Chapter 17
Transcriptome in Human Mycoses

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17.1 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, resulting in enormous economic losses. Humans are constantly in contact with fungi by inhaling spores in the air and ingesting them as nutritional sources. Human mycoses have increased in incidence due to the high prevalence of immunocompromised patients, becoming a major public-health concern. According to the Global Action Fund for Fungal Infections (GAFFI – https://www.gaffi.org), fungal diseases affect more than 300 million people, leading to the death of approximately 1.6 million people

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G. A. Passos (ed.), *Transcriptomics in Health and Disease*,
https://doi.org/10.1007/978-3-030-87821-4_17

annually worldwide. 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; Rodrigues and Nosanchuk 2020). Fungi cause a wide spectrum of diseases, 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. The increasing clinical reports of fungal coinfections among hospitalized patients, especially those with respiratory infections such as the recent COVID-19 pandemic caused by the SARS-CoV-2 virus (Zhu et al. 2020; Silva et al. 2021; Alanio et al. 2020), highlight the importance of investments in basic and clinical research exploring fungal mechanisms and pathways. Therefore, more data about the life cycle of pathogenic fungi and the pathogenesis of these 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 infect several anatomical sites, resulting in different clinical symptoms. The most prevalent are cutaneous, mucosal, subcutaneous, 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 and microbiota enable its invasive behavior, leading to tissue damage and dissemination through the bloodstream to other organs (d'Enfert et al. 2020). More recently, the emerging pathogen *Candida auris* poses a threat causing nosocomial infections and hospital outbreaks, increasing global concern owing to its high resistance to antifungal agents and disinfectant chemical compounds (Du et al. 2020).

Deep fungal infections are mainly caused by *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Cryptococcus gattii*, *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

Table 17.1 Main fungal pathogens and their associated diseases in humans

Fungi	Main species	Disease
Aspergillus	*A. fumigauts*	Pulmonary infections (invasive aspergilosis and aspergilloma) Allergy
Blastomyces	*B. dermatitis*	Skin lesions Pulmonary infections
Candida	*C. albicans* *C. glabrata* *C. parapsilosis* *C. auris*	Cutaneous infections – skin and nails oropharyngeal candidiasis (thrush) Vulvovaginal candidiasis Nosocomial infections, fungemia
Coccidioides	*C. immitis* *C. posadasii*	Pulmonary infections
Cryptococcus	*C. neoformans* *C. gattii*	Meningitis Meningoencephalitis Pulmonary infections (pneumonia)
Dermatophytes	*Trichophyton rubrum* *Trichophyton mentagrophytes* *Microsporum canis*	Cutaneous infections – skin, nail, and hair (tinea or ringworms)
Histoplasma	*H. capsulatum*	Pulmonary infections
Malassezia	*M. furfur*	Cutaneous infections – skin (pityriasis versicolor)
Paracoccidioides	*P. brasiliensis* *P. lutzii*	Pulmonary and systemic infections
Penicillium	*P. marneffeii*	Pulmonary infections
Pneumocystis	*P. jirovecii*	Pneumonia
Sporothrix	*S. brasiliensis* *S. schenkii*	Subcutaneous infections

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. *Cryptococcus* spp. may also migrate into the central nervous system, causing meningitidis and meningoencephalitis. Table 17.1 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. 2018).

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 multicellular structure, and cells may be 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, high-throughput RNA sequencing (RNA-seq), and RT-qPCR (Reverse Transcription – quantitative Polymerase Chain Reaction). Fungal transcriptomics has 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, antifungal resistance, and pathogenicity as well as the host's immune response. Recent advances in molecular biology methods and bioinformatics tools have enabled the study of the whole transcriptome, providing meaningful insights into the functionality of the genome, revealing interaction networks and 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 noncoding 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 (Stark et al. 2019).

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 between 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.

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

Fungal diseases can result from poor immune responses or from exacerbated activation of the immune system, such as the inflammatory response. Therefore, the interplay of the innate and adaptive immune mechanisms and their appropriate activation are crucial for successful pathogen clearance and cellular homeostasis. 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 (AMPs) 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), such as α- and β-glucans, mannans, lipopolysaccharides, and phospholipomannan in the fungal cell wall. 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, Fcγ receptor, Dectin-1 and 2, Galectin, Macrophage-Inducible C-type Lectin (mincle), and Dendritic Cells - Specific Intercellular adhesion molecule Grabbing Non-integrin (DC-SIGN) (Romani 2011). Pathogen recognition by macrophages leads to their differentiation into the classic (M1) and alternatively (M2) activated macrophages. While M1 macrophages have microbicidal and pro-inflammatory properties, playing a role in fungal clearance, M2 macrophages have anti-inflammatory activities implicated in fungal persistence (Pathakumari et al. 2020). Furthermore, besides fungal PAMPs, recent studies on fungal extracellular vesicles have demonstrated their role in modulating the host immune response (Bitencourt et al. 2018; Bielska and May 2019).

In general, a Th1 response is correlated with protective immunity against fungi, and is characterized by the production of interferon gamma (IFN-γ), among other cytokines, leading 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 the production of signature cytokines. Moreover, Th17 cells support Th1 cellular responses, playing an important role in promoting neutrophil recruitment (Bedoya et al. 2013; Pathakumari et al. 2020; Romani 2011). Regulatory T cells (Treg) control tissue damage by reducing the inflammatory response. However, this also causes immune suppression, thereby allowing fungal persistence. Th2 response, characterized by the production of IL-4, IL-5, IL-10, TGF-β, and IL-13, is correlated with an increased fungal burden. However, IL-4 may play a protective role in the early stages of fungal infections, and the balance between Th1 and Th2 is crucial for the outcome of the infection (Pathakumari et al. 2020). 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 Treg, establishing 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. However, yeast cells use alveolar macrophages as vehicles to spread to different organs, such as the liver, spleen, lymph nodes, and bone marrow (Mittal et al. 2019). Microarray analysis revealed that in response to conidia, macrophages specifically upregulated type I IFN-induced genes, including IFN-β and a classic type 1 IFN signature, in addition to general inflammatory genes. This effect was dependent on interferon regulatory factor 3 (IRF3) and independent of the TLR signaling pathway. IFNAR1 (type I IFN receptor) knockout mice showed a decreased fungal burden in the lungs and spleen after intranasal infection with conidia and yeast cells, compared to wild-type mice. Therefore, IFNAR1 signaling might contribute to disease and fungal burden rather than conferring protection, through the modulation of cytokines, 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 and decreased survival, 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. IFNAR1 and IFN-β knockout mice also displayed a lower survival rate and increased fungal burden in the kidneys, showing that type 1 IFN response plays a protective role against *C. albicans* (Biondo et al. 2011).

The major virulence factor of *C. neoformans* is its polysaccharide capsule, which interferes with recognition by immune cells. Microarray analysis showed that a nonencapsulated strain induced the expression of genes involved in DC maturation, chemokines, and cytokines, characterizing an immunostimulatory response. Among the proteins encoded by these upregulated genes 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. Several chemokines were also upregulated in DCs stimulated with the nonencapsulated 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. In contrast, an

encapsulated strain caused 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 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-κB signaling, respectively (Lupo et al. 2008). Additionally, *C. neoformans* profoundly alters the expression profiles of both polarized macrophages M1 and M2 to a naive phenotype (Subramani et al. 2020) under *in vitro* conditions. RNA-seq comparing pulmonary infection by *C. neoformans* in mice and monkeys revealed species-specific responses. The expression of genes coding for IL-1a and IL-1b and those involved in iron acquisition, transport, and storage was upregulated in the lungs of monkeys. However, the expression of calcium homeostasis-related genes was repressed in mice but remained unchanged in monkeys. Genes related to the TLR, TNF, IL-17 pathways, and copper homeostasis were upregulated in both models; the insulin signaling pathway was also modulated in response to *C. neoformans* infection (Li et al. 2019).

P. brasiliensis causes pulmonary and systemic infections. 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-κB 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). Also, TNF-α deficient mice 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*; however, it 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-12p40 knockout mice displayed decreased survival, higher fungal burden, and decreased production of IFN-γ (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-κB, concomitantly with the downregulation of the NF-κB inhibitor Nκ-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). There is a significant difference between the transcriptome profile of DCs derived from susceptible and resistant mice infected *in vitro* with *P. brasiliensis*. This observation highlights that a high activation of the inflammatory responses and downregulation of autophagy, lysosome, and apoptosis are involved in the disease. A low activation of these pathways is related to infection resistance and a proper immune response (de-Souza-Silva et al. 2020).

The dynamics of the molecular response triggered by *C. albicans* in human monocytes identified a pattern of gene expression related to recruitment, activation, and viability of phagocytes, as well as the enhancement of chemotaxis and inflammation (Kim et al. 2005). Increased expression of genes encoding TNF-α, IL-6, and IL-1α was correlated with neutrophil infiltration at the site of infection. There was an upregulation of genes encoding the chemokines CCL3, CCL4, CCL20, CCL18, CXCL1, CXCL-3, 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. 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 (Johns et al. 2021). Further, RNA-seq of mononuclear cells challenged with *C. albicans* and *C. auris* showed unique and species-specific transcriptional signatures. *C. auris* induced the expression of type I and II IFNs, IFN-related genes, IL-1RA, IL-10, IL-9, and IL-27, thus triggering a stronger host response than *C. albicans*. This may be attributed to differences in the cell wall mannoproteins, leading to different phagocytic indices and clinical outcomes between these two species (Bruno et al. 2020).

Neutrophils display a potent set of hydrolytic enzymes, antimicrobial peptides, and oxidative species within their intracellular granules, having an immediate and pronounced effect on *C. albicans* (Fradin et al. 2005). Granulocyte-like cells phagocytose and kill *C. albicans*, 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-1β, 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 adheres and invades 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 involved in 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). *C. albicans* induces several genes that are targets of the pro-inflammatory transcription factor NF-κB, and chemokines, including IL-8, CXCL1, CXCL2, CXCL3, CXCL5, and CXCL6, indicating that ECs help to recruit neutrophils and monocytes to the infection site (Muller et al. 2007). The overexpression of genes involved in stress and wound healing, 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 antiapoptotic 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*. In general, human umbilical vein ECs infected with high densities of *C. albicans* displayed a stronger and broader transcriptional response than cells infected with low densities, which may be 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. Dual transcriptomics is used to identify molecular patterns of the pathogen and the host simultaneously, providing insights into the dynamics of the infectious process (Westermann et al. 2017). Dual transcriptomics by RNA-seq was performed during DC phagocytosis of *C. albicans*, and gene interactions were predicted using systems biology. RNA-seq identified 545 *C. albicans* and 240 DCs genes differentially expressed, 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 the 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, revealing expression patterns indicating the 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. IL-6, a potent pro-inflammatory cytokine, was highly expressed in response to *A. fumigatus* conidia, 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). In addition, genes involved in nucleosome organization and chromatin assembly were overexpressed. Genes involved in mitosis and cell cycle progression were downregulated, suggesting 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-1β, 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). Recently, a triple RNA-seq analysis of DCs coinfected with *A. fumigatus* and cytomegalovirus (CMV) revealed unique transcription profiles of the host in response to each pathogen alone or during coinfection along with distinct profiles of both pathogens during infection and coinfection. The gene expression profile of DCs showed a different pattern in response to each pathogen. *A. fumigatus* induced Th17 expression, whereas CMV infection led to a Th1 response. However, coinfection led to downregulation of the expression of these genes, with each pathogen attenuating the effect of the other on the molecular signature of DCs and thereby interfering with the host response (Seelbinder et al. 2020).

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 represent an important barrier against pathogens and help mediate the immune response (Burstein et al. 2020). The zoophilic dermatophyte *Arthroderma benhamiae*, and the anthropophilic species *Trichophyton tonsurans* induced different cytokine expression profiles in keratinocytes, correlating with the inflammatory response. In infected keratinocytes, the zoophilic species induced the upregulation of pro-inflammatory genes and the concomitant secretion of cytokines IL-1β, IL-6, IL-6R, and IL-17 and chemokines IL-8 and CCL2. This effect may promote the infiltration of inflammatory cells in the skin during infection, and the upregulation of IL-6, IL-6R, and granulocyte-colony stimulating factor (G-CSF) may lead to 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 *A. benhamiae* displayed an infiltration of PMNs, macrophages, and DCs in the skin as well as increased levels of TGF-β, IL-1β, IL-6, and IL-22 mRNA in skin biopsies (Cambier et al. 2014). This pro-inflammatory profile was also observed in keratinocytes challenged with the zoophilic dermatophyte *Microsporum gypseum*, with the upregulation of the expression of IL-6, IL-8, IL-1β, TNF-α, and c-Jun and enrichment of the NF-kB, TNF, and MAPK signaling pathways. However, the gene network of keratinocytes response to *Trichophyton rubrum* was based on metabolic pathways such as steroid, fatty acid, and isoprenoid biosynthesis (Deng et al. 2020). *In vitro* infection of keratinocytes with *T. rubrum* induced the expression of genes coding for the AMPs RNase 7, beta-defensin 3, and the natural resistance-associated macrophage protein 1, in addition to that of cytokine-related genes (Firat et al. 2014); Petrucelli et al. 2018). The expression of genes involved in epidermal cell differentiation, such as

caspase 14 and laminin subunit gamma 2, and cell migration, such as metalloproteinase 9, was upregulated, whereas that of genes involved in skin barrier maintenance, such as keratin 1 and filaggrin, was downregulated. This may also account for the tissue damage and antifungal response during *T. rubrum* infection (Petrucelli et al. 2018). Further, the inflammatory response during infection may also be regulated by microRNAs, owing to their upregulation in macrophages challenged with heat-inactivated *T. rubrum* conidia (Gonzalez Segura et al. 2020).

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 expression 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 to better characterize the immune components that contribute to the outcome of fungal infections.

17.3 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 (Brown et al. 2014). In particular, nutrient availability can be limited in host niches, especially inside phagocytes. Host cues and tissue nutrients substantially affect the outcome of the infection by triggering the activation of different fungal signaling pathways that govern germination, cell wall remodeling, and morphological cell type switch, as well as of those regulating the production of enzymes involved in transcription regulation and metabolic adjustments to improve growth and host invasion and dissemination (Johns et al. 2021). Thus, fungi undergo metabolic adaptations to control, for example, glycolysis, gluconeogenesis, 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). To avoid immune recognition, fungal cells monitor host cues through plasma membrane receptors. Subsequently, they mask cell wall components such as β-glucans and melanin, either by the encapsulation or formation of titan cells, as shown for *C. neoformans*, or by the activation of the transcription factors Crz1 and Ace2 that govern the cell wall remodeling in *C. albicans*, aiding fungal colonization and reducing neutrophil recruitment (Ballou et al. 2016). Moreover, it is well known that phagocytes produce reactive oxygen and nitrogen species (ROS and RNS), which induce oxidative and nitrosative stress

as an attempt to 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 Jesus-Berrios et al. 2003), and superoxide dismutases, catalases, and peroxidases, which counteract oxidative stress. Nonenzymatic 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; Martinez-Rossi et al. 2017). The pH affects enzymatic activities; the alkaline pH of human tissues influences nutrient uptake because the solubility of essential elements, such as iron and zinc, are pH dependent (Amich et al. 2010). Iron is a critical micronutrient in both the host and pathogen, as it is required for several metabolic processes, including respiration and DNA replication. In the form of heme and iron-sulfur compounds, iron is an essential cofactor in various cellular enzymes, oxygen carriers, and electron-transfer systems. Iron homeostasis plays a key role in host–pathogen interactions. Similarly, zinc is essential for pathogenic fungi because it is a constituent of many transcription factors and acts as a cofactor for enzymes involved in cell signaling. For instance, host tissues can restrict free iron and zinc availability to prevent infection. Accordingly, fungal pathogens have adapted strategies for iron uptake, including the production of metalloreductases, ferroxidases, and siderophores (Silva et al. 2011) and uptake of zinc through the production of zincophore such as Pra1 and its ortholog Aspf2 (Amich et al. 2010; Citiulo et al. 2012) to survive in iron and zinc-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 fungal pathogens' transcriptional profiles during infection and interaction with host cells. These studies have helped to elucidate the pathogenesis of superficial, deep, and bloodstream fungal infections. In this sense, 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 (*ACS1*). Genes involved in fermentation, such as those encoding alcohol dehydrogenases (*ADH1* and *ADH2*), were also upregulated. Importantly, *C. albicans* isolated from infected mice exhibited a similar transcription profile, thus validating some of the *in vitro* results. Moreover, these data suggested that *C. albicans* use 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.

Besides, during the interaction of *C. albicans* with neutrophils, the activation of nitrogen- and carbohydrate-starvation responses was observed, 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 *ACS1*), amino acid transporters, and proteins involved in amino acid metabolism. Moreover, *C. albicans* internalized by murine macrophages *in vitro* displayed growth arrest and downregulation of the expression of genes associated with translation machinery and glycolysis. In contrast, there was an upregulation of genes encoding enzymes involved in the gluconeogenesis (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. 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). The interaction with host cells also triggered the upregulation of oxidative stress response genes such as superoxide dismutases (*SOD1* and *SOD5*) and catalase (*CAT1*) (Fradin et al. 2005), flavohemoglobin, cytochrome *c* peroxidase, peroxidases, reductases, stress response (heat shock protein HSP78), metal homeostasis, and DNA repair (Lorenz et al. 2004). In accordance with this data, a previous work evaluated the transcriptional profile of *C. albicans* using biopsies of infected oral mucosa from 11 HIV-positive patients showed changes that reflected fungal protective responses toward 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. From this evidence in association with literature support, glyoxylate genes have been considered an important virulence factor. Indeed, Δ*icl1* 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, is involved in hyphal elongation during infection (Zakikhany et al. 2007). Time-course microarray analysis of the wild type and Δ*eed1* strains interacting with RHE showed the downregulation of seven genes throughout infection, including the hyphae-associated genes *ECE1* and *HYR1* and those encoding proteins involved in polarized growth, such as CDC42, RDI1, MYO2, CDC11, CYB2, MOB1, and MLC1.

Another study showed the coordinated host and fungal transcriptional response during macrophage infection with *C. albicans* (Munoz et al. 2019). In this study, sorted cells were analyzed with respect to different infection stages, and a single-cell approach was employed to track different trajectories in the course of infection. Important changes in metabolic pathways were evidenced by the expression of genes modulated in phagocytosed *C. albicans*. Alternative pathways were activated at early infection stages to cope with the macrophage environment and favor fungal survival with limited glucose availability in the phagosome, including genes belonging to the glyoxylate cycle and beta-oxidation. In contrast, the expression of genes related to chaperones, transcription factors that regulate translation, and peptide synthesis was downregulated. A shift in gene expression occurred at a later infection time. The central carbon pathway was reactivated with the expression of genes involved in morphological changes, such as cell wall assembly and filamentation. The genes related to phagocytosis and innate immune response activation were enriched in macrophages, including those associated with IL-6, IL-8, and NF-κB signaling pathways. Moreover, the production of nitric oxide, reactive oxygen species (ROS), and pattern recognition receptors was also induced. Notably, there was a shift during the time of the infection, with significant repression of the immune response. Interestingly, morphological changes in *C. albicans* and induction of filamentation were followed by repression of the immune response. Beyond that, the single-cell analysis also provided new insights into infection outcomes. It demonstrated that bimodality in gene expression was observed in about 15% of differentially expressed genes (DEGs) mainly involved in pathogen recognition and pro-inflammatory pathways within 2 h and 4 h of interaction. In *C. albicans,* about 23% of DEGs presented bimodality in gene expression, evidenced mainly in genes related to metabolism and virulence, and hyphae transition. Finally, this study evaluated the alternative splice (AS) as exon skipping in these sorted cells, highlighting the occurrence of two isoforms for a gene that encodes a dectin potentially involved in the Th17 response. From that perspective, both cells trigger mechanisms to promote stochastic diversification to favor the phenotype and infection outcome during the interaction.

In order to investigate expression changes in *C. albicans* during systemic infection, transcriptional profiling was performed *in vivo* on mice infected 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 stress 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. Also, among genes identified during the comparison of transcriptional profile of an invasive *C. albicans* strain with a noninvasive strain 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). Moreover, in rabbits, the infected kidneys with *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 *ACS1*), 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 infection models, fatty acid degradation is not essential for the 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 (Cavalheiro and Teixeira 2018). *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. However, a recent comprehensive analysis in the circuit of biofilm formation demonstrated that the biofilm/hyphae regulatory network shows a more profound variation in accordance to genotype from each isolate, which was partly attributed to the occurrence of single nucleotide polymorphisms in cis-regulatory elements of BRG1 that influences its control by

BCR1 (Huang et al. 2019). Moreover, the biofilm network targeted approximately 15% of the entire genome (Nobile et al. 2012). The enriched GO terms of EFG1 responsive genes involve biofilm formation and cell surface. It is remarkable that the involvement of carbohydrate metabolism, mainly glycolytic and gluconeogenesis, processes as an important set of EFG1-activated genes (Huang et al. 2019).

In *C. auris,* resistance to different antifungal compounds in association with the capability to form biofilm and rapid dissemination between patients urge for efforts aiming to unveil its physiology. Recent reports have profiled the gene expression of *C. auris* during biofilm formation and exposure to caspofungin (Kean et al. 2018; Zamith-Miranda et al. 2020). In biofilms, the over-enriched GO terms involved translation, siderophore transport, and iron homeostasis. Moreover, in biofilms, the expression of glycosylphosphatidylinositol (GPI)-anchored cell wall genes, potentially involved with adhesion properties, such as *IFF4*, *CSA1*, *PGA26*, and *PGA52*, was upregulated. During the intermediate to mature biofilm formation phase, the expression of some genes encoding efflux pumps, such as *RDC3*, *SNQ2*, *CDR1*, and *YHD3*, was upregulated. Similarly, in mature biofilms, the expression of two adhesin-encoding genes, *HYR3* and *ALS5,* was upregulated (Kean et al. 2018). Another recent study assessed the transcriptional profile of *C. auris* after caspofungin exposure and compared the effect on extracellular vesicles (EVs) secretion. The enriched GO terms involved cell wall biogenesis, cell cycle, oxidative stress response, and protein transport. In addition, morphological topography of yeast cells was affected after caspofungin treatment, and clumps were evidenced, suggesting a transition from yeast to hyphae as a compensatory mechanism to overcome disturbances in the cell wall. Regarding EV production, there was a shift in the content of small RNAs (Zamith-Miranda et al. 2020).

Microarray analyses were used to profile *C. neoformans* transcription profile 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*). 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 Jesus-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. 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. Moreover, the upregulation of several stress-response genes, including flavohemoglobin denitrosylase, superoxide dismutase, *HSP12*, *HSP90*, and other virulence factors were evidenced. 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).

The gene expression profile was compared among seven isolates of *C. neoformans* var. *grubii,* including the VNI and VNB lineages, comprising four clinical and three environmental isolates grown in five different *in vitro* and *in vivo* conditions (Yu et al. 2020). The conditions corresponded to synthetic media, such as YPD and a restrictive low iron medium supplement with an inductor of ROS, infection models like macrophage-like murine cells infected with yeasts, cerebrospinal fluid (CSF) obtained from intracisternal yeast-infected rabbit, and pigeon media guano, which is a niche environment in terms of nitrogen composition for VNI isolates. Genes that favor fungal virulence and survival within the host were modulated. Typically, genes involved in oxidative stress response, acquisition and reduction of iron, capsule production, glycosylation pathway, ATP-binding cassette transporters, *APP1, CXD3,* and *SRX1* were regulated. *APP1* encodes a secreted anti-phagocytic protein. Although APP1 deletion does not impair the growth, capsule, or melanin production of *C. neoformans*, it increased the dissemination of *C. neoformans* in hosts with compromised immune response, and the AP1 administration inhibited phagocytosis of fungal cells (Luberto et al. 2003). *CXD3* encodes a carboxypeptidase D that seems to participate in nitrogen metabolism and capsule formation (Frazzitta et al. 2013). The sulfiredoxin SRX1 is a virulence factor of *C. neoformans,* which also plays a protective role by counteracting the stress caused by peroxide (Upadhya et al. 2013). Notably, *in vivo* conditions highlighted the expression of metabolic and stress adaptive mechanisms. The functional enrichment of DEGs demonstrated genes involved in amino acid biosynthesis and nitrogen metabolism, cell cycle, DNA repair, stress responses, inositol phosphate metabolism, and

inositol lipid modifications. As the brain microenvironment has limited glucose availability, it is conceivable that *C. neoformans* utilize inositol as a carbon source. This study evidenced the upregulation of the expression of virulence-associated genes. Almost half of such genes consisted of capsule production genes. Moreover, DEGs are involved in sodium efflux transport (*ENA1* and *NHA1*), oligopeptide transport, and quorum sensing (*OPT1)*. The expression of stress-responsive genes (*SRE1*and *SREBP*) was upregulated. Similarly, the expression of genes involved in signaling pathways that respond to thermal stress and pH, including Bck1-Mkk2-Mpk1 and the pH-response transcription factor, *RIM101* was upregulated. Beyond that, the targeted genes of RIM101 include *CDA1* and *KRE6*, which are responsible for regulating the levels of chitosan and β-glucan synthesis, respectively, in the cells (O'Meara et al. 2013).

In a murine model of pulmonary aspergillosis, *A. fumigatus* exhibited downregulation of genes related to ribosomal biogenesis and protein biosynthesis and upregulation of approximately 150 genes related to siderophore biosynthesis and transport, including ferric-chelate reductases, amino acid 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, and several genes encoding antioxidant enzymes, including a Mn-superoxide dismutase and the bifunctional catalase-peroxidase CAT2 (Oosthuizen et al. 2011). 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, which is a toxic product generated from the degradation of the host aminoacids methione, valine, and isoleucine (McDonagh et al. 2008). Moreover, an *A. fumigatus* strain deficient in methylcitrate synthase displayed attenuated virulence (Ibrahim-Granet et al. 2008). Besides, a current study assessed the transcriptional profile of *A. fumigatus* in a model of invasive pulmonary infection by NanoString nCounter. Among the evaluated genes, the expression of 125 genes was upregulated whereas that of 85 was downregulated, representing genes potentially involved in the response to environmental cues, with an extensive list of transcription factors. Upregulated genes were involved in iron acquisition (*fre2*, *hapX*, *sidA*, *sidD, mirB*, and *sit1),* zinc uptake *(zrfC*, *zrfA*, *aspf2*, and *zafA),* and nitrogen uptake *(nrtB* and *area)*. Simultaneously, the expression of *sreA* was downregulated, whose codified product represses iron uptake and siderophore synthesis. A prominent induction of the expression of genes involved in secondary metabolism, such as *gliG*, *gliP, gliZ* (belonging to gliotoxin biosynthesis pathway), and *mtfA*, which acts in both gliotoxin and extracellular proteases synthesis, was verified. Notably, this study highlighted the role of *rlmA,* which is involved in the ability of fungus to proliferate in the lung, *ace1*, which controls gene clusters related to multiple secondary metabolites, and mycotoxin, which is paramount for full virulence (Liu et al. 2021). Conceivably, the invading hyphae in the lungs trigger neutrophil recruitment, and as a consequence, the fungus activates a stress-responsive mechanism, including the induction of *sebA, mkk2,* and *sho1*. Furthermore, 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) (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. Albeit the involvement of ROS release 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).

Transcriptional profiling was performed on the dermatophyte *A. benhamiae* during an *in vivo* skin infection in guinea pigs. During acute infection, *A. 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. 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 their function in generating short peptides and amino acid breakdown products allows 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). In addition, the crosstalk of PacC with different pathways for the maintenance of cellular homeostasis has been demonstrated. In *T. interdigitale* the regulation of *egr2* that encodes a C2H2 transcription factor involved in ion homeostasis, and *P-type ATPase* gene, putatively involved in the extrusion of Na+ and K+, is influenced by *pacC* background (da Silva et al. 2020). Moreover, the ortholog of PacC, Pac3 in *N. crassa* is involved in a myriad of processes. The responsive genes involve those that encode catalase 1 and catalase 3, cell wall protein PhiA, C6 transcription factor, calcium-transporting ATPase 3, cyclin, and ornithine N5 oxygenase (Martins et al. 2020a).

Indeed, many aspects of dermatophyte physiology were understood based on studies performed using protein sources like keratin and elastin to mimic the dermatophyte superficial and deep infection, respectively, or even human molecules such as nail fragments and skin explants (Peres et al. 2016). In this sense, a study that assessed the transcriptional profile of *T. rubrum* mycelium grown in keratin or elastin through oligonucleotide microarray displayed the modulation of a large set of proteases, with a significant upregulation of *mep4* and *lap1* genes as well as genes

encoding heat shock proteins, including Hsp 70 like-protein, Hsp 88-like protein, and Hsp 90 like-protein (Bitencourt et al. 2019a). Besides, this study showed the equal importance of lipases and keratinases for dermatophyte infection during interaction with elastin. It also revealed the modulation of a large set of genes involved in carbon and nitrogen metabolism. In this context, another study evaluating the transcriptional profile of *T. rubrum* conidia during growth in keratin and elastin revealed the modulation of genes involved in conidia dormancy. Moreover, the expression of protease genes including *lap1, lap2, sub1, sub3, sub6*, and *mep4*, as well as genes belonging to the respiratory chain and tricarboxylic cycle was primarily induced during growth in keratin. In contrast, the expression of approximately 40 genes involved in metabolic processes was downregulated in both protein sources, including genes related to nitrogen and fatty acid metabolism. This study unveiled adaptative mechanisms related to conidia survival and germination and characterized a putative adhesin potentially involved in the initial phases of dermatophyte infection (Bitencourt et al. 2016). In addition, a recent study evaluated the transcriptional profile of *T. rubrum* time-course mycelial growth in minimal medium supplement with glucose or keratin, revealing that keratin growth led to the repression of genes related to glycolysis, nitrogen catabolism, and TCA cycle, and induction of glyoxylate genes, such as *icl* (Martins et al. 2020b). This study also showed that keratin degradation is followed by an accumulation of ammonium, and as a consequence, mechanisms related to glutamine and urea metabolism are activated for ammonium utilization and extrusion.

During interaction with human keratinocytes, *A. benhamiae* upregulated the *hypA* 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 *hypA gene* increased the susceptibility of *A. benhamiae* to human neutrophils and DCs. Compared to wild type, the Δ*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 prevents *A. fumigatus* recognition by neutrophils (Aimanianda et al. 2009). Furthermore, in *T. rubrum*, the *hypA* gene is regulated by the transcription factor StuA, which belongs to the APSES family of transcription factors. In comparison to the wild type, the Δs*tuA* mutant strain displayed a significant reduction in *hypA* transcript levels during growth in keratin, and as a consequence, altered other mechanisms that ultimately influence germination, stress response, and fungal mechanosensing (Lang et al. 2020). Conceivably, the interaction with host cells and the dampening of host recognition might also be affected. Within this context, LysM-domain proteins influence fungal infection and immune response by masking fungal chitin recognition by host cells and controlling fungal growth. These proteins have garnered interest in dermatophytes due to high copies in the genome and diversification in domain organization, suggesting LysM family evolution in these pathogenic fungi (Martinez et al. 2012; Persinoti et al. 2014). Moreover, a recent work characterized LysM-domain proteins in *T. rubrum* and showed the transcriptional profile of 14 LysM-encoding genes during the growth of *T. rubrum* in host molecules. In this

study, two genes (TERG 03756 and TERG 05625) demonstrated marked changes in transcription levels during *T. rubrum* growth in keratin, displaying a signal peptide, hydrophobic region, and two LysM domains without glycosylation sites (Lopes et al. 2019).

Recently, the transcriptional profiles of HaCat keratinocyte cell line and *T. rubrum* during fungus–host interaction have been identified simultaneously. In this respect, dual RNA seq data showed the induction of the expression of glyoxylate cycle genes (malate synthase and isocitrate lyase), *erg6,* and a carboxylic acid transporter gene that probably enhances the assimilation of nutrients (Petrucelli et al. 2018). Furthermore, deletion of *hacA* impaired the hyphal development during interaction with HaCat and altered immune responses, with an increase in TNF-α secretion and a decrease in IL-8 levels (Bitencourt et al. 2020).

Transcriptome data from various human fungal pathogens have identified global responses and survival strategies during interaction with host cells and substrates. Moreover, a deeper understanding of the core in transcriptional responses obtained by analyzing the dynamic and coordinate behavior of fungi and hosts during the infection and tackling aspects of niche association and adaptation is critical for identifying vulnerabilities. Accordingly, some pathways have been implicated in mycotic diseases, fungi can 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 interaction with host cells are potentially important for virulence (Table 17.2), and the functional characterization of mutant strains has been performed for some of them. Thus, genome-wide transcriptional analyses combined with genetic approaches have provided significant insight into fungal responses, adaptive processes, virulence, and pathogenesis.

17.4 Transcriptome of Drug Response and Resistance

Microorganisms respond to sublethal doses of chemical and physical agents by synthesizing various 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. 2018). Thus, analyses of transcriptional changes in response to cytotoxic drugs have elucidated the mechanisms by which fungi adapt to physiological stress and the mechanisms of drug action (Table 17.3).

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. Polyenes, a class of antifungal drugs including amphotericin B (AMB) and nystatin, bind to ergosterol and form pores in the membrane, which leads to the

Table 17.2 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 *C. albicans, C. neoformans*, *A. fumigatus, A. benhamiae,* and *T. rubrum.* Gene inactivation attenuates virulence in *C. albicans* but not in *C. neoformans* and *A. fumigatus.*	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), Schobel et al. (2007), Lorenz and Fink (2001), Wachtler et al. (2011), and Martins et al. (2020b)
Malate synthase (glyoxylate cycle enzyme)	Upregulated in *C. albicans, C. neoformans*, *A. fumigatus,* and *A. benhamiae.* Gene inactivation does not attenuate virulence in *C. neoformans.*	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), and Cairns et al. (2010)
Acetyl-coenzyme-A-synthetase (glyoxylate cycle enzyme)	Upregulated in *C. albicans, C. neoformans,* and *A. fumigatus.* Gene inactivation attenuates virulence in *C. neoformans.*	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), and Lorenz et al. (2004)
Aconitase (tricarboxylic acid cycle enzyme)	Upregulated in *C. albicans, C. neoformans,* and *A. fumigatus*.	Lorenz et al. (2004), Walker et al. (2009), Hu et al. (2008), and Sugui et al. (2008)
Malate dehydrogenase (tricarboxylic acid cycle enzyme)	Upregulated in *C. albicans, C. neoformans,* and *A. fumigatus*.	Lorenz et al. (2004), Hu et al. (2008), Cairns et al. (2010), and Sugui et al. (2008)
Phosphofrucktokinase (glycolysis enzyme)	Upregulated in *C. albicans* and *C. neoformans*	Fradin et al. (2003), Thewes et al. (2007), and Hu et al. (2008)
Enolase (glycolysis enzyme)	Upregulated in *C. albicans* and *C. neoformans*	Fradin et al. (2003), Thewes et al. (2007), and Hu et al. (2008)
Phosphoenolpyruvate carboxykinase (gluconeogenesis enzyme)	Upregulated in *C. albicans* and *C. neoformans*. Gene inactivation attenuates virulence in *C. albicans*.	Zakikhany et al. (2007), Lorenz et al. (2004), Thewes et al. (2007), Hu et al. (2008), and Barelle et al. (2006)
Flavohemoglobin denitrosylases (RNS detoxification)	Upregulated in *C. albicans* and *C. neoformans*. Gene inactivation attenuates virulence in *C. albicans* and *C. neoformans*.	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), and Brown et al. (2009)
Superoxide dismutases (ROS detoxification)	Upregulated in *C. albicans, C. neoformans,* and *A. fumigatus*. Gene inactivation attenuates virulence in *C. albicans* and *C. neoformans* but not in *A. fumigatus.*	Hu et al. (2008), McDonagh et al. (2008), Morton et al. (2011), Fradin et al. (2003, 2005), Lorenz et al. (2004), Lambou et al. (2010), Missall et al. (2004), and Brown et al. (2009)

(continued)

Table 17.2 (continued)

Protein description	Gene expression modulation and functional analysis	References
Hydrophobin (cell surface protein)	Upregulated in *A. fumigatus, A. benhamiae,* and *T. rubrum.* Gene inactivation in *A. fumigatus* and *A. benhamiae* increases the susceptibility to the host immune response.	Cairns et al. (2010), Burmester et al. (2011), Heddergott et al. (2012), Aimanianda et al. (2009), and Lang et al. (2020)
HacA transcription factor (Unfolded protein response)	Gene inactivation in *A. fumigatus* and *T. rubrum* leads to attenuation in virulence traits and increases susceptibility to antifungal agents	Richie et al. (2009) and Bitencourt et al. (2020)
StuA transcription factor (APSES-family of the transcriptional regulators)	Upregulated in *T. rubrum*. Gene inactivation in *A. benhamiae* and *T. rubrum* impaired the growth on host molecules.	Krober et al. (2017) and Lang et al. (2020)
RlmA transcription factor (MPK1 mitogen-activated protein kinase pathway)	Upregulated in *A. fumigatus*. Gene inactivation in *A. fumigatus* decreases pathogenicity in mice.	Liu et al. (2021)

leakage of intracellular contents and fungal cell death. AMB also induces oxidative damage to cellular membranes via the generation of ROS. Some antifungal drugs target proteins involved in the ergosterol biosynthetic pathway (Martinez-Rossi et al. 2008) (Fig. 17.1). 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 14α-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), responsible for converting squalene to lanosterol. Inhibition of ERG1 decreases the production of ergosterol and increases the accumulation of squalene to toxic levels (Sagatova 2021) (Fig. 17.1).

Other antifungal drugs target DNA/RNA metabolism. Flucytosine is a cytosine analog that was first used as an antitumor agent. It also exhibits 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.

Table 17.3 Mechanism of action and mechanisms underlying antifungal resistance in fungi

Drug	Mechanism of action	Putative resistance mechanisms and drug response	References
Acriflavine	Topoisomerase inhibition/DNA intercalation. Nonspecific cellular interactions	Drug efflux, stress response, oxidative stress, decreases virulence	Fachin et al. (2006), Paiao et al. (2007), Persinoti et al. (2014), and Martinez-Rossi et al. (2016)
Amphotericin B	Binds irreversibly to ergosterol, resulting in disruption of membrane integrity	Drug efflux, stress response	Yu et al. (2007b), Martins et al. (2016), Mendes et al. (2016), and Bitencourt et al. (2019b)
Caspofungin	(1,3)-β-D-glucan synthase inhibition (encoded by FKS1/FKS2)	Mutations in FKS genes, posttranscriptional regulation of cell wall biosynthesis, stress response	Imtiaz et al. (2012), Perlin (2015), Bitencourt et al. (2019b), and Kalem et al. (2021)
Fluconazole	Cytochrome P450 14 α-lanosterol demethylase inhibition	Drug efflux, stress response, alteration of the drug target	Cervelatti et al. (2006), Fachin et al. (2006), Paiao et al. (2007), and Shapiro et al. (2011)
5-Flucytosine	DNA synthesis and nuclear division inhibition	Decreased drug uptake, alteration in enzyme activity, cell wall remodeling	Costa et al. (2015)
Griseofulvin	Mitosis inhibition	Drug efflux, stress response	Fachin et al. (1996, 2001, 2006), Cervelatti et al. (2006), Paiao et al. (2007), and Martins et al. (2016)
Imazalil	Cytochrome P450 14 α-lanosterol demethylase inhibition	Drug efflux	Cervelatti et al. (2006) and Fachin et al. (2006)
Itraconazole	Cytochrome P450 14 α-lanosterol demethylase inhibition	Drug efflux, alteration of the drug target	Cervelatti et al. (2006), Fachin et al. (2006), and Shapiro et al. (2011)
Ketoconazole	Cytochrome P450 14 α-lanosterol demethylase inhibition	Drug efflux	Cervelatti et al. (2006), Fachin et al. (2006), and Wang et al. (2021)
Terbinafine	Squalene epoxidase inhibition (encoded by Erg1)	Drug efflux, stress response, mutations in Erg1, drug metabolism	Graminha et al. (2004), Osborne et al. (2005, 2006), Rocha et al. (2006), Fachin et al. (2006), Paiao et al. (2007), Martins et al. (2016), Martinez-Rossi et al. (2016), Yamada et al. (2017), Santos et al. (2018), Petrucelli et al. (2019), and Kano (2021)

(continued)

Table 17.3 (continued)

Drug	Mechanism of action	Putative resistance mechanisms and drug response	References
Tioconazole	Cytochrome P450 14 α-lanosterol demethylase inhibition	Drug efflux	Fachin et al. (1996, 2001, 2006)
Undecanoic acid	Nonspecific cellular interactions	Stress response, drug metabolism, oxidative stress, decreases virulence	Paiao et al. (2007), Mendes et al. (2018), and Rossi et al. (2021)

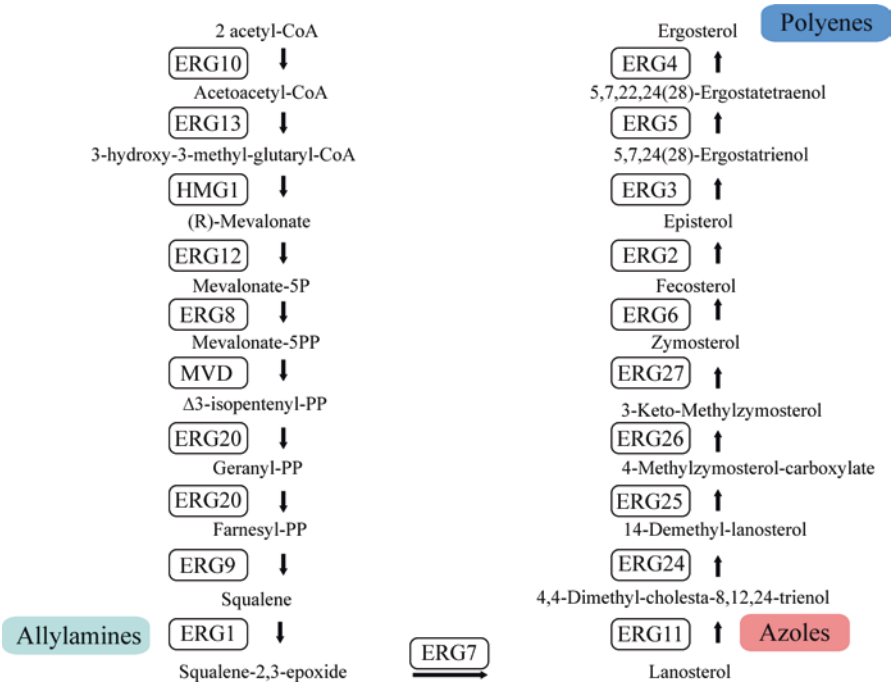

Fig. 17.1 Schematic representation of the ergosterol biosynthetic pathway

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; Billmyre et al. 2020). Griseofulvin, another antifungal drug, interacts with microtubules affecting the mitotic spindle formation, thereby inhibiting the mitosis in fungi. This drug serves as a fungistatic agent against dermatophytes. However, griseofulvin is not effective against dimorphic fungi, yeast, or chromomycosis-causing agents (Gupta et al. 2018).

The fungal cell wall is 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 synthases (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* spp., *C. neoformans*, or *Fusarium* spp. (Perlin 2015; Kalem et al. 2021).

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, undecanoic acid, and echinocandins (Yu et al. 2007b; da Silva Ferreira et al. 2006; Gautam et al. 2008; Diao et al. 2009; Zhang et al. 2009; Peres et al. 2010b; Mendes et al. 2018; Cervelatti et al. 2006; Liu et al. 2005; Paiao et al. 2007). These studies revealed that the modulation of genes in the ergosterol biosynthetic pathway varies significantly among species and drugs. 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). 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, as well as the multidrug-resistance gene encoding ABC1, which is a homolog of *C. albicans* CDR1 (Yu et al. 2007a). Transcriptome sequencing revealed that ketoconazole may also change cell membrane permeability, destroy the cell wall, and inhibit mitosis in *Microsporum canis* (Wang et al. 2021).

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*. Besides, 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 an 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 upregulated genes include those involved in purine and pyrimidine biosynthesis, such as YNK1, a nucleoside diphosphate kinase, and FUR1, an uracil phosphoribosyltransferase (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 biosyntheses, such as *erg2*, *erg4*, *erg24*, and *erg25*, and increased the expression of genes involved in lipid metabolism. 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 *mdr*2 likely causes the efflux of TRB, since deletion of *mdr*2 increased dermatophyte susceptibility to TRB (Fachin et al. 2006). Interestingly, *in T. interdigitale*, the transcription of *mdr4* was downregulated in the Δ*mdr2* mutant challenged with amphotericin B or terbinafine, indicating that the transcription of *mdr4* is dependent on the function of *mdr2* in response to these drugs. However, when the Δ*mdr2* mutant was challenged with griseofulvin, the high expression of the *mdr4* gene seemed to compensate for the inactivation of the *mdr2* gene. These results suggest that these ABC transporter genes act synergistically, and they may compensate for one another when challenged with antifungal drugs (Martins et al. 2016; Martins et al. 2019). These results also indicate the existence of a network interaction responsible for the failure of antifungal therapeutics. An intriguing mechanism of resistance to TRB in *A. nidulans* (Graminha et al. 2004) and *T. rubrum* (Santos et al. 2018) involves the *salA* gene, which encodes a salicylate 1-monooxygenase. TRB contains a naphthalene nucleus in its molecular structure that might be degraded by salicylate 1-monooxygenase, an enzyme in the naphthalene degradation pathway in *Pseudomonas* (Bosch et al. 2000).

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 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; Rosam et al. 2020). Overexpression of efflux pumps is associated with antifungal resistance in *C. albicans*. CDR1 and CDR2 confer resistance to multiple azoles, while MDR1 confers fluconazole resistance (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, dermatophytes overexpressed *mdr1* and *mdr2*, which encode ABC transporters (Cervelatti et al. 2006; Fachin et al. 2006). The zinc cluster transcription factor TAC1 regulates genes encoding the ABC transporters CDR1 and CDR2 in azole-resistant *C. albicans*, and deletion of *TAC1* gene prevented the upregulation of *cdr* genes (Coste et al. 2004). Furthermore, ChIP-chip experiments demonstrated that TAC1 directly binds to the promoter region of several genes, including CDR1, CDR2 (Liu et al. 2007).

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, 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 (Morschhauser et al. 2007). 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 and 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 (Dhamgaye et al. 2012).

The transcription profile of a *C. auris* isolate susceptible to AMB and voriconazole showed upregulation of the expression of 39 genes in response to AMB, 21 in response to voriconazole, and 14 being upregulated in response to both drugs (Munoz et al. 2018). AMB-responsive genes included those involved in arginine synthesis (ARG1/ARG3), ergosterol biosynthesis (ERG24), fatty acid metabolism (FAS1/FAS2), GPI-linked surface proteins (PGA7 and RBT5), and iron transporters, such as SIT1. In response to both drugs, there was an upregulation of the expression of transmembrane transport and iron transport-related genes, such as the high-affinity iron transporter FTH1, ferric reductase, glucose transporter, N-acetylglucosamine transporter, and OP1-like oligopeptide transporter. In a *C. auris*-resistant strain to AMB and voriconazole, AMB-responsive genes were enriched in translation and transcription processes and the sterol biosynthetic pathway. Voriconazole response induced the expression of genes related to RNA processing and transcription. Furthermore, both strains induced the expression of the genes *ARG1*, *CSA1*, *MET15*, and OPT1-like transporter in response to AMB. The authors also showed an intrinsic expression profile of polyene resistance genes, such as D-xylulose reductase, phosphoenolpyruvate carboxykinase, and several transporters and stress response genes in the resistant strain (Munoz et al. 2018).

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 susceptibly to fluconazole even in resistant clinical isolates. Transcriptional analysis was performed on the Δ*goa1* and Δ*ndh51* mutant strains, which are associated with CI-induced susceptibility to fluconazole. GOA1 is required for the function of the electron transport chain, and the Δ*goa1* 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*goa1* mutant (Sun et al. 2013). NDH51 is conserved among eukaryotes, including mammals; nevertheless, GOA1 is conserved only in some *Candida* species. Therefore, fungi-specific mitochondrial genes may be targets for the development of novel antifungal drugs. Indeed, acriflavine, an acridine derivative with 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). Transcriptomic analysis of the effect of acriflavine on *T. rubrum* showed that the expression of genes involved in cellular detoxification was upregulated, protecting the cell against oxidative stress and reactive oxygen species. Furthermore, this drug interferes with the establishment and maintenance of the fungal infection (Persinoti et al. 2014).

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 HPS90 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, leading to cell death. The development of an inhibitor selective for fungal HSP90 and inactive against human HSP90 has been challenging. Nevertheless, HSP90 is a promising target for the treatment of resistant fungal diseases and may combat the emergence of drug resistance (Cowen 2009; Martinez-Rossi et al. 2016). Additionally, chemical inhibition of Hsp90 of *T. rubrum* increased the susceptibility to itraconazole and micafungin and decreased its ability to grow on human nail fragments. These results suggest the role of Hsp90 in the pathogenicity and drug susceptibility in *T. rubrum* (Jacob et al. 2015), reinforcing its potential as a target for the treatment of fungal infections.

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. To understand their mechanisms of resistance, mature biofilm 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). 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 the structural organization of biofilms (Gibbons et al. 2012). The complex gene network involved in biofilm formation is consistent with the fact that *C. albicans* can form biofilms in different niches, such as the bloodstream, oral cavity, or medical devices serving as reservoirs of drug-resistant cells (Mamouei et al. 2021; Li et al. 2021). This highlights the challenge inherent to treating these infections as well as the importance of searching for new antifungal targets.

Furthermore, posttranscriptional regulation has been described as a fine adjustment for some fungi to adapt to antifungal exposure. This phenomenon occurs in *N. crassa* through alternative splicing of pre-mRNA transcripts of genes encoding asparagine synthetase 2, C6-zinc-finger regulator, and farnesyltransferase in response to amphotericin B and ketoconazole (Mendes et al. 2016). Additionally, RNA-Seq analysis of *T. rubrum* exposed to undecanoic acid revealed alternative splicing in several genes, including *hsp*s (Mendes et al. 2018; Neves-da-Rocha et al. 2019). These results show the complexity of the metabolic modulation triggered by antifungal signaling.

In conclusion, analyses of the transcriptional changes in response to cytotoxic drugs have identified genes with known biological functions, suggesting novel effects of antifungal drugs. Besides, some of the drug-responsive genes are shared across multiple classes of antifungal agents in *C. albicans* (Liu et al. 2005), dermatophytes (Peres et al. 2010b; Persinoti et al. 2014; Mendes et al. 2018; Fachin et al. 2006; Paiao et al. 2007), and other fungi. Nonspecific responses to stress are also known that allow fungi to adapt to several drugs and environmental challenges, highlighting the broad range of fungal responses to cope with stress.

17.5 Concluding Remarks

The pathogenesis of fungal infections involves gene expression changes and metabolic pathways, which enable fungal invasion, survival, and dissemination. 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 the interaction. It has provided insights into the adaptive responses that occur during the establishment of infection, antifungal resistance, and exposure. The combination of large-scale transcriptomic analysis and systems biology approaches has enabled the development of regulatory molecular models that can aid in the assessment of dynamic behaviors 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 the knockout strains under *in vitro*, *ex vivo*, and *in vivo conditions*. Furthermore, transcriptomics is a valuable source of data on gene expression regulation, gene structure and function, and 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.

Acknowledgments This work was supported by grants from Brazilian funding agencies FAPESP (Grant No. 2019/22596-9), National Council for Scientific and Technological Development (CNPq) (Grants No. 305797/2017-4 and 304989/2017-7); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Finance Code 001; and Fundação de Apoio ao Ensino, Pesquisa e Assistência (FAEPA).

FAPESP supported the postdoctoral fellowships to N.P. (2009/08411-4), T.B. (2015/23435-8), G.P. (2012/22232-8 and 2013/19195-6), and E.L. (2011/08424-9).

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