis of dermatophytoses. *Mycoses* 55(5):381–387
- Mayer FL, Wilson D, Hube B (2013) *Candida albicans* pathogenicity mechanisms. *Virulence* 4(2):119–128
- McDonagh A, Fedorova ND, Crabtree J et al (2008) Sub-telomere directed gene expression during initiation of invasive aspergillosis. *PLoS Pathog* 4(9):e1000154
- Missall TA, Lodge JK, McEwen JE (2004) Mechanisms of resistance to oxidative and nitrosative stress: implications for fungal survival in mammalian hosts. *Eukaryot Cell* 3(4):835–846
- Monod M (2008) Secreted proteases from dermatophytes. *Mycopathologia* 166(5–6):285–294
- Morschhauser J, Barker KS, Liu TT et al (2007) The transcription factor Mrr1p controls expression of the MDR1 efflux pump and mediates multidrug resistance in *Candida albicans*. *PLoS Pathog* 3(11):e164
- Morton CO, Varga JJ, Hornbach A et al (2011) The temporal dynamics of differential gene expression in *Aspergillus fumigatus* interacting with human immature dendritic cells *in vitro*. *PLoS One* 6(1):e16016
- Muller V, Viemann D, Schmidt M et al (2007) *Candida albicans* triggers activation of distinct signaling pathways to establish a proinflammatory gene expression program in primary human endothelial cells. *J Immunol* 179(12):8435–8445

- Mullick A, Elias M, Harakidas P et al (2004) Gene expression in HL60 granulocytoids and human polymorphonuclear leukocytes exposed to *Candida albicans*. *Infect Immun* 72(1):414–429
- Nett J, Lincoln L, Marchillo K et al (2007) Beta-1,3 glucan as a test for central venous catheter biofilm infection. *J Infect Dis* 195(11):1705–1712
- Nobile CJ, Solis N, Myers CL et al (2008) *Candida albicans* transcription factor Rim101 mediates pathogenic interactions through cell wall functions. *Cell Microbiol* 10(11):2180–2196
- Nobile CJ, Fox EP, Nett JE et al (2012) A recently evolved transcriptional network controls biofilm development in *Candida albicans*. *Cell* 148(1–2):126–138.
- O’Meara TR, Norton D, Price MS et al (2010) Interaction of *Cryptococcus neoformans* Rim101 and protein kinase A regulates capsule. *PLoS Pathog* 6(2):e1000776
- O’Meara TR, Xu W, Selvig KM et al (2013) The *Cryptococcus neoformans* Rim101 transcription factor directly regulates genes required for adaptation to the host. *Mol Cell Biol* 34(4):673–684
- Ok M, Latge JP, Baeuerlein C et al (2009) Immune responses of human immature dendritic cells can be modulated by the recombinant *Aspergillus fumigatus* antigen Aspfl. *Clin Vaccine Immunol* 16(10):1485–1492
- Oosthuizen JL, Gomez P, Ruan J et al (2011) Dual organism transcriptomics of airway epithelial cells interacting with conidia of *Aspergillus fumigatus*. *PLoS One* 6(5):e20527
- Paião FG, Segato F, Cursino-Santos JR et al (2007) Analysis of *Trichophyton rubrum* gene expression in response to cytotoxic drugs. *FEMS Microbiol Lett* 271(2):180–186
- Peres NTA, Maranhao FCA, Rossi A et al (2010a) Dermatophytes: host-pathogen interaction and antifungal resistance. *An Bras Dermatol* 85(5):657–667
- Peres NTA, Sanches PR, Falcão JP et al (2010b) Transcriptional profiling reveals the expression of novel genes in response to various stimuli in the human dermatophyte *Trichophyton rubrum*. *BMC Microbiol* 10:39–48
- Petzold EW, Himmelreich U, Mylonakis E et al (2006) Characterization and regulation of the trehalose synthesis pathway and its importance in the pathogenicity of *Cryptococcus neoformans*. *Infect Immun* 74(10):5877–5887
- Prigneau O, Porta A, Poudrier JA et al (2003) Genes involved in beta-oxidation, energy metabolism and glyoxylate cycle are induced by *Candida albicans* during macrophage infection. *Yeast* 20(8):723–730
- Ramirez MA, Lorenz MC (2007) Mutations in alternative carbon utilization pathways in *Candida albicans* attenuate virulence and confer pleiotropic phenotypes. *Eukaryot Cell* 6(2):280–290
- Rogers PD, Barker KS (2002) Evaluation of differential gene expression in fluconazole-susceptible and -resistant isolates of *Candida albicans* by cDNA microarray analysis. *Antimicrob Agents Chemother* 46(11):3412–3417
- Rogers PD, Barker KS (2003) Genome-wide expression profile analysis reveals coordinately regulated genes associated with stepwise acquisition of azole resistance in *Candida albicans* clinical isolates. *Antimicrob Agents Chemother* 47(4):1220–1227
- Romani L (2011) Immunity to fungal infections. *Nat Rev Immunol* 11(4):275–288
- Rossi A, Cruz AHS, Santos RS et al (2013) Ambient pH sensing in filamentous fungi: pitfalls in elucidating regulatory hierarchical signaling networks. *IUBMB Life* 65(11):930–935
- Rude TH, Toffaletti DL, Cox GM et al (2002) Relationship of the glyoxylate pathway to the pathogenesis of *Cryptococcus neoformans*. *Infect Immun* 70(10):5684–5694
- Sanguinetti M, Posteraro B, Fiori B et al (2005) Mechanisms of azole resistance in clinical isolates of *Candida glabrata* collected during a hospital survey of antifungal resistance. *Antimicrob Agents Chemother* 49(2):668–679
- Schobel F, Ibrahim-Granet O, Ave P, Latge JP, Brakhage AA, and Brock M (2007) *Aspergillus fumigatus* does not require fatty acid metabolism via isocitrate lyase for development of invasive aspergillosis. *Infect Immun* 75:1237–1244
- Segato F, Nozawa SR, Rossi A et al (2008) Over-expression of genes coding for proline oxidase, riboflavin kinase, cytochrome c oxidase and an MFS transporter induced by acriflavin in *Trichophyton rubrum*. *Med Mycol* 46(2):135–139
- Shapiro RS, Robbins N, Cowen LE (2011) Regulatory circuitry governing fungal development, drug resistance, and disease. *Microbiol Mol Biol Rev* 75(2):213–267

- Shiraki Y, Ishibashi Y, Hiruma M et al (2006) Cytokine secretion profiles of human keratinocytes during *Trichophyton tonsurans* and *Arthroderma benhamiae* infections. *J Med Microbiol* 55(Pt 9):1175–1185
- Silva SS, Tavares AHFP, Passos-Silva DG et al (2008) Transcriptional response of murine macrophages upon infection with opsonized *Paracoccidioides brasiliensis* yeast cells. *Microbes Infect* 10(1):12–20
- Silva MG, Schrank A, Bailao EF et al (2011) The homeostasis of iron, copper, and zinc in *Paracoccidioides brasiliensis*, *Cryptococcus neoformans* var. *grubii*, and *Cryptococcus gattii*: a comparative analysis. *Front Microbiol* 2:49
- Silveira HCS, Gras DE, Cazzaniga RA et al (2010) Transcriptional profiling reveals genes in the human pathogen *Trichophyton rubrum* that are expressed in response to pH signaling. *Microb Pathog* 48(2):91–96
- Silver PM, Oliver BG, White TC (2004) Role of *Candida albicans* transcription factor Upc2p in drug resistance and sterol metabolism. *Eukaryot Cell* 3(6):1391–1397
- Singh SD, Robbins N, Zaas AK et al (2009) Hsp90 governs echinocandin resistance in the pathogenic yeast *Candida albicans* via calcineurin. *PLoS Pathog* 5(7):e1000532
- Souto JT, Figueiredo F, Furlanetto A et al (2000) Interferon-gamma and tumor necrosis factor-alpha determine resistance to *Paracoccidioides brasiliensis* infection in mice. *Am J Pathol* 156(5):1811–1820
- Staib P, Zaugg C, Mignon B et al (2010) Differential gene expression in the pathogenic dermatophyte *Arthroderma benhamiae* *in vitro* versus during infection. *Microbiology* 156(Pt 3):884–895
- Steen BR, Zuyderduyn S, Toffaletti DL et al (2003) *Cryptococcus neoformans* gene expression during experimental cryptococcal meningitis. *Eukaryot Cell* 2(6):1336–1349
- Sugui JA, Kim HS, Zarembk KA et al (2008) Genes differentially expressed in conidia and hyphae of *Aspergillus fumigatus* upon exposure to human neutrophils. *PLoS One* 3(7):e2655
- Sun N, Fonzi W, Chen H et al (2013) Azole susceptibility and transcriptome profiling in *Candida albicans* mitochondrial electron transport chain complex I mutants. *Antimicrob Agents Chemother* 57(1):532–542
- Tavares AH, Derengowski LS, Ferreira KS et al (2012) Murine dendritic cells transcriptional modulation upon *Paracoccidioides brasiliensis* infection. *PLoS Negl Trop Dis* 6(1):e1459
- Thewes S, Kretschmar M, Park H et al (2007) *In vivo* and *ex vivo* comparative transcriptional profiling of invasive and non-invasive *Candida albicans* isolates identifies genes associated with tissue invasion. *Mol Microbiol* 63(6):1606–1628
- Tierney L, Linde J, Muller S et al (2012) An Interspecies regulatory network inferred from simultaneous RNA-seq of *Candida albicans* invading innate immune cells. *Front Microbiol* 3:85
- Vediyappan G, Rossignol T, d'Enfert C (2010) Interaction of *Candida albicans* biofilms with antifungals: transcriptional response and binding of antifungals to beta-glucans. *Antimicrob Agents Chemother* 54(5):2096–2111
- Vermes A, Guchelaar HJ, Dankert J (2000) Flucytosine: a review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. *J Antimicrob Chemother* 46(2):171–179
- Viriyakosol S, Singhanua A, Fierer J et al (2013) Gene expression in human fungal pathogen *Coccidioides immitis* changes as arthroconidia differentiate into spherules and mature. *BMC Microbiol* 13:121
- Wachtler B, Wilson D, Haedicke K et al (2011) From attachment to damage: defined genes of *Candida albicans* mediate adhesion, invasion and damage during interaction with oral epithelial cells. *PLoS One* 6(2):e17046
- Walker LA, Maccallum DM, Bertram G et al (2009) Genome-wide analysis of *Candida albicans* gene expression patterns during infection of the mammalian kidney. *Fungal Genet Biol* 46(2):210–219
- Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet* 10(1):57–63

- Westermann AJ, Gorski SA, Vogel J (2012) Dual RNA-seq of pathogen and host. *Nat Rev Microbiol* 10(9):618–630
- White TC, Holleman S, Dy F et al (2002) Resistance mechanisms in clinical isolates of *Candida albicans*. *Antimicrob Agents Chemother* 46(6):1704–1713
- Woodfolk JA, Platts-Mills TA (1998) The immune response to dermatophytes. *Res Immunol* 149(4–5):436–445
- Yu L, Zhang W, Liu T et al (2007a) Global gene expression of *Trichophyton rubrum* in response to PH11B, a novel fatty acid synthase inhibitor. *J Appl Microbiol* 103(6):2346–2352
- Yu L, Zhang W, Wang L et al (2007b) Transcriptional profiles of the response to ketoconazole and amphotericin B in *Trichophyton rubrum*. *Antimicrob Agents Chemother* 51(1):144–153
- Zakikhany K, Naglik JR, Schmidt-Westhausen A et al (2007) *In vivo* transcript profiling of *Candida albicans* identifies a gene essential for interepithelial dissemination. *Cell Microbiol* 9(12):2938–2954
- Zaugg C, Monod M, Weber J, Harshman K, Pradervand S, Thomas J et al. (2009) Gene expression profiling in the human pathogenic dermatophyte *Trichophyton rubrum* during growth on proteins. *Eukaryot Cell* 8:241–250
- Zhang W, Yu L, Yang J et al (2009) Transcriptional profiles of response to terbinafine in *Trichophyton rubrum*. *Appl Microbiol Biotechnol* 82(6):1123–1130