Chapter 15
Molecular Mechanisms of Fungal Adaptive Evolution

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1 Introduction

Traditionally, fungi refer to the group of heterotrophic eukaryotes with thick cell walls made of chitin and cellulose, containing mitochondria but not chloroplasts. With the broad application of DNA sequencing in the systematics and taxonomy of cellular organisms over the last two decades, our understanding of what a fungus is has changed significantly and continues to be refined and debated. While most fungi as revealed based on DNA sequencing still have the traditional structural and genetic traits, some of the "old fungi" such as the structurally similar myxomycetes (slime molds) and oomycetes (water molds that include the agent responsible for the Irish Potato Famine) are no longer considered true fungi (Kirk et al. 2008; Alexopoulos et al. 1996). Because some scientists studying slime molds and oomycetes still identify them as mycologists, when appropriate, we will include examples of these organisms in this chapter. Conversely, many newly discovered fungi exhibit variations in cell wall structures and in whether they contain functional mitochondrial genomes. The emergence of these novel fungal lineages discovered through genomic and metagenomic sequencing has significantly influenced not only our understanding of the evolutionary history of fungi but also their potential novel mechanisms for survival, reproduction, and adaptation (Xu 2016).

Fungal ecological niches are very diverse and include not only those commonly associated with humans and human activities such as agricultural soil, forests, fresh

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and marine water, indoor and outdoor air, households, and workplaces but also niches in extreme environments such as deserts, deep-sea sediments, hydrothermal vents, and areas with high salt concentrations or ionizing radiations (e.g., Dadachova et al. 2007; Raghukumar and Raghukumar 1998; Le Calvez et al. 2009). Indeed, the applications of genomic and metagenomic tools to analyze fungi, especially fungal extremophiles, are bringing researches on fungal adaptation into an exciting new era (Xu 2016).

Though traditionally studied in Botany departments instead of Zoology departments, evolutionarily, the true fungi (Eumycota) are more closely related to animals than to plants. Collectively, fungi share a fundamental feature with animals in that they are unable to photosynthesize but acquire their carbohydrates by absorbing dissolved energy-rich organic molecules. This method of nutrient acquisition means that wherever energy-rich organic compounds are present, there is a possibility that some fungi may live there. This method of nutrient acquisition is also found in water molds (the oomycetes), a group of filamentous protists previously considered as fungi. However, unlike true fungi, the oomycetes lack chitin in their cell walls but contain a mixture of cellulosic compounds and glycan.

Having an efficient means to obtain nutrients is essential for the survival and reproduction of any organism, including fungi. However, fungi differ from animals in several aspects that can impact their nutrient acquisition, survival, and reproduction. In animals, the food is ingested either actively or passively before being digested to release the absorbable forms that are further taken up by cells. In contrast, fungi typically obtain their food by secreting digestive enzymes into their environments to degrade complex organic compounds into monomeric units such as monosaccharides and amino acids before they can be absorbed. Thus, the fungal nutrient-acquisition pathway is more similar to those of heterotrophic prokaryotes than to animals, and the feast or famine condition is likely the norm for most environmental fungi. Another major difference between animals and fungi is in their dispersal abilities. In animals, the somatic body is typically mobile, allowing the animals actively forage for nutrients. In contrast, fungi are typically not mobile except by hyphal growth or spore dispersal. The limited mobility of fungal hyphae means that each fungus often develops specific adaptations to the dominant, local ecological niche that the fungus occupies.

Ecologically, fungi can be broadly classified into four types based on their interaction patterns with other organisms: (a) saprophytes where the fungi feed on dead organic matter and interactions with other living organisms are not required components of their regular life cycle in nature; (b) mutualists where the fungi form mutually beneficial symbiotic relationships with other organisms such as plants, animals (including humans), and other microbes (including other fungi), obtaining organic compounds from and contributing nutrients to their interacting partners; (c) commensals where the fungi benefit from being associated with a biotic partner (plants, animals, and other microbes) for nutrient access while exerting little or no detrimental effect to the partner; and (d) parasites where the fungi benefit from the interaction at the expense of their interacting partners. While some of the above associations are obligate, many are loose, with the fungal partner capable of living in

more than one type of ecological niches. Furthermore, in these diverse situations, the ecological conditions can vary widely in their physical/chemical/biological parameters. Aside from growing in most of the "normal" ecological niches, fungal niches also include those with extreme temperatures, high and low water activities, high salinity, extreme pH values, antifungal drugs, lack of oxygen, biotic competitors, and host defense mechanisms (for fungal parasites) (e.g., Brem and Lips 2008). While the fundamental issue is the same for the fungi (i.e., to obtain sufficient nutrients to survive and reproduce), living in these extreme environments would require the fungi to have specific adaptations for each of the extreme factors.

Their ubiquitous distributions and abilities to digest a variety of organic compounds mean that ecologically, fungi play essential roles in nutrient cycling, environmental protection, plant and animal health, and human welfare such as issues relating to food security and infectious diseases. Thus, understanding the factors involved in their adaptations will have tremendous implications for managing these fungi in many fields including the conservation and sustainable use of biodiversity, ecological monitoring, and the prevention and control of fungal pathogens, including human fungal pathogens.

Compared to plants and animals, fungi are often considered simple. However, fungi exhibit great diversities not only ecologically as mentioned above but also in morphology and life cycles (Kirk et al. 2008; Alexopoulos et al. 1996). Morphologically, fungi can range from microscopic single-celled yeasts to filamentous molds and macroscopic multicellular mushrooms. Some fungi can switch morphological forms depending on environmental condition or in response to specific environmental cues. Reproductively, they can propagate both asexually and/or sexually in a variety of structures such as conidiophores and mushroom fruiting bodies, often producing sexual and/or asexual spores that can survive extreme environmental stresses and disperse long distances (Kirk et al. 2008; Alexopoulos et al. 1996; Xu 2005). Indeed, sporulation in fungi represents a major adaptation in the face of environmental adversity, and fungal spores are among the most stress-resistant biological forms.

In this chapter, we first review the basic concepts of adaptation and adaptive evolution as well as the approaches that have been used to study fungal adaptation and adaptative evolution. This is then followed by the specific examples on how fungi adapt to several environmental stresses, including extreme temperatures, drought/low water activity, antifungal drugs, and host defense mechanisms. We draw examples from all four major ecological groups (saprophytes, commensals, mutualists, and parasites), with a special emphasis on the *Basidiomycota* and *Ascomycota*, the two dominant phyla in the fungal kingdom (Kirk et al. 2008; Alexopoulos et al. 1996; Xu 2005, 2016).

2 Adaptive Evolution and Approaches to Study Them in Fungi

In biological studies, the term "adaptation" typically refers to genetic and/or physiological changes leading to a population's increased survival and/or reproduction in a particular environment. The population's genetic changes could be due to the changing frequency of existing alleles and/or the emergence of new alleles through mutations. Genetic mutations are the ultimate sources of phenotypic variations we see among organisms within and among species in Earth's biosphere. Mutations can occur every time DNA replicates and transcribes, with the rate related to adjacent base pairs, transcription levels, replication timing, chromatin states, meiotic crossover rates, and GC content (Chen et al. 2017). Those mutations that cause protein sequence changes can influence protein's structure and function, potentially contributing to organisms' survival and reproduction in the specific environment. If the particular environmental pressure persists, this can lead to fixation of the allele(s) for that trait in the population. In contrast to genetic changes that can permanently change the genetic makeup of the population, physiological adaptations due to epigenetic modifications are temporary and may include DNA methylation/demethylation, increased/decreased expression of specific genes, alternative splicing of messenger RNA transcript(s), and modification of proteins through phosphorylation/dephosphorylation and/or acetylation/deacetylation.

While adaptation refers to genetic and/or physiological changes that benefit the population's survival and reproduction, evolution is generally a neutral term and commonly defined as the change in allele frequencies in a population over time. Such allelic frequency changes are produced by mutation, genetic drift, migration, and selection. Consequently, several factors can influence the direction and speed of allele frequency changes in a population, including mutation rate, population size, gene flow, mating system, and the types and intensities of selection pressure. Selection pressure is often classified into three categories: natural selection, sexual selection, and artificial selection imposed by humans, either intentionally or unintentionally. These allelic frequency changes may be beneficial, neutral, or even (slightly) deleterious. In this chapter, we refer the genetic changes associated with increased survival and reproduction in a population over time as adaptive evolution. Some of these changes may be historical, happened in the distant past but are present in all individuals of a population/species but absent in other populations of the same species or sister species.

To fully understand the mechanism(s) of the adaptation of a fungus to a specific environmental factor, several approaches and methods may be necessary (Singh et al. 2012). Below we briefly describe the general approaches that have been used to elucidate the potential mechanisms underlying fungal adaptations in a diversity of traits. The applications of these specific methods for understanding fungal adaptations will be further described in Sects. 3, 4, 5, 6, and 7 using specific examples.

For evolutionary and population biologists, one common approach to investigate the molecular mechanism of adaptation is to compare the genotypes and phenotypes

among strains/species that have adapted to different environments. Depending on the research objectives, the specific investigative methods may differ. For example, if the study is on comparing strains and populations within an individual species, a sufficient number of strains that represent the different phenotypes will be needed. These strains and populations are then subjected to genotyping at candidate loci or even at the whole-genome level. The relationships between genotypes and phenotypes can be statistically investigated to determine whether certain genetic polymorphisms are associated with specific phenotypic values. In humans, genome-wide association studies have helped reveal many candidate genes or genomic regions associated with diseases. While this approach can be applied to any organism, it is especially useful for those where genetic crosses and genetic manipulations can't be performed due to ethical and/or biological constraints.

For organisms where experimental crosses and genetic manipulations can be performed, both the forward and reverse genetics approaches could be applied to understand the genetic basis of phenotypic variations and adaptation. Briefly, the forward genetics (or a forward genetic screen) is an approach used to identify genes (or sets of genes) responsible for a particular phenotype of an organism. This approach typically involves crossing individuals with divergent phenotypic trait values, obtaining and genotyping sexual offspring, constructing a genetic linkage map, and identifying the genetic marker(s) or genomic region(s) that co-segregate with phenotypic trait values (Vogan et al. 2016). Depending on the genetic basis of a specific trait, a large progeny population (e.g., hundreds to thousands) may be required to identify the specific gene(s) controlling the phenotypic trait. In contrast, reverse genetics (or a reverse genetic screen) analyzes the phenotype of an organism following the disruption or targeted modification of a known gene. The CRISPR-cas9 system has significantly facilitated the reverse genetic screening in a diversity of organisms, including fungi (Krappmann 2017).

For organisms that can be grown in lab conditions, there is another approach for investigating the genetic basis of adaptation. In this approach, the investigator can directly create experimentally adapted populations from non-adapted ones and compare the genes and genomes between the derived strains and the ancestral strain(s) (Kohn and Anderson 2014). Using this approach to investigate fungal adaptation, the fungal isolates are typically placed in a specific environmental condition (e.g., high temperature or high drug concentration environment) to clonally select for mutants capable of growing in such an environment. Because of their known and extremely close genetic relatedness between the ancestral and evolved strains, any novel genetic or genomic differences between the original non-adapted and the derived adapted strains would represent candidate loci potentially contributing to their phenotypic differences.

In recent years, comparative transcriptomics, proteomics, and metabolomics have become an increasingly common approach for investigating the differences between fungal strains grown under different stress conditions and to help identify the genes, proteins, and metabolites involved in stress response. The increasing availability and affordability of whole-genome sequencing services make this approach extremely attractive for organisms with relatively small genomes and fast rates of reproduction

under laboratory conditions (e.g., prokaryotes and fungi). Below we provide examples on our understanding of the genetic bases related to fungal adaptation to extreme temperatures, low water activity, antifungal drugs, and host immune system attacks.

3 Adaptation to High and Low Temperatures

While most known fungi grow at temperatures from 15 to 35 °C, some have adapted to high-temperature habitats (e.g., human bodies and compost) and others have adapted to low-temperature habitats (e.g., glaciers and snow). Fungi growing in such diverse temperatures have evolved different mechanisms of adaptation. Below we separately summarize our current knowledge with regard to molecular mechanisms of fungal adaptation to high and low temperatures (Table 15.1).

The heat-shock (HS) response is a common mechanism that organisms use in their adaptation to elevated temperatures. Elevated temperatures can cause substantial changes in the composition of cellular membranes, proteins, and soluble carbohydrates. To protect the cellular macromolecules, thermophilic organisms have evolved mechanisms of persistent thermotolerance. During thermal stress response, the synthesis of heat-shock proteins (HSPs) and their elevated expressions have been well documented in multiple fungi. At present, five major families of HSPs are recognized: HSP100, HSP90, HSP70, HSP60, and small HSP (sHSP), and these proteins are broadly distributed in many fungi. However, the number of HSP proteins and the relative importance of each HSP family in stress tolerance can vary among organisms. For example, sHSPs are important in the acquisition of thermotolerance in the ectomycorrhizal fungus *Pisolithus* sp. under thermal stress (Ferreira et al. 2005), while HSP60 orchestrates the adaptation of the human pathogenic fungus *Histoplasma capsulatum* to high-temperature stress (Guimaraes et al. 2011).

Aside from HSPs, other genes and proteins have also been reported as associated with cold and heat-shock responses and that the responses can differ among fungi. For example, using 2D gel protein electrophoresis, Tesei et al. (2012) found that rock-inhabiting black fungi likely used a different strategy to cope with nonoptimal temperature compared with the cosmopolitan and mesophilic hyphomycete *Penicillium chrysogenum*. In this study, three black rock-inhabiting fungi were chosen, *Exophiala jeanselmei*, *Coniosporium perforans*, and *Friedmanniomyces endolithicus*, and they were incubated at different temperatures, ranging from 1 °C, 15 °C, and 28 °C to 40 °C. 2D protein gel electrophoresis patterns revealed that *P. chrysogenum* expressed the highest number of proteins at 40 °C, whereas when exposed to temperatures far above their growth optimum, black fungi decreased the number of expressed proteins, suggesting a downregulation of their metabolism and the lack of a heat-shock response at the protein level. In contrast, at the low temperature of 1 °C, there was an increased number of expressed proteins in all fungi, with the exception of *P. chrysogenum*. At present, the specific genes and proteins that cause such expression differences are not known.

Table 15.1 Examples of molecular mechanisms of fungal adaptation to extreme environments

Environmental stress	Molecular mechanisms	Reference
High temperature	Increased transcription of heat-shock genes	Steen et al. (2002)
	Elevated expression of heat-shock proteins	Ferreira et al. (2005), Guimaraes et al. (2011)
	Decreased expression of nonessential proteins	Tesei et al. (2012)
	Increased trehalose concentration	Oberson et al. (1999)
	More saturated fatty acids	Oberson et al. (1999)
Low temperature	Expansion of gene families coding for antifreeze proteins	Hu et al. (2013)
	Decreased sterol and glycolipids	Tereshina and Memorskaya (2005)
	Increased non-saturated fatty acids	Tereshina and Memorskaya (2005)
	Increased glycerol	Tereshina and Memorskaya (2005)
	Increased arabitol and trehalose	Tereshina and Memorskaya (2005)
	Increased metabolites from TCA cycles	Tsuji (2016)
	Expression of antifreeze proteins	Hoshino et al. (2009)
	Cytoskeleton rearrangements	Blasi et al. (2015)
	Increased expression of nonessential proteins	Tesei et al. (2012)
Drought/low water activity	Increased intracellular concentration of glycerol and erythritol	Pettersson and Leong (2001)
	Production of walleminol and walleminone	Jancic et al. (2016a, b)
	Loss of gene clusters involved in secondary metabolite production	Leong et al. (2015)
	Increased expression of genes involved in fatty acid oxidation and the glyoxylate cycle	Singh et al. (2005)
	Increased catalase expression	Franca et al. (2005)
	Increased autophagy	Ratnakumar et al. (2011)
	Mycelial compartmentalization and interconnectivity	Guhr et al. (2015)
	Overall metabolic modulation	Wang et al. (2015)
Antifungal drug—flucytosine	Mutations in genes Fcy1, Fcy2, and Fur1 that either render the cell unable to take flucytosine up or unable to convert flucytosine to its toxic form	Espinel-Ingroff (2008)
Antifungal drug—azoles	Synthesis of alternative sterols	Pemán et al. (2009)
	Elevated expression of the target gene ERG11/CYP51	Pemán et al. (2009)
	Mutation in the target gene ERG11/CYP51	Pemán et al. (2009)
	Increased expression of efflux pumps	Pemán et al. (2009)

(continued)

Table 15.1 (continued)

Environmental stress	Molecular mechanisms	Reference
Antifungal drug—echinocandins	Mutations in FKS1 and FKS2, the target genes of echinocandins	Perlin (2007)
Antifungal drug—polyenes	Low ergosterol content	Pemán et al. (2009)
	Defects in genes involved in ergosterol biosynthesis	Pemán et al. (2009)
	Enhanced catalase activity	Pemán et al. (2009), Blum et al. (2008)
Plant host defense	Effectors	Dong et al. (2015), Kemen et al. (2011)
	Plant cell-wall degrading enzymes	Ma et al. (2017)
Animal host defense	Chromatin remodeling	O'Meara et al. (2010)
	Hydrolytic enzymes such as extracellular proteases	Ortiz-Urquiza and Keyhani (2013)
	Adhesins and specialized adhesive structures	Ortiz-Urquiza and Keyhani (2013)
	Metabolites facilitating infection	Ortiz-Urquiza and Keyhani (2013)
	Acquisition of host genes involved in immune response	Wang et al. (2016)
	Reduction/expansion of gene families	Wichadakul et al. (2015)
	Melanin and capsule	Alspaugh (2015)
Anoxia	Expression of genes involved in sterol and glycerol transport	Snoek and Steensma (2007)
	Genes for posttranslational fucosylation	Youssef et al. (2013)
	Lack of mitochondria but presence of hydrogenosome	Youssef et al. (2013)
	Expansion of gene families coding for glycoside hydrolases	Youssef et al. (2013)
Heavy metal	Increased concentration of intracellular malondialdehyde, intracellular thiol, and proline	Mukherjee et al. (2010)
	Increased ROS scavenging ability	Mukherjee et al. (2010)
High salt	Expression of the pentose phosphate pathway	Kashyap et al. (2016)
	Elevated expression of heat-shock proteins	Kashyap et al. (2016)
	Increased HOG expression	Padamsee et al. (2012)
	Increased expression of Na^+ efflux proteins	Ma et al. (2015)
Domestication	Selective enrichment of specific genes related to human-created environments	Xiao et al. (2016)
	Expansion of gene families related to substrate transport and utilization	Ropars et al. (2015)
UV irradiation	Melanin pigment	Wang and Casadevall (1994)

Aside from changes at the protein level, changes in metabolites have been intensively investigated for their roles in acquired thermotolerance in fungi (i.e., thermotolerance acquired after being exposed to HS). The accumulation of trehalose (a membrane-stabilizing cytosolic carbohydrate) and changes in membrane composition are often observed and that differences may be observed among closely related fungi. For example, a comparative study of the response to HS between two closely related fungi, the mesophilic *Chaetomium brasiliense* and thermophilic *Chaetomium thermophilum* var. *thermophilum*, revealed that fatty acids of the thermophilic fungus were more saturated than those of the mesophilic fungus. However, under optimal conditions, both fungi synthesized comparable amounts of trehalose, and in response to HS, both fungi increased similar amounts of trehalose (Oberson et al. 1999). In a different study of two thermophilic fungi *Rhizomucor tauricus* and *Myceliophthora thermophila*, in response to HS, the proportions of phosphatidic acids and sterols increased, while the amounts of phosphatidylcholines and phosphatidylethanolamines decreased (Ianutsevich et al. 2016). However, there was no increase in the degree of fatty acid saturation in the major phospholipids under HS in these two species. Furthermore, these two fungi did not show any "acquired" thermotolerance as a result of the HS probably due to their inability to further increase the synthesis of trehalose, already at 8–10% of dry weight at their optimal growth temperatures (Ianutsevich et al. 2016).

Similar to those observed at HS, changes in the profiles of proteins, lipids, carbohydrates, and other metabolites have also been observed in fungal response to cold stress. For example, changes in membrane lipids were observed during the adaptation of the white-rot fungus *Flammulina velutipes* to hypothermia (5 to −5 °C) in natural environments (Tereshina and Memorskaya 2005). Specifically, the levels of sterols and glycolipids decreased, and the proportion of phospholipids with a high degree of non-saturation (2.2) increased. Similarly, glycerol, known to have antifreeze properties, accumulated in the cell cytosol along with arabitol and trehalose (Tereshina and Memorskaya 2005). By analyzing two strains of the Antarctic basidiomycetous yeast *Mrakia blollopis* that exhibited distinct growth characteristics under subzero conditions, Tsuji (2016) found that these two strains also showed different cold adaptation mechanisms. In response to cold shock, strain SK-4, which grew well under subzero temperatures, accumulated high levels of TCA-cycle metabolites as well as lactic acid, aromatic amino acids, and polyamines. In contrast, in strain TKG1-2, which did not grow as efficiently under subzero temperatures, cold stress strongly induced the TCA cycle, but other metabolites did not show significantly increased accumulation within the cells (Tsuji 2016). These results suggest that closely related species and even different strains within the same species can have very different metabolic responses to cold stresses.

For fungi that are historically adapted to cold environments, their genetic and physiological features associated with cold adaptation have also been investigated and reviewed (e.g., Hoshino et al. 2009). Different strategies have been found in different taxa of snow molds. For example, basidiomycetous snow molds produce extracellular antifreeze proteins to keep the immediate extracellular environment ice-free. However, the psychrophilic ascomycete *Sclerotinia borealis* does not

produce extracellular antifreeze proteins but instead increased its osmotic stress tolerance in order to grow at subzero temperatures. Like most psychrophilic fungi, *S. borealis* grows faster under cold/frozen conditions than under normal unfrozen conditions.

Aside from differences in cold-resistant mechanisms between strains and species, the cell's developmental states can also exert effects on the types of physiological response to low temperatures. For example, Cheawchanlertfa et al. (2011) showed that after being similarly acclimated to low temperatures, mycelia and phenethyl alcohol-induced yeast cultures of the dimorphic fungus *Mucor rouxii* had different fatty acid profiles due to different fatty acid desaturations through cooperative upregulation of the desaturase genes.

In addition to the above-described physiological mechanisms, results from genomic, transcriptomic, and proteomic analyses are also increasing our understanding on fungal adaptation to high/low temperatures. For example, Hu et al. (2013) found that the caterpillar fungus *Ophiocordyceps sinensis*, which is endemic to high altitudes on the Tibetan Plateau and adapted to extreme cold, had enriched gene families encoding putative antifreeze proteins and mechanisms for increasing lipid accumulation and fatty acid unsaturation. In the opportunistic pathogen *Cryptococcus neoformans* that causes meningitis in humans and other animals, growth at a lower temperature (25 °C) increased transcript levels for histone-encoding genes, indicating a general influence of temperature on chromatin structure. At a higher temperature of 37 °C, there were elevated transcript levels for several genes encoding heat-shock proteins and translation machinery (Steen et al. 2002). Transcriptome analysis of the pathogenic oomycete *Pythium insidiosum*, which can infect both humans and animals, revealed a total of 1074 genes either significantly upregulated (625 genes) or downregulated (449 genes) at body temperature (37 °C), in comparison with those grown at room temperature (28 °C) (Krajaejun et al. 2014). These 1074 genes can be divided into 309 gene product groups, with the biggest group consisting of 408 genes. Murata et al. (2006) studied the genome-wide transcriptional response in *Saccharomyces cerevisiae* in the presence of a cold shock at 4 °C. They found that genes related to energy production, metabolism, cell rescue, defense, and virulence were upregulated, while those related to protein synthesis were generally downregulated. In the truffle fungus *Tuber melanosporum*, compared to those at 25 °C, a total of 423 genes were differentially expressed (>2.5-fold; P value <0.05) when the mycelia were exposed to cold (7 days at 4 °C) (Zampieri et al. 2011). Among these 423 genes, 187 were upregulated, while 236 were downregulated. Sixty-six and fifty-one percent, respectively, of the up- or downregulated transcripts had no KOG classification.

Through an integrated analysis using genomic, transcriptomic, and proteomic tools, Su et al. (2016) analyzed the mechanisms of both cold adaptation and inability to grow at above 20 °C in the obligate psychrophilic fungus *Mrakia psychrophila*. They found that several strategies used by *M. psychrophila* are shared with other psychrophiles, including the upregulation at 4 °C of desaturase and glycerol 3-phosphate dehydrogenase, which are involved in biosynthesis of unsaturated fatty acid and glycerol, respectively. The lack of growth of the fungus at above

20 °C was at least partially due to the accumulation of unfolded proteins in the endoplasmic reticulum. However, differences with other psychrophiles were also observed, including codon usage bias and alternative splicing events that were unique to *M. psychrophila*. Codon usage bias and alternative splicing events might contribute to the cold adaptation of *M. psychrophila*.

Blasi et al. (2015) presented the functional analysis of the transcriptional response of the black fungus *Exophiala dermatitidis*, a human pathogen, at 1 °C, 37 °C, and 45 °C at two different exposure time points. At 1 °C, *E. dermatitidis* activated several mechanisms to acclimatize, such as lipid membrane fluidization, trehalose production, or cytoskeleton rearrangement, and allowed the fungus to remain metabolically active. At 45 °C, the fungus drifts into a replicative state and increases the activity of the Golgi apparatus. In addition to expression differences in protein-coding genes, this study also found differential expressions in noncoding RNAs, circular RNAs, as well as fusion transcripts among the temperature treatments, suggesting potentially novel mechanisms involved in low- and high-temperature adaptations.

In the model microbial eukaryote *Neurospora crassa*, Ellison et al. (2011) discovered two cryptic and recently diverged populations, one in the tropical Caribbean and the other endemic to subtropical Louisiana, USA. Comparison of the genomes of the two populations revealed two "islands of differentiation." The subtropical Louisiana population has a higher fitness at low temperature (10 °C), and several of the genes within these distinct regions have functions related to low-temperature adaptation. These results suggest the divergent genomic islands may be the result of local adaptation to the 9 °C temperature difference in the average yearly minimum temperature between these two populations.

4 Adaptation to Drought/Low Water Activity

Drought is a common phenomenon in nature. In microbial ecology, including fungal ecology, drought typically refers to an environment with an insufficient water activity to sustain microbial growth. Quantitatively, water activity (a_w) is defined as the ratio between the partial vapor pressure of water in a substance (e.g., soil) over the partial vapor pressure of pure water, everything else being equal. Water activity or the availability of usable water is one of the most significant variables for fungi in natural ecosystems. In typical terrestrial environments, seasonal, monthly, or even daily changes of water activity are common, often with short periods of high a_w interspersed with long periods of low a_w, including complete desiccation in certain environments such as the desserts under midday sun. While the presence of water typically facilitates the growths of fungi, the absence of water can elicit a diversity of physiological and/or life cycle responses. Thus, adaptation to low water activity, including desiccation, represents a common type of physiological response in fungi. Indeed, in combination with other factors, low water activity often leads to vegetative growth arrests and the initiation of sexual reproduction in fungi. Below we

briefly describe the molecular mechanisms of fungi living under drought stress (Table 15.1).

Fungi can be divided into two groups based on their growth abilities around water activity of 0.85 a_w: those that can't grow at or below this water activity are called xerophobic fungi and those that can grow are commonly called xerophilic fungi. Under low water activity condition, xerophilic fungi can synthesize compatible solutes (e.g., sugar alcohols, especially glycerol and erythritol) to balance the internal water activity with the outside and enable their enzyme systems to function (Pettersson and Leong 2001). As a group widely spread on the fungal tree of life, xerophiles are extremely important in the spoilage of many processed foods and stored commodities and in indoor environments (Micheluz et al. 2015; Oetari et al. 2016; Jancic et al. 2016a, b; Skrinjar et al. 2012; Vytrasova et al. 2002). Some xerophiles have a preference for salt or sugar substrates, whereas other species can be isolated from both jam and salterns. The most xerophilic fungi include *Xeromyces bisporus*, *Aspergillus penicillioides*, and *Wallemia* species. The latter species also produce secondary metabolites (e.g., walleminol, walleminone) which may enhance their competitive advantages in environments where there is insufficient water but rich sugar or salt (Jancic et al. 2016a, b). Several other genera such as *Eurotium* and *Penicillium* also include xerophilic species.

Among the xerophilic fungi, *Xeromyces bisporus* is regarded as the most xerophilic to date, capable of growing on sugary substrates down to an extremely low a_w of 0.61 (Leong et al. 2011). Genome sequencing of the fungus revealed the apparent loss of all gene clusters to produce secondary metabolites, key molecules for competition, and interaction with other organisms. Transcriptomes at optimal (approximate to 0.89) versus low a_w (0.68) revealed differential expression of only a few stress-related genes; among these, certain (not all) steps for glycerol synthesis were upregulated (Leong et al. 2015).

Singh et al. (2005) analyzed the transcriptional response of the budding yeast *Saccharomyces cerevisiae* to desiccation and rehydration under glucose-limiting conditions. They found that expression of genes involved in fatty acid oxidation and the glyoxylate cycle increased during drying and remained in this state during the rehydration phase. Franca et al. (2005) found that cytoplasmic catalase of *S. cerevisiae* plays a role in the maintenance of the intracellular redox balance during dehydration and, therefore, in tolerance against a water stress.

Saccharomyces cerevisiae is more desiccation tolerant during stationary phase (one in five cells surviving desiccation) than exponential phase (only one in a million cells) (Calahan et al. 2011). Welch et al. (2013) exploited the desiccation sensitivity of exponentially dividing cells to understand the stresses imposed by desiccation and their stress response pathways. They found that a transient heat shock induced a 5000-fold increase in desiccation tolerance in desiccation-sensitive, exponential-phase cells, whereas hyper-ionic, -reductive, -oxidative, or -osmotic stresses induced much less. They provided evidence that the Sch9p-regulated branch of the TOR (target of rapamycin) and Ras-cAMP signaling pathway inhibited desiccation tolerance by inhibiting Gis1p, Msn2p, and Msn4p (transcription factors critical for heat stress response) and by activating Sfp1p (a ribosome biogenesis transcription factor).

Among the 41 mutants defective in ribosome biogenesis, a subset defective in the 60S subunit showed a dramatic increase in desiccation tolerance independent of growth rate. Their results suggest that reduction of a specific intermediate in 60S biogenesis, resulting from conditions such as heat shock and nutrient deprivation, increases desiccation tolerance.

Another survival analysis of a mixture of approximately 4800 mutant strains of *S. cerevisiae* subjected to desiccation, each deleted for a different nonessential gene, suggested that about 653 genes (constituting about 14% of nonessential genes in the yeast and about 10% of its genome) may be important for desiccation tolerance of cells growing after diauxic shift (Ratnakumar et al. 2011). Desiccation of yeast cells in the post-diauxic phase of growth induced changes in transcription of 12% (814 genes) of the yeast genome, activating expression of 484 genes (7%) and downregulating 330 (5%). Autophagy processes were significantly overrepresented, indicating the importance of the clearance of protein aggregates/damaged organelles and the recycling of nutrients for the survival of desiccation in yeast (Ratnakumar et al. 2011).

Lichens are well known to survive severe drought conditions (Sancho et al. 2007). In drought resistance, the mycobiont partner in lichens seems to be the main contributor (Zhang and Wei 2011), probably due to the fact that within lichens, the photobionts are housed by the fungal cells, providing protection for their photosynthetic partners from drought environments. Comparative transcriptome analysis of the lichen-forming fungus *Endocarpon pusillum* showed that a total of 1781 genes were differentially expressed between samples cultured under normal and PEG (polyethylene glycol)-induced drought stress conditions. Among these 1781 genes, 1004 were significantly upregulated, while 777 were downregulated. A large number of differentially expressed genes were classified into metabolism process, which suggests that *E. pusillum* had active metabolism under PEG-induced drought stress. This phenomenon is different from several other drought-resistant organisms, where metabolic processes were largely suppressed during desiccation including PEG-induced stress and suggests that *E. pusillum* is intrinsically adapted to water limitations and drought (Wang et al. 2015).

The desiccation of upper soil horizons is a common phenomenon, leading to a decrease in soil microbial activity and mineralization. In general, fungal communities and fungal-based food webs are less sensitive and more resilient to soil desiccation than bacterial communities and bacterial-based food webs (de Vries et al. 2012; Six 2012). Part of the reason for the greater resilience of fungal communities might be related to the presence of mycelial networks where the loss of water in part of the colony could be compensated for by interconnected mycelia in another part of the colony. Indeed, a recent study on a saprotrophic fungus *Agaricus bisporus* demonstrates that hydraulic redistribution can partly compensate water deficiency if water is available in other zones of the mycelia network (Guhr et al. 2015).

5 Adaptation to Antifungal Drugs

In natural environments such as soil, antibiotic compounds are produced by a diversity of microbes. These antibiotics can inhibit the growth of other microbes and help the producers gain an advantage in their competition against others for nutrients. In return, the nonproducing microbes can develop resistant mechanisms to overcome their competitive disadvantage. Different microbes may produce different compounds or use different strategies against others. Indeed, such arms races among microbes have long existed and are widespread in natural environments (Pawlowski et al. 2016). However, it was not until the last 50 years that antibiotic resistance started attracting worldwide attention. According to data from the World Health Organization, antibiotic resistance is among the most urgent issues facing public health in the world.

While most of our attention on antibiotic resistance has focused on bacteria, there is increasing indication that antifungal drug resistance is also on the rise, threatening the continued use of many of the existing antifungal drugs (Pfaller 2012). Compounding this problem is the increasing spectrum of fungal pathogens capable of causing diseases in humans (Köhler et al. 2015). Among the diversities of human fungal pathogens, two yeast species *Candida glabrata* and *Candida krusei* are causing increasing proportions of invasive fungal infections. These two species are intrinsically more resistant than others to two common groups of antifungal drugs, the triazoles and amphotericin B (Pfaller 2012). Similarly, over the last 10 years, multiple triazole-resistant strains of *Aspergillus fumigatus* have been reported from many countries, including the Netherlands, Britain, India, China, and the USA (Ashu et al. 2017). Infections caused by drug-resistant strains are typically associated with high morbidity and mortality. In order to help control the rapid emergence of antifungal resistance, it's essential to understand the mechanisms of resistance. Below we focus on the main types of antifungal drugs and summarize the main resistance mechanisms (Table 15.1). Most of the molecular mechanisms of resistance described below were identified by comparing drug-susceptible and drug-resistant isolates from the same patients over the course of infection time using genetic, transcriptomic, proteomic, and/or metabolomic approaches. However, a number of studies have also employed QTL mapping of progeny from genetic crosses and laboratory experimental evolution analyses, followed by genome comparisons (e.g., Vogan et al. 2016; Kohn and Anderson, 2014).

There are four major groups of systemic antifungal drugs used in clinics: (a) antimetabolites such as flucytosine, (b) azoles such as fluconazole and voriconazole, (c) echinocandins such as caspofungin and micafungin, and (d) polyenes such as amphotericin B and nystatin.

Flucytosine is a pyrimidine analog that can inhibit the synthesis of DNA and RNA, causing cell death. The molecular mechanisms of flucytosine resistance among fungal pathogens are commonly associated with mutations in three genes (Espinel-Ingroff 2008). The first is the Fcy2 gene, encoding the purine-cytosine permease that is also responsible for the uptake of flucytosine into the cell. The

second is the Fcy1 gene, encoding the cytosine deaminase enzyme responsible for converting flucytosine to 5-flurouracil. The third is the Fur1 gene, encoding the kinase responsible for converting 5-flurouracil to 5-flurouridine monophosphate. Mutations in these three genes can either render the cell unable to take flucytosine up or unable to convert flucytosine to its toxic form inside the cells, resulting in flucytosine resistance.

The second group of antifungal drugs is the azoles. Azole drugs inhibit fungal growth by interfering with the biosynthesis of ergosterol, the key fungal cell membrane sterol. The specific target of azole drugs is the enzyme lanosterol-14-α-demethylase encoded by ERG11 or CYP51, and the binding of azole drugs to this enzyme leads to failed conversion of lanosterol into ergosterol, resulting in reduced ergosterol content, altered membrane structure and functions, and ultimately inability to grow. The high solubility and low side effects of azole drugs on humans make these drugs the first-line antibiotic for treating most of the invasive fungal infection. However, like many other categories of antibiotics, the use of azoles has also resulted in frequent azole resistance among fungal pathogens. Based on our current understanding, the mechanisms of resistance can be grouped into four major types; however, more than one mechanism may be present in a given resistant strain, and these mutations can act either additively or synergistically to enhance the strains' resistance level (Pemán et al. 2009; Oliver et al. 2005; Kohn and Anderson 2014). Briefly, the first major type of azole resistance mechanism involves the development of alternate pathways and the synthesis of alternative sterols to negate the membrane-disruptive effects of azole drugs on the depletion of ergosterol. The second common azole resistance mechanism is through elevated expression of the target enzyme. The third major mechanism involves the induction of efflux pumps that reduce intracellular drug concentration. The dominant efflux pumps involved in azole resistance are those encoded by either the major facilitator gene MDR1 or the ATP-binding cassette transporter genes CDR1 and CDR2. It should be noted that the genes and their specific involvements might differ among species and azole drugs. For example, upregulation of the MDR1 gene is predominantly related to fluconazole resistance, while the upregulation of CDR1 and to a lesser extent the CDR2 gene is commonly found in strains resistant to multiple triazoles (Pemán et al. 2009; Oliver et al. 2005). The fourth type of azole resistance mechanism is due to non-synonymous substitutions in the gene encoding for the target enzyme (ERG11 or CYP51). A diversity of mutations have been found, and such mutations can either completely eliminate or reduce the drugs' affinity with the enzymes and maintain its enzymatic activity in the presence of the drugs (Pemán et al. 2009; Chang et al. 2016; Oliver et al. 2005; Vogan et al. 2016).

The third major class of antifungal drugs is the echinocandins represented by anidulafungin, caspofungin, and micafungin. These drugs target the 1,3-β-D-glucan synthetase, a fungal-specific enzyme involved in the synthesis of the fungal cell wall. The inhibiting of cell wall synthesis by echinocandins leads to cell rupture and/or aberrant hyphal growth. As with the azoles and flucytosine, reduced susceptibility to echinocandins has been observed in several *Candida* species, including *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. dubliniensis*. The dominant resistant mechanisms

have been linked to point mutations in the presumed catalytic domains of genes FKS1 and FKS2, the genes encoding the major subunits of 1,3-β-D-glucan synthase (Perlin 2007). However, different from azole resistance mechanisms, overexpression of the MDR and CDR genes seemed to have little effect on the susceptibility of fungal pathogens to echinocandins, suggesting that echinocandins are probably not good substrates for these efflux pumps (Pemán et al. 2009).

The fourth major group of clinically important systemic antifungal drugs is the polyenes, including nystatin and amphotericin B. Polyenes inhibit fungal growth by intercalating with each other and with membrane ergosterol to form aqueous pores, leading to leakage of cytoplasmic materials. Though resistance to amphotericin B is low, the analyses of target strains have found generally lower levels of ergosterol in cell membrane and the presence of alternative, probably less effective sterols to maintain fungal growths. Indeed, defects in genes (e.g., *ERG3*, *ERG6*, and *ERG11*) involved in ergosterol biosynthesis are among the major mechanisms of amphotericin B resistance in human fungal pathogens (Pfaller 2012; Pemán et al. 2009). It should be noted that in such strains, due to the absence or limitation of their preferred sterol (i.e., the ergosterol), their growths are typically impaired compared to the wild-type strains in the absence of amphotericin B. In addition, aside from intercalating with ergosterol, amphotericin B can also cause oxidative damage to cell membrane through generating reactive oxygen species. Thus, fungal strains with high levels of resistance to amphotericin B typically also have enhanced catalase activity to counter the oxidative stress. Indeed, in *Aspergillus terreus*, amphotericin B resistance was not due to reduced ergosterol content but to significantly upregulated catalase activity (Blum et al. 2008).

As shown above, the development of antifungal resistance is predominantly through mutation of existing genes and is quite different from those in bacterial pathogens where antibiotics resistance genes are often acquired by horizontal gene transfers. However, the impacts of the emergence of drug resistance on our healthcare systems are very similar among microbial pathogens, from viruses to bacteria, protozoa, and fungi. Understanding the mechanisms of resistance and their epidemiological patterns will have significant practical implications in helping develop rapid diagnosis and targeted treatments for patients with fungal infections.

6 Adaptation to Host Defenses

Fungi are common pathogens of plants and animals, including humans. However, both plants and animals possess a diversity of protective and defense mechanisms. In order for pathogenic fungi to successfully colonize and invade host tissues, they need specific adaptations to overcome such host defenses. Below we highlight a few examples in this broad area (Table 15.1).

As one of the major sources of organic compounds, plant materials, both living and dead, are among the favored substrates for fungal growth. Indeed, each year, fungal pathogens cause tens of billions dollars worth of crop loss. In order for fungal

pathogens to cause plant diseases, the pathogens have to colonize and invade plant tissues and overcome plant defense mechanisms. Effectors are specific types of proteins secreted into plants by phytopathogens to interfere with plant defenses. By applying genomic and transcriptomic analytical tools, 134 candidate effectors were identified in the rice blast pathogen *Magnaporthe oryzae*; overexpression of two effectors, Iug6 and Iug9, suppressed defense-related gene expression in rice (Dong et al. 2015). Studies on the obligate biotroph white rust pathogen (*Albugo laibachii*, Oomycota) of *Arabidopsis* found biotrophy also requires "effectors" to suppress host defense. Two effectors, RXLR and Crinkler, in *A. laibachii* were found to be shared with other oomycetes. In addition, a novel class of effectors that share a CHXC motif within 50 amino acids of the signal peptide cleavage site was discovered and experimentally verified (Kemen et al. 2011).

A recent study by Ma et al. (2017) revealed that during the soybean-oomycete pathogen *Phytophthora sojae* interaction, an intriguing molecular war takes place at the plant's extracellular space, the apoplast. In this interaction, the pathogen releases the virulence factor, the apoplastic xyloglucan-specific endoglucanase PsXEG1. The soybean counters by producing a PsXEG1 inhibitor, GmGIP1, that binds to PsXEG1 and abolishes its function. However, the pathogen also secretes a PsXEG1-like protein, PsXLP1, as a decoy. PsXLP1 does not have an enzymatic activity but has a highly binding affinity to GmGIP1 than PsXEG1, thus freeing PsXEG1 to degrade host plant tissue and release nutrients for their growth. Similar to the RXLR and Crinkler effectors, homologs of the PsXEG1 and PsXLP1 pair are widely distributed in *Phytophthora* species.

The basidiomycete yeasts *Cryptococcus neoformans* species complex and *Cryptococcus gattii* species complex are a group of opportunistic human and other animal pathogens (Kwon-Chung et al. 2017). They are broadly distributed in nature, including soil, bird excreta, and plant debris. Through inhalation of propagules from the environment by the host, *C. neoformans* can survive and replicate within macrophages in vivo. Two cellular components are critically for *C. neoformans*' survival within host, capsule and melanin, and many genes have been found influencing their syntheses and production (Steenbergen et al. 2001; Alspaugh 2015). To better understand the origin of *C. neoformans* virulence, Derengowski et al. (2013) compared the transcriptional profiles of *C. neoformans* 6 h after phagocytosis by the amoeba *Acanthamoeba castellanii* and by murine macrophages. The results revealed 656 and 293 genes in *C. neoformans* ingested by amoebae and macrophages, respectively, whose expression changed at least twofold relative to non-phagocytosed cells. Despite their differences on the number of modulated genes, the overall categorization of the protein-coding genes revealed a very similar gene expression profile of the fungus inside either phagocyte. Genes related to nutrient transport, general metabolism, and oxidative stress response showed increased expression, while genes involved in transcription, translation, and ergosterol biosynthesis were suppressed. Overall, these results are consistent with the view that cryptococcal virulence for mammals originated from fungus-protozoan interactions in the environment. Another study in *C. neoformans* demonstrates that chromatin remodeling by the conserved histone acetyltransferase Gcn5 is important

in regulating the expression of specific genes that allow *C. neoformans* to respond appropriately to the human host (O'Meara et al. 2010).

Entomogenous fungi are able to parasitize susceptible hosts via direct penetration of the cuticle with the initial and potentially determining interaction occurring between the fungal spore and the insect epicuticle. Entomogenous fungi have evolved mechanisms for adhesion and recognition of host surface cues that help direct an adaptive response that includes the production of: (a) hydrolytic, assimilatory, and/or detoxifying enzymes including lipase/esterases, catalases, cytochrome P450s, proteases, and chitinases; (b) specialized infectious structures, e.g., appressoria or penetrant tubes; and (c) secondary and other metabolites that facilitate infection (Ortiz-Urquiza and Keyhani 2013).

Wang et al. (2016) sequenced the genome of *Zancudomyces culisetae*, formerly known as *Smittium culisetae*. The species has been shown to benefit the in vivo development of infested mosquito larvae under specific conditions (Horn and Lichtwardt 1981). In contrast, *Z. culisetae* can also lead to the death of mosquito larvae, in situations where the host's hindgut becomes overgrown with this fungus (Williams 2001). An insect-like polyubiquitin chain was encoded by the fungus. Ubiquitin and ubiquitin-like proteins are universally involved in protein degradation and regulation of immune response in eukaryotic organisms. Multiple lines of evidence support this polyubiquitin gene in *Z. culisetae* was obtained via a horizontal gene transfer event from the host. The acquired polyubiquitin gene in *Z. culisetae* may be useful during the invasive processes of the fungus, to induce the hosts' ubiquitin-proteasome systems by labeling and degrading host cell membrane proteins. *Z. culisetae* may also use it as a defense against bacteria, viruses, or other microbes that coexist in the insect guts, whether for its own competitive advantage or as an ally of the host.

Wichadakul et al. (2015) sequenced the genome of *Ophiocordyceps polyrhachis-furcata*, a species in the *Ophiocordyceps unilateralis* species complex specialized in colonizing the ant *Polyrhachis furcata*, and performed a comparative genomic analysis of insect fungi. They found evidence for genome contractions for species with narrow host ranges. Interestingly, the sizes of several gene families, including cuticle-degrading genes (proteases, carbohydrate esterases) and some families of pathogen-host interaction (PHI) genes, were reduced for specialized obligate fungal parasites. However, two gene families also showed evidence of expansions: (1) the genes involved in the production of bacteria-like toxins in *O. polyrhachis-furcata*, compared with other entomopathogenic fungi, and (2) retrotransposable elements. The loss of various genes involved in the pathogenesis for *O. unilateralis* would result in a reduced capacity to exploit larger ranges of hosts and therefore in the different level of host specificity. In contrast, the expansions of other gene families suggest an adaptation to particular environments with unexpected strategies like oral toxicity to its host insects, through the production of bacteria-like toxins, or sophisticated mechanisms underlying pathogenicity mediated by genes within or mobilized by retrotransposons.

7 Molecular Mechanisms of Adaptation to Other Stressors

Aside from our understanding of fungal adaptations to stressors mentioned above, studies have also analyzed the molecular mechanisms of fungal adaptations to other environmental factors, including anoxic environments, heavy metal contaminations, high-salt condition, and human domestications. Below we briefly review examples of our current understanding of these adaptations (Table 15.1).

Anoxia The ascomycete yeast *Saccharomyces cerevisiae* is a model eukaryote for research and is commonly used by the baking and fermentation industry. This unicellular fungus can grow under both aerobic and anaerobic conditions. Our understanding of fungal adaptation to anaerobic environments has mainly come from research conducted using this yeast. Different molecules have to be transported into and out of the cell using pathways not commonly expressed under aerobic conditions (Snoek and Steensma 2007). In *S. cerevisiae*, this adaptation is mainly controlled at the transcriptional level. About 500 genes showed differential expression when transcriptomes from aerobic and anaerobic cultures are compared (ter Linde et al. 1999; Kwast et al. 2002; Tai et al. 2005). Among these, 23 genes were expressed only under anaerobic conditions. Apart from *ARV1* (functioning in sterol metabolism/transport), *NPT1* (nicotinate phosphoribosyl transferase), and *GUP1* (glycerol transporter), the 20 other genes have no obvious function in anaerobic metabolism (Snoek and Steensma 2007). In addition, posttranscriptional regulation also contributed to the adaptation of *S. cerevisiae* to anaerobic growth (Bruckmann et al. 2009).

Anaerobic gut fungi represent a distinct early-branching fungal phylum (Neocallimastigomycota) and reside in the rumen, hindgut, and feces of ruminant and nonruminant herbivores. Youssef et al. (2013) sequenced the genome of an anaerobic fungal isolate, *Orpinomyces* sp. strain C1A. Comparative genomic analysis identified multiple genes and pathways that are absent in Dikarya genomes but present in early-branching fungal lineages and/or nonfungal Opisthokonta. These included genes for posttranslational fucosylation, the production of specific intramembrane proteases and extracellular protease inhibitors, the formation of a complete axoneme and intraflagellar trafficking machinery, and a near-complete focal adhesion machinery. The mitochondrial reductive evolution to a hydrogenosome, the apparent replacement of ergosterol with tetrahymanol in the cell membrane, and the sole dependence on a mixed-acid fermentation pathway for pyruvate metabolism and energy production in strain C1A are clear adaptations to anaerobiosis. The development of cellulosomes and the acquisition of many glycoside hydrolases could be viewed as an adaptation to improve the access, speed, and efficacy of biomass degradation.

Heavy Metals The toxicity of heavy metals to microorganisms has attracted considerable research attention in recent years. Mukherjee et al. (2010) reported an *Aspergillus niger* strain that was able to thrive even in a medium with 100 mg/L arsenate, an unusually high concentration for most other living organisms to survive.

To understand the possible cellular strategy toward tolerance of arsenate-induced toxicity, the responses evoked to counter arsenate toxicity were analyzed by assaying alterations of certain enzymes and several biomolecules. MDA (malondialdehyde), intracellular thiol, and proline contents increased up to a certain level. Activities of GR (glutathione reductase), SOD (superoxide dismutase), and CAT (catalase) declined following a rise at low concentrations; SDH (succinate dehydrogenase) activity decreased gradually with increased arsenate stress. These results showed that cells of *A. niger* are equipped with an elaborate network of antioxidative enzymes, which are involved in scavenging ROS (reactive oxygen species) and other oxidative products generated by arsenate insult. By sequencing cDNA libraries of the aquatic fungus *Blastocladiella emersonii* submitted to heat shock and cadmium stress, Georg et al. (2009) found that environmental stresses, particularly cadmium treatment, inhibit intron processing (2.9% ESTs containing introns from stress library vs. 0.2% from the unstressed library), revealing a new adaptive response to cellular exposure to this heavy metal.

High-Salt Environments Kashyap et al. (2016) isolated a *Penicilliopsis clavariiformis* culture AP from mangrove in India. The fungus is salt tolerant, being able to tolerate up to 10% (w/v) NaCl. To understand the mechanism of adaptation to high salinity, activities of the key enzymes regulating glycolysis, pentose phosphate pathway, and tricarboxylic acid cycle were investigated under normal (0% NaCl) and saline stress environment (10% NaCl). The results revealed a rerouting of carbon metabolism away from glycolysis to the pentose phosphate pathway, a common pathway related to saline stress tolerance in fungi. In addition, several other genes such as *Hsp98*, *Hsp60*, *HTB*, and *RHO* were significantly upregulated under saline stress, suggesting that they likely play significant roles under such conditions.

The ability to tolerate environments with reduced water activity and high salt concentrations are rare in Basidiomycota. However, species of the basidiomycetous genus *Wallemia*, most commonly found as food contaminants, have been isolated from hypersaline environments. The diverse habitats from which strains of *Wallemia sebi* have been isolated (e.g., jam, dried fish, marine sponges, and house dust) suggest that it can adjust its physiology to adapt to different environments. Recently, the genome of *W. sebi* was sequenced in order to understand its adaptations for surviving in osmotically challenging environments. *W. sebi* has a compact genome (9.8 Mb), with few repeats and the largest fraction of genes with functional domains compared with other Basidiomycota. In silico analyses identified 93 putative osmotic stress proteins; homology searches showed the HOG (high-osmolarity glycerol) pathway to be mostly conserved. Despite the highly reduced genome size, several gene family expansions (esp., HSP20, Dabb, and AA_trans) and a high number of transporters (549) were found that also provide clues to the ability of *W. sebi* to colonize harsh environments (Padamsee et al. 2012).

In fungi, the gene encoding ENA ATPase (ENA is from "exitus natru: exit of sodium") is phylogenetically broadly distributed, and this enzyme plays a central role in Na^+ efflux and Na^+ tolerance. Like in all cellular organisms, the K^+ and Na^+

concentrations within fungal cells are strictly regulated to maintain constant concentrations, relatively high for K^+ and low for Na^+. However, in high saline environments, the high Na^+ concentrations outside of the cell will create a high Na^+ influx and elevate the intracellular Na^+ concentration. To counter such an influx, Na^+ effluxes need to be activated. Indeed, Ma et al. (2015) demonstrated that the deletion of the ENA ATPase gene from the entomopathogenic fungus *Metarhizium acridum* (Δ*MaENA1*) resulted in reduced tolerance to high salt concentration. In addition, the deletion strain was also less tolerant to other stressors such as heat and UV radiation than its wild-type counterpart. Transcriptome profiling showed a large number of differentially expressed genes between the WT and Δ*MaENA1* strains, including 6 cytochrome P450 superfamily genes, 35 oxidoreductase genes, 24 ion-binding genes, 7 DNA repair genes, and 8 genes involved in 5 stress response pathways (the Ras-cAMP PKA pathway, the RIM101 pathway, the Ca^{2+}/calmodulin pathway, the TOR pathway, and the HOG/Spcl/Styl/JNK pathway). These results are consistent with *MaENA1* playing a very important role in the adaptation and survival of this entomopathogenic fungi in stressful conditions.

Comparative genomics was conducted to analyze the genomes of eight *Aspergillus* spp. (*A. nidulans*, *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. oryzae*, *A. terreus*, and *Neosartorya fischeri*) to identify stress response proteins. All genomes harbored elements of the SskA-HogA/SakA stress signaling pathway, suggesting the importance of SskA-HogA/SakA signaling in different types of stress responses (e.g., responses to osmotic, oxidative, starvation, and even heat stress in germinating conidia) in the aspergilli. The abundance of annotated histidine kinases, MAPKs (HogA/SakA, MpkC), response regulators (two SskAs in *A. flavus*), and transcriptional regulators, e.g., AtfA, AtfB, NapA (AfYap1), MsnA, and RpdA (two orthologues in *A. flavus*), may be indicative of a complex and robust stress defense system controlled by a high-complexity regulatory network in these filamentous fungi (Miskei et al. 2009).

Melanin as a Common Stress Response Factor Melanin is a pigment produced by laccase, a phenoloxydase enzyme. Several experiments suggest that melanin serves a protective role against a variety of harmful stimuli in a diversity of fungi. For example, melanin can protect *Cryptococcus neoformans* against ultraviolet light (Wang and Casadevall 1994), suggesting a role in protection against solar radiation. Melanized *C. neoformans* cells are usually less susceptible to antimicrobial drugs and oxidant agents; in addition they are able to trick the host immune system by inactivating the drugs normally used on therapeutics (Mauch et al. 2013). Melanization also affects susceptibility of *C. neoformans* to heat and cold, with melanized cells being less susceptible than non-melanized cells (Rosas and Casadevall 1997).

Domestication Some fungi were domesticated to produce useful products. Domestication is an excellent model for studies of adaptation because it involves recent and strong selection on a few identified traits. By comparing the genomes of 10 *Penicillium* species, Ropars et al. (2015) reported that adaptation to cheese was associated with multiple recent horizontal transfers of large genomic regions carrying crucial metabolic genes. They identified seven horizontally transferred regions (HTRs)

spanning more than 10 kb each, flanked by specific transposable elements, and displaying nearly 100% identity between distant *Penicillium* species. Two HTRs carried genes with functions involved in the utilization of cheese nutrients or competition and were found nearly identical in multiple strains and species of cheese-associated *Penicillium* fungi, indicating recent selective sweeps; they were experimentally associated with faster growth and greater competitiveness on cheese and contained genes highly expressed in the early stage of cheese maturation.

The effect of human domestication on genomic variation was also revealed in the cultivated edible mushroom *Lentinula edodes*. In the study by Xiao et al. (2016), they compared the genome sequences of 39 wild and 21 cultivated strains and identified three distinct genetic groups in the Chinese *L. edodes* population with the majority of the cultivated strains in one genetic cluster. Interestingly, this genetic cluster had enriched non-synonymous nucleotide substitutions in genes related to stress response and in fruiting body formations. These genes include those encoding a protein kinase Pbs2-like MAPKK protein, a cofactor (DnaJ) of heat-shock protein HSP70, a zinc-finger DNA binding protein PriB correlated with mushroom fruiting, a cyclopropane fatty acid synthase that triggers reproductive shift from vegetative to sexual reproduction, and a DEAD-box ATP-dependent RNA helicase related to cold stress response.

8 Conclusions and Perspectives

The fungal kingdom is phylogenetically very ancient and ecologically broadly distributed. Fungi can survive and reproduce in a diversity of environments and have evolved a variety of mechanisms to cope with environmental stresses. In this chapter, we selectively reviewed the molecular mechanisms of fungal adaptation to several common stresses such as extreme temperatures, desiccation, antifungal drugs, host defenses, and osmotic stress such as high salt concentrations. Our surveys identified that some of the genes and pathways are involved in responses to multiple stresses (e.g., the efflux pumps such as the ENA ATPase), and others may be unique to a specific stressor (e.g., a mutation in a drug target). While significant progresses have been made over the last couple of decades, much remains unknown. Increasingly, comparative genomics have been used to identify the genomic differences among species living under different environments to infer the potential signatures of adaptation. Similarly, information from transcriptome profiling for the same species/strains but under different conditions is used to infer the regulatory mechanisms of a variety of adaptations. Together, such data are generating abundant hypotheses for further experimental tests using targeted approaches.

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