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Regulatory Circuitry Governing Fungal Development, Drug Resistance, and Disease

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INTRODUCTION

The remarkably diverse fungal kingdom boasts a collection of spectacularly successful decomposers, symbionts, pathogens, and parasites. Fungi have indispensable roles as the earth's preeminent degraders of organic matter, as domesticated industrial manufacturers of food and antibiotics, and as the best-characterized eukaryotic model systems for scientific and biomedical research. Of the estimated 1.5 million species of fungi (215, 216), only approximately 150 are able to cause disease in mammals, and even fewer are commonly observed clinical pathogens (300, 453). It has been proposed that virulence in fungi may have evolved as a result of selection pressures imposed by environmental predators, including amoebae, slime molds, and worms (95, 553–555). Phylogenetic analyses revealed that pathogenic fungi are not distinctly clustered from other fungal species and suggested that numerous evolutionary transitions to pathogenic lifestyles have occurred (249). Accordingly, pathogenic fungi exploit a variety of niches and hosts and exhibit diverse strategies of pathogenesis.

The three predominant pathogenic fungi of humans, *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*, have evolved a diverse repertoire of strategies to survive in hostile host environments and thrive when the host's immune system is compromised, causing life-threatening disease. These strategies rely on complex signaling cascades that pair environmental sensing with critical cellular responses. Recent studies have revealed that the circuitry mediating morphogenetic responses to host factors such as temperature and serum share common components with circuitry mediating responses to cellular stress exerted by antifungal drugs. In light of that, this review emphasizes recent work that implicates many of the same regulators in both fungal development and drug resistance. This review discusses the core circuitry governing fungal morphogenetic programs and responses to drug-induced stress and the impact of perturbing this circuitry on fungal virulence. We emphasize the conservation and divergence among these

three pathogenic fungi with regard to regulatory circuitries controlling development, drug resistance, and disease.

WHAT IS ANTIFUNGAL DRUG RESISTANCE?

Since a major focus of this review is the regulatory circuitry that governs fungal drug resistance, this introductory section will provide a brief overview of key concepts for an understanding of drug resistance and will summarize the mechanisms of action of antifungal drugs used in the clinic. Microbial pathogens are a leading cause of human mortality worldwide, at least in part due to their ability to thwart therapeutic regimens by rapidly evolving resistance to antimicrobials. Eukaryotic pathogens such as fungi pose a particular therapeutic challenge since they share a close evolutionary relationship with their human hosts, minimizing the number of drug targets that can be exploited to selectively kill the pathogen. The cost to the health care system of treating invasive fungal infections is estimated to exceed $2.6 billion annually in the United States alone (632). This is partially due to the increasing frequency of fungal infections worldwide in recent decades. Fungi have emerged as a major cause of human disease, especially in immunocompromised individuals, such that the number of acquired fungal bloodstream infections has increased by 207% (456, 457). However, the number of antifungal drugs with novel targets developed over the past few decades has been limited to one class: the echinocandins. The fact that the emergence of antifungal drug resistance dramatically outpaces the development of new antifungal compounds emphasizes the importance of an understanding of the evolutionary mechanisms that govern the development of resistance (111).

Drug resistance can be defined in distinct ways from clinical and laboratory perspectives. From a clinical perspective, drug resistance is the persistence or progression of an infection despite appropriate drug therapy (112, 623). In a laboratory setting, drug resistance is quantified by using an MIC assay, in which the growth of the pathogen is measured with a series of drug concentrations over a defined period of time according to standard protocols (106). The lowest concentration of a drug that inhibits growth, generally by either 50% or 90%, is defined as the MIC. Although MIC assays provide a measure of how the pathogen will respond to drug treatment, they are not always accurate predictors of the response *in vivo* or even of how the resistant pathogen will compete against a drug-sensitive counterpart *in vitro* (110, 114). MIC assays do not account for how pharmacokinetics, host-pathogen interactions, microbial interactions, and environmental factors affect drug resistance and clinical outcomes. Furthermore, MIC assays do not distinguish cidal from static drug activity. Different fungal species often show distinct intrinsic levels of drug resistance, and within a species, specific resistance mechanisms can be acquired to confer resistance (13, 111, 120, 623). Even in the absence of specific resistance mechanisms, species and strains differ in their capacities to survive and reproduce during drug exposure, independent of changes in the MIC, often referred to as drug tolerance (111, 120). Tolerance can enable the evolution of drug resistance, as it allows a population of proliferating cells to respond to selection imposed by the drug.

Monitoring the evolution of drug resistance in real time can be accomplished by two main experimental approaches. The first approach examines fungal populations collected from a patient undergoing antifungal treatment over time. The strength of this approach is the clinical relevance, in that it enables the identification of mutations that accumulate in a pathogen under drug selection *in vivo* (13). The major limitation is that population dynamics cannot easily be monitored or controlled, such that there can be quiescent reservoirs of the pathogens, infection with multiple genotypes, and variable selection pressure across anatomical sites and over time; furthermore, parameters such as mutation rate, recombination rate, intensity of selection, and population size cannot be controlled, and therefore, their influence on the evolution of resistance can only be inferred (13, 112). The second approach monitors the evolution of drug resistance in artificial populations in real time. The advantages of this method are that experimental parameters can be controlled and experiments can be replicated. A limitation is the clinical relevance of these populations that have evolved under simplified laboratory conditions. However, studies of *C. albicans* have shown that the mechanisms of resistance arising in experimental populations are also found in clinical isolates that evolved resistance in a human host (116). Thus, both experimental approaches have important and distinct roles in dissecting the molecular mechanisms that contribute to the development of fungal drug resistance.

MECHANISM OF ACTION OF ANTIFUNGAL DRUGS

To minimize host toxicity, antifungal drugs must act upon targets that are not well conserved between fungi and their human hosts. The vast majority of the antifungal drugs in clinical use target ergosterol in the fungal cell membrane, the biosynthesis of ergosterol, or the biosynthesis of (1,3)-β-D-glucan, a major component of the fungal cell wall (Fig. 1). Ergosterol is the functional fungal analogue of cholesterol in mammalian cells and functions by modulating membrane fluidity and integrity and the function of many membrane-bound enzymes (623). The majority of antifungal drugs in clinical use target ergosterol or its biosynthesis and thus exploit the distinct plasma membrane composition of fungal cells. Fungal cell walls are rigid structures consisting of (1,3)-β-D-glucans covalently linked to (1,6)-β-D-glucans and chitin. These polymers form hydrogen bonds between adjacent polysaccharide chains to generate a tough three-dimensional network of microfibrils (143). Although fungi are eukaryotes, the cell wall is not shared by mammalian cells and therefore provides another target for antifungal drugs.

Azoles

The azoles, including both imidazoles and triazoles, are a class of five-membered, nitrogen-containing, heterocyclic compounds that have been the most widely deployed group of antifungals for approximately 2 decades (120, 441). Currently, there are four triazole drugs available for clinical use, fluconazole, itraconazole, voriconazole, and posaconazole, each with its own pharmacokinetic properties. The azoles function by targeting the ergosterol biosynthetic enzyme lanosterol demethylase (also referred to as cytochrome P450), encoded by *ERG11* in *C. albicans* and *C. neoformans* and by *cyp51A* and *cyp51B* in *A. fumigatus.* In many fungal species, they enter the fungal cell by facilitated diffusion (363) and act through an unhindered nitrogen atom in the azole ring, which binds to an iron atom in the heme group located in the active site of Erg11

Structure **Mechanism of Action**

A. Azoles

Fluconazole

Voriconazole

Posaconazole

Ergosterol

Toxic Sterol

Membrane Stress

Ergosterol

Erg11
Cyp51A/
Cyp51B

Erg3

Azole

Erg11

Erg3

Erg3

Toxic
Sterol

B. Polyenes

Amphotericin B

Ergosterol

Polyene-Ergosterol
Complex

C. Echinocandins

Micafungin

Caspofungin

Fks

(1,3) β-D-glucan synthase

Fks

Echinocandin

Cell Wall Stress

(623). This inhibits the activation of oxygen, which is necessary for the demethylation of lanosterol, causing a block in the production of ergosterol and the accumulation of 14-α-methyl-3,6-diol, a toxic sterol produced by the Δ-5,6-desaturase encoded by *ERG3* (347). This toxic sterol exerts severe membrane stress on the cell (Fig. 1A). Recently, it was also shown that the azoles impair the function of vacuolar membrane H^+ ATPases, thereby disrupting cation homeostasis within the cell and providing a mechanistic insight into the cellular consequences of ergosterol depletion (647). The azoles generally act in a fungistatic manner against yeasts, including *Candida* species, and in a fungicidal manner against molds, such as *Aspergillus* species (120). The fungistatic nature of azoles toward *Candida* imposes strong directional selection on surviving populations to evolve drug resistance (13, 120).

Polyenes

The fungicidal polyenes are amphipathic drugs, having both hydrophobic and hydrophilic sides, that function by binding strongly to ergosterol to create drug-lipid complexes, which intercalate into the fungal cell membrane to form a membrane-spanning channel (Fig. 1B) (441). This causes cellular ions, such as potassium ions, to leak out of the cell, thereby destroying the proton gradient (211, 441). The polyene amphotericin B is effective against systemic fungal disease and has *in vitro* and *in vivo* activities against several *Candida* species as well as *C. neoformans* and *Aspergillus* species. Although the polyenes have been used in the clinic for over 50 years, the major limitation of their use is host toxicity, such as renal dysfunction, which is likely due to the structural similarities between ergosterol and cholesterol in the mammalian cell membrane (176, 441). The introduction of lipid-complexed formulations of amphotericin B has managed to considerably reduce the incidence of host toxicity, and efforts are ongoing to develop and test novel lipid-complexed polyenes against diverse pathogenic fungi in experimental animal models (139, 516).

Echinocandins

The echinocandins are the only new class of antifungal to reach the clinic in decades. There are currently three drugs belonging to this class that are available for clinical use: caspofungin, micafungin, and anidulafungin. The echinocandins are large lipopeptide molecules that act as noncompetitive inhibitors of (1,3)-β-D-glucan synthase, an enzyme involved in fungal cell wall synthesis (143). The disruption of this polysaccharide results in the loss of cell wall integrity and severe cell wall stress on the fungal cell (Fig. 1C). The activity of the echinocandins is generally opposite that of the azoles in that they are fungicidal against yeasts and fungistatic against molds. Good antifungal activity against *Candida* and *Aspergillus* has been reported, but the echinocandins show no antifungal activity against *C. neoformans* (143). The safety profile of this drug class is impressive and is likely due to the fact that it targets a specific component of fungi that is not conserved in mammalian cells (441). Due to their relatively short duration of use in the clinic, there have not been extensive long-term studies on the mechanisms of echinocandin resistance.

CANDIDA ALBICANS

The opportunistic pathogen *C. albicans* is a natural member of the human mucosal microbiota and can thrive in diverse niches within a healthy host, from the oral cavity to the gastrointestinal tract (429). Within immunocompetent or immunocompromised hosts, it is capable of causing superficial infections through the invasion of oral and vaginal epithelial surfaces; in individuals who are immunocompromised or otherwise compromised (for example, by severe underlying disease, surgery, or hospitalization), it can also cause lethal systemic infections through dissemination in the bloodstream and invasion of internal organs (88). Species of *Candida* are the fourth most common cause of hospital-acquired infectious diseases in the United States, costing the health care system an estimated $1 billion annually (120, 382). More than 90% of invasive *Candida* infections are attributed to five species: *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* (456, 457). These organisms are the most prevalent cause of opportunistic mycoses worldwide, with crude mortality rates of ~40% (456). *C. albicans* reigns as the most common causal agent of invasive candidiasis. Its pathogenic prowess is due to various factors, including the expression of multiple surface structures that mediate adherence to epithelial cells (655), the secretion of hydrolytic enzymes that induce host cell damage (408), phenotypic switching between states with distinct gene expression and physiology (544), the capacity to produce biofilms that are resistant to antifungal drugs (142), metabolic and stress adaptation during infection (76, 242), and the ability to transition between yeast and filamentous growth (293, 625). In this introductory section, we focus on the basic biology of this organism to set the stage for subsequent sections on morphogenesis and drug resistance.

For more than a century, *C. albicans* was thought to be asexual, existing only as an obligate diploid. However, the identification of *C. albicans* genes with homology to the *Saccharomyces cerevisiae* mating-type genes through the genome-sequencing project sparked intense investigation into a cryptic

FIG. 1. Antifungal drugs and their targets. (A) The azoles function by targeting the ergosterol biosynthetic enzyme lanosterol demethylase, encoded by *ERG11* (*C. albicans* and *C. neoformans*) or *cyp51A* and *cyp51B* (*A. fumigatus*), causing a block in the production of ergosterol and the accumulation of a toxic sterol produced by Erg3. This toxic sterol exerts a severe membrane stress on the cell. (B) The fungicidal polyenes are amphipathic drugs that function by binding to ergosterol to create drug-lipid complexes, which intercalate into the fungal cell membrane to form a membrane-spanning channel. This causes cellular ions to leak out of the cell, destroying the proton gradient and culminating in osmotic cellular lysis. (C) Fungal cell walls are composed of (1,3)-β-D-glucans covalently linked to (1,6)-β-D-glucans as well as chitin, mannans, and cell wall proteins. The echinocandins act as noncompetitive inhibitors of (1,3)-β-D-glucan synthase (encoded by *FKS1* in *C. albicans*, *C. neoformans*, and *A. fumigatus* and by *FKS1* and *FKS2* in *C. glabrata* and *S. cerevisiae*) and thereby cause a loss of cell wall integrity and severe cell wall stress. (Adapted from reference 111 with permission of Nature Publishing Group.)

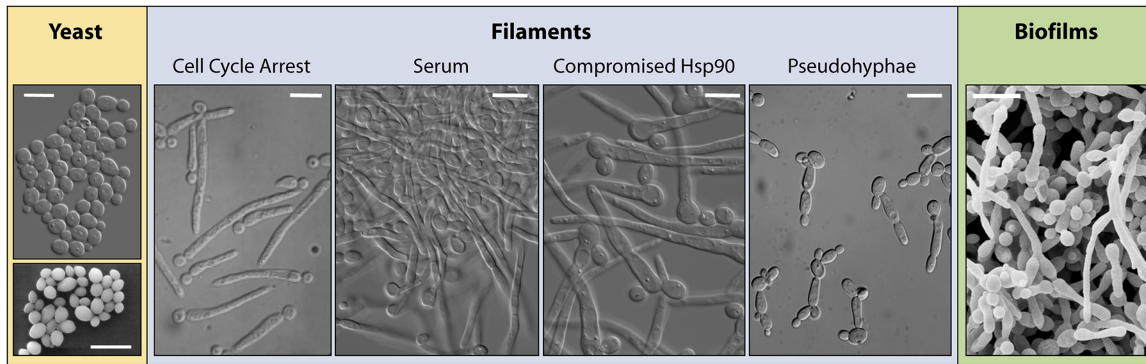

FIG. 2. The distinct morphogenetic states of *C. albicans*, including yeast, filaments, and biofilms. *C. albicans* transitions between yeast and filamentous growth states and also forms biofilms, which are complex surface-associated communities composed of multiple cell types. Yeast cells are shown under differential interference contrast (DIC) microscopy (top left) or scanning electron microscopy (SEM) (bottom left) (644). Filaments are heterogeneous structures, which vary greatly depending on the signaling cue that induces them. The filaments depicted (middle panels) were induced by different environmental cues: cell cycle arrest upon the depletion of *CDC5* (19), 10% serum at 37°C, compromised Hsp90 function by treatment with geldanamycin, and pseudohyphae produced in medium at pH 6.0 at 35°C (570). The biofilm image on the right is an SEM image of a mature (48-h) biofilm composed of yeast and filamentous cells (477). Scale bars indicate 10 μm. (Image of yeast cells reprinted from reference 644 with permission from the Society for General Microbiology; image of cell cycle-arrested filaments reprinted from reference 19 with permission from John Wiley and Sons; image of pseudohyphae reprinted from reference 570 with permission from Elsevier; image of biofilm reprinted from reference 477 with permission.)

sexual cycle. The *C. albicans* mating-type genes are organized into two nonhomologous mating-type (*MTL*) loci, *MTL***a** and *MTL*α, on chromosome 5 (244). Mating typically occurs between diploid *MTL***a** and *MTL*α strains to generate an **a**/α tetraploid both under laboratory conditions and in a mammalian host (162, 245, 354). The detection of mating events remained challenging until the discovery that phenotypic switching regulates mating such that only the opaque form is competent for efficient mating, and switching to this state requires *MTL* homozygosity (383). A meiotic program remains elusive, and tetraploid mating products undergo chromosome loss to return to a near-diploid state, although aneuploidies are common. Most progeny from this parasexual cycle have undergone extensive genetic recombination between homologous chromosomes (183). Further complexities of *C. albicans* mating are illustrated by the discovery of two distinct same-sex mating pathways (4). A comparison of the genome sequences of six *Candida* species revealed that key components of the mating and meiotic machineries are missing from multiple species (83). The diversity in the apparent capacity of these species to mate and the genomic plasticity of both mating and meiotic machineries suggest that cryptic meiotic cycles may exist. With recombination during the parasexual cycle and mating between cells of different or the same mating types, *C. albicans* can harness multiple mechanisms to generate genetic diversity.

The success of *C. albicans* as a pathogen depends in large part on its ability to generate diversity not only at the genetic level but also at the morphological and physiological levels. One example mentioned in the context of mating is phenotypic switching, an epigenetic transition between white and opaque cellular states (544). These two cell types are remarkably different in their cell and colony morphologies, metabolic states, preferred host niches, and interactions with the host immune system (343). White-opaque differentiation is governed by the master transcriptional regulator Wor1 (240, 658), which imparts precise and complex transcriptional regulation via 5′ and 3′ untranslated region specificity, intergenic binding, and the transcription of coding, noncoding, and antisense transcripts (587). Each cell type is heritable for many generations, with multiple feedback loops regulating their stability. While switching appears largely stochastic, environmental cues influence the frequency of switching (3).

Environmental cues also govern transitions among the morphological states of *C. albicans*, including yeast, pseudohyphae, and hyphae, with morphogenetic flexibility being implicated as an important virulence trait. These morphological states also enable the formation of biofilms on medical devices. *C. albicans* biofilms are complex surface-associated communities of multiple cell types surrounded by an extracellular matrix (Fig. 2) (60, 413, 422, 476). The generation of a *C. albicans* biofilm is a stepwise process initiated by adherence to a foreign substrate, followed by the proliferation of yeast cells and the beginning of hyphal development and culminating with a maturation stage in which yeast growth is repressed, hyphal growth is enhanced, and the biofilm is encased by an extracellular matrix. The final dispersal stage is ultimately characterized by the budding of nonadherent yeast cells that have properties distinct from those of their planktonic counterparts, including enhanced adherence, filamentation, biofilm formation, and virulence (591). In the clinical context, *C. albicans* biofilms are extremely resistant to antifungal therapy (142), such that the removal of the infected device is often required to avert potentially fatal consequences.

Given its clinical relevance, *C. albicans* has been the subject of extensive research to dissect the mechanisms governing fungal virulence and drug resistance. However, due to a limited repertoire of molecular and genetic techniques, many studies have relied on the genetic tractability and genomic resources of the model yeast *S. cerevisiae*. Facets of *C. albicans* biology that make it challenging to genetically manipulate in the laboratory include its obligate diploid state; its lack of a conventional meiotic cycle; that plasmid maintenance is limited to those that carry autonomously replicating sequences (ARSs), which can

Signalling Cue	Proteins/Pathways
Serum/Peptidoglycans	cAMP - PKA Pathway
Nutrient Limitation	Mep2, MAPK Pathway
Amino Acid Metabolism	Gpr1, Csh3, Gcn4, Cdc53
Alkaline pH	Rim101 Pathway
Embedded Conditions	Czf1, Rac1, Dck1
Elevated [CO_2]	cAMP - PKA Pathway
Elevated Temperature	Hsp90, cAMP - PKA Pathway
Cell Cycle Arrest	Clb2, Clb4, Hgc1 - Cdc28
Yeast ⟷ Filament	
Hyperosmotic Stress	HOG Pathway
	Pes1, Pde2, Srv2
Quorum Sensing Molecules	cAMP - PKA Pathway

FIG. 3. Environmental cues and corresponding pathways that mediate morphogenesis in *C. albicans*. *C. albicans* transitions between distinct morphogenetic states, including yeast, pseudohyphae, and hyphae, as depicted. Numerous environmental signals mediate the transitions between yeast and filamentous forms. Cues and pathways at the top mediate filament-to-yeast morphogenesis, and cues and pathways at the bottom mediate yeast-to-filament morphogenesis.

integrate randomly into the genome; and an unconventional codon usage that translates the CUG codon as serine rather than the universal leucine (51). Despite the divergence of ~200 to 800 million years of evolution between *S. cerevisiae* and *C. albicans* (48, 222), comparative analyses have provided a powerful platform for identifying both conserved and divergent cellular circuitries.

INTRODUCTION TO *CANDIDA ALBICANS* MORPHOGENESIS: YEAST, PSEUDOHYPHAE, AND HYPHAE

C. albicans is a polymorphic organism that transitions between distinct morphological states: the yeast form and the filamentous pseudohyphal and hyphal forms (Fig. 2). The morphogenetic plasticity of *C. albicans* is an important virulence trait, as mutants that are unable to undergo morphogenesis are often attenuated in virulence. Diverse environmental signals regulate morphogenesis, and complex and interconnected signaling cascades are responsible for sensing and responding to these cues (Fig. 3). This section will present an introduction to the characteristics of the different morphological states, a summary of how *C. albicans* morphogenesis is linked to virulence, a description of the various environmental signals modulating morphogenesis, and, finally, an overview of the major signaling pathways involved.

C. albicans yeast cells are readily distinguished from filamentous growth forms and generally resemble the budding yeast *S. cerevisiae*. Filamentous growth results in the production of elongated cells relative to the yeast form. Pseudohyphae and hyphae can be differentiated based on their general sizes and shapes (525) and based on a morphological index (MI) according to the relative lengths and septal diameters of the cells (381). Hyphal cells are narrower than pseudohyphal cells and have parallel walls with no constriction at the site of septation, while pseudohyphal cells are wider and form constrictions between elongated buds (Fig. 2) (570).

These three morphologies can also be distinguished based upon discrepancies in cell cycle regulation (50). Yeast cells grow and bud asymmetrically, with septin rings forming before bud emergence and nuclear division occurring across the mother-bud neck (571, 615). In yeast, bud emergence is coordinated with the transition from G_1 to S phase of the cell cycle, with mother and daughter buds separating after the completion of the cell cycle (220). The pseudohyphal cell cycle closely resembles that of budding yeast, in that septin rings form before bud emergence and nuclear division occur at the bud neck (571, 615). However, unlike yeast, pseudohyphal cells bud in a unipolar fashion, remain attached after cytokinesis, and form branched chains of elongated buds. The buds become elongated after a prolonged G_2 phase compared to yeast (50). Hyphal cells, however, show a distinct cell cycle progression. Unlike yeast and pseudohyphae, hyphae divide asynchronously. Hyphal growth begins with germ tube formation, which occurs before the G_1-to-S-phase transition and continues through cytokinesis (50). Nuclear division in hyphal cells occurs within the germ tube itself following nuclear migration (180). Hyphal cells also posses a unique structure called the Spitzenkörper, which is located behind the growing hyphal tip and mediates growth directionality and hyphal tip morphogenesis (605). Spitzenkörper-located proteins are highly dynamic, and secretory vesicles accumulate in this region before delivery to the hyphal tip or cell surface (257).

These distinct characteristics of pseudohyphae and hyphae are often drawn upon to suggest that these filamentous morphologies are discrete forms (570). This is reinforced by the fact that pseudohyphae and hyphae show different patterns of septin ring localization (571) and different patterns of germ tube formation or bud emergence (571, 615). Nevertheless, recent evidence suggests that pseudohyphae may in fact represent an intermediate morphology between yeast and hyphae. Most notably, it has been shown that expression levels of the transcription factor Ume6 are correlated directly with morphology: high levels of *UME6* expression promote hyphal growth, intermediate levels promote pseudohyphal growth, and low levels produce yeast (93).

Morphogenesis and Its Association with Virulence

The capacity for *C. albicans* to undergo morphogenesis is important for virulence, such that mutants that cannot undergo a morphological transition often have reduced pathogenicity. For instance, numerous mutants that grow exclusively as yeast are avirulent in mouse models of systemic candidiasis (312, 342, 490), as is the case with numerous mutants that grow exclusively in the filamentous form (31, 68, 71, 305, 407). This finding suggests that both yeast and filamentous forms contribute to virulence. The current paradigm suggests that whereas filaments are responsible for tissue invasion and deep-seated infection, yeasts play a crucial role in early dissemination and infection processes (511).

Hyphal cells are further implicated in virulence, as they

specifically express virulence factors such as adhesins and proteases. Importantly, numerous genes expressed during yeast-to-hypha morphogenesis are dispensable for cellular morphology but are necessary for virulence (262, 293, 304, 410). Specific examples include the expression of genes encoding secreted aspartic proteases (178, 409) as well the adhesins *HWP1* and *ALS3* (193, 550, 573, 651). In a reconstituted epithelial cell infection model, the upregulation of *C. albicans* hypha-specific virulence genes is evident within 30 min postinoculation, coinciding with filamentation and damage to the epithelial cells (547). To date, the only example of a hypha-specific gene that is critically required for *C. albicans* morphogenesis in addition to virulence is that encoding the G_1 cyclin Hgc1 (652). Hgc1 provides a fascinating link between morphogenesis and virulence, as the *hgc1* mutant is defective in yeast-to-hypha morphogenesis but still expresses hypha-specific genes implicated in virulence, including *HWP1* and *ECE1* (652).

Morphogenesis is coupled to biofilm formation, which plays an important role in virulence. *C. albicans* can colonize a substrate and produce a biofilm on medical implants such as vascular catheters. These biofilms are significant risk factors for infection and disease (158, 159). Morphogenesis plays a crucial role in biofilm formation, as *C. albicans* biofilms are composed of a diverse population of yeast, pseudohyphae, and hyphae (60, 158). In accordance with this, numerous hypha-defective mutants are unable to colonize plastic surfaces and form robust biofilms (288). Additionally, a screen for biofilm-defective mutants revealed that all of the mutants were also defective in hyphal development, emphasizing the link between morphogenesis and biofilm formation (486). More recently, it was found that the hypha-to-yeast ratio of a biofilm influences its compression strength, and biofilms with a hyphal content of over 50% have a higher compressive strength and are significantly more difficult to disrupt (445). Proteins produced in response to filamentous growth cues, including cell wall proteins such as adhesins, have unique and specific functions in biofilm formation. The expression of *C. albicans* proteins in a strain of *S. cerevisiae* that does not form biofilms confers substrate-specific binding and biofilm formation. For instance, the expression of Als1 allows binding to epithelial cells (194), while the expression of the Als3 and Hwp1 proteins allows binding to fibrinogen and the salivary pellicle, and the expression of Eap1 proteins allows binding to polystyrene surfaces (420). Expression of both Als3 and Eap1 confers the ability to bind *Streptococcus gordonii*, which is a primary colonizing bacterium often found in biofilms with *C. albicans* (420).

Another way in which morphogenesis is linked to *C. albicans* virulence is through the interaction of different cellular morphologies with the host immune system. It was shown that the yeast-to-hypha morphogenetic transition promotes escape from macrophages and neutrophils (284, 342). Furthermore, dendritic cells are able to differentiate between yeast and hyphal morphotypes, and while both are phagocytosed, this occurs via different receptors and stimulates different immune responses (156). Similarly, yeasts and hyphae have differential susceptibilities and responses to macrophage-derived compounds (62). With macrophages, the receptor Dectin-1 recognizes β-glucan on the surface of *C. albicans* cells. *In vitro*, Dectin-1 mediates the specific macrophage recognition of yeast but not of hyphal cells (198). This is due to the fact that yeast cells expose β-glucan during budding, while hyphal cells do not (198). Notably, the antifungal drug caspofungin exposes *C. albicans* β-glucan *in vitro* and *in vivo*, although it preferentially unmasks β-glucan on filamentous cells, thereby exposing the cell wall component to immune effectors (620, 621). A further connection between morphology and the host immune response is apparent in the host's biphasic mitogen-activated protein kinase (MAPK) response upon *C. albicans* infection. The first host MAPK phase is triggered by fungal cell wall recognition and occurs independently of fungal morphology; however, the second MAPK phase is specifically dependent on *C. albicans* hyphal formation (397).

Although there are important links between morphogenesis and virulence, the relationship can be more complex. A systematic screen of homozygous mutants representing ~11% of the *C. albicans* genome revealed uncoupling between morphogenetic switching and pathogenicity (425). Of 115 mutants that were attenuated in infectivity in a mouse model, nearly half displayed normal morphological switching under the one environmental condition tested as well as normal proliferation rates (425). Similarly, not all mutants with morphological defects had reduced infectivity (425). This reveals the presence of other important mechanisms of virulence that function independent of morphogenesis to regulate *C. albicans* pathogenicity.

Environmental Signals That Regulate Morphogenesis

Complex and interconnected signaling cascades regulate morphogenesis in *C. albicans*. In order to dissect the networks involved in morphogenetic regulation, it is critical to introduce the different environmental signals that control *C. albicans* morphogenesis. As summarized below, many different cues have been found to induce or block morphogenesis in *C. albicans* (75, 426).

Serum is one of the most common cues for stimulating *C. albicans* morphogenesis. The combination of serum and an elevated temperature of 37°C will promote yeast-to-hypha morphogenesis (Fig. 2 and 3). For decades, the component of serum responsible for triggering morphogenesis remained elusive. For some time, the glucose in serum was suggested to be responsible for morphogenesis (243); however, it was recently shown that bacterial peptidoglycans in serum trigger hyphal growth by directly activating the adenylyl cyclase Cyr1 (635). RPMI and M199 media also stimulate yeast-to-hypha morphogenesis at elevated temperatures and are thought to act through a common pathway with serum.

Conditions of nutrient limitation can also induce filamentation. It has been known for some time that media containing poor carbon and nitrogen sources, such as *N*-acetylglucosamine or Spider medium, can stimulate yeast-to-hypha morphogenesis at elevated temperatures (374, 582). Nitrogen starvation-induced filamentous growth occurs via the ammonium permease Mep2 (59, 129).

Amino acid metabolism provides another link to *C. albicans* morphogenesis. The amino acids proline and methionine have both been shown to activate yeast-to-hypha morphogenesis under certain conditions via the G protein-coupled receptor (GPCR) Gpr1 (357). Furthermore, defects in amino acid sensing have been correlated with morphogenetic defects. For instance, the

inactivation of the amino acid sensor Ssy1 blocks amino acid uptake and causes defects in yeast-to-hypha morphogenesis (72). Similarly, the deletion of the endoplasmic reticulum (ER) packaging chaperone Csh3 inhibits amino acid uptake and hyphal morphogenesis (371). Amino acid starvation has been shown to stimulate pseudohyphal growth in a Gcn4-dependent manner (583). It was recently proposed that Cdc53, a component of a ubiquitin-ligase complex, directly regulates Gcn4 and, therefore, amino-acid-induced morphogenesis (584). The depletion of *CDC53* promotes pseudohyphal growth as well as an amino acid starvation-like transcriptional response (584).

Morphogenesis is also regulated by environmental pH. When *C. albicans* is shifted to more alkaline conditions (pH $>$6.5) at an elevated temperature, it will undergo a yeast-to-hypha transition (81, 316). The measurement of the internal pH of *C. albicans* cells undergoing morphogenesis has confirmed that the internal pH rises to around pH 7.0 before the outgrowth of the germ tube in response to different hypha-inducing cues. This internal alkalinization of the cell does not occur during the growth of budding yeast or in cells that are defective in hypha formation under hypha-stimulating conditions (563, 564).

The physical environment of *C. albicans* cells can also have an impact on morphogenesis. Cells embedded in soft agar form hyphae in many different growth media, even at lower temperatures (25°C) (77). It was suggested that filamentation in response to embedded conditions occurs as a results of low oxygen concentrations, although this remains contentious. Nevertheless, hypoxic stress can promote hyphal growth and the upregulation of hypha-specific genes (155, 524).

Another important environmental factor that regulates *C. albicans* morphogenesis is CO_2. CO_2 concentrations within the mammalian host are $\sim$5%, much higher than atmospheric concentrations ($<$0.1%) (30). These physiologically relevant concentrations of CO_2, or the addition of bicarbonate to laboratory media, can promote pseudohyphal growth in different media at elevated temperatures. This occurs via the activation of the core catalytic domain of Cyr1 (276). Furthermore, the carbonic anhydrase Nce103, which converts CO_2 to bicarbonate, is essential for the pathogenicity of *C. albicans* in environments with low concentrations of CO_2 (276).

The transition from yeast to filamentous morphologies is often contingent upon elevated temperatures. For instance, many of the morphogenetic signals listed above, including serum, pH, and CO_2, depend on a concurrent increase of the temperature to 37°C before morphogenesis can occur. The essential molecular chaperone Hsp90 was recently implicated in temperature-dependent yeast-to-filament morphogenesis in *C. albicans* (527). An elevated temperature relieves the Hsp90-mediated repression of the morphogenetic program and induces the yeast-to-filament transition via cyclic AMP (cAMP)-protein kinase A (PKA) signaling (Fig. 2) (527).

The focus to this point has been on stimuli that promote yeast-to-filament morphogenesis; however, distinct cues inhibit yeast-to-filament morphogenesis (Fig. 3). For instance, quorum-sensing molecules act to repress morphogenesis in *C. albicans*. Farnesol, a quorum-sensing molecule produced by *C. albicans* itself, represses hyphal growth and biofilm formation in dense populations (232, 478) and inhibits the expression of filament-specific transcripts (168). 3-Oxo-C_{12} homoserine lactone, a signaling molecule produced by *Pseudomonas aeruginosa*, and the structurally similar molecule dodecanol both repress *C. albicans* hyphal formation (227). Both farnesol and dodecanol function by repressing cAMP-PKA signaling (137). Recently, the *Streptococcus mutans* quorum-sensing molecule competence-stimulating peptide (CSP) has been implicated in the repression of *C. albicans* morphogenesis (251). Signals distinct from quorum-sensing molecules can also repress filamentation, as is the case with hyperosmotic stress (8). Specifically, the high-osmolarity glycerol (HOG) pathway represses the serum-induced yeast-to-hypha transition in *C. albicans* (8).

Along with cues that block yeast-to-filament morphogenesis, there are signals that actively regulate the transition from filament to yeast (Fig. 3). Pseudohyphal and hyphal cells can produce yeast cells on their subapical segments, which are referred to as lateral yeast (278). Far less is known about the filament-to-yeast transition than about the yeast-to-filament transition; however, certain genes have been implicated. *C. albicans* mutants lacking the phosphodiesterase Pde2 have decreased levels of production of lateral yeast, while mutants lacking the adenylyl cyclase-associated protein Srv2 have increased lateral yeast growth (31, 32). More recently, the *C. albicans pescadillo* homologue Pes1 has been implicated in lateral yeast growth on filaments as well as the normal growth of yeast cells, indicating that Pes1 is critical for the filament-to-yeast transition (530).

Major Morphogenetic Signaling Cascades

cAMP-protein kinase A. The cAMP-PKA signaling cascade is of central importance for *C. albicans* morphogenesis (Fig. 4 and see Fig. 8). Many components of this pathway are required for filamentation under a variety of different conditions. Intracellular levels of cAMP positively regulate yeast-to-hypha morphogenesis. In brief, cAMP is synthesized from ATP by the adenylyl cyclase Cyr1 and is converted to AMP by the phosphodiesterases Pde1 and Pde2. Cyr1 itself is activated by the guanine nucleotide binding protein Ras1 as well as the Gα protein Gpa2. Once produced, cAMP activates the PKA complex, which consists of two catalytic subunits, Tpk1 and Tpk2, as well as the regulatory subunit Bcy1. PKA phosphorylates and activates downstream transcription factors, which regulate the expression of filament-specific genes. These transcription factors, as well as the other components of this pathway, will be discussed in detail below.

The cAMP-PKA pathway is activated by different upstream receptors that sense and respond to environmental cues. For instance, Mep2 is a transmembrane ammonium permease that regulates yeast-to-hypha morphogenesis in response to nitrogen starvation. When ammonium is absent or present only at low concentrations, Mep2 activates cAMP-PKA signaling as well as a MAPK signaling cascade, thereby inducing filamentous growth (59). Conversely, when ammonium is abundant in the environment, Mep2 is engaged in ammonium transport, and the activation of these cascades, along with filamentous growth, is blocked (59). Recently, the G protein Rhb1 and its GTPase-activating protein (GAP) Tsc2 have been implicated in nitrogen starvation-induced filamentation and have been proposed to regulate the expression of *MEP2* (585).

Another upstream component of cAMP-PKA signaling is

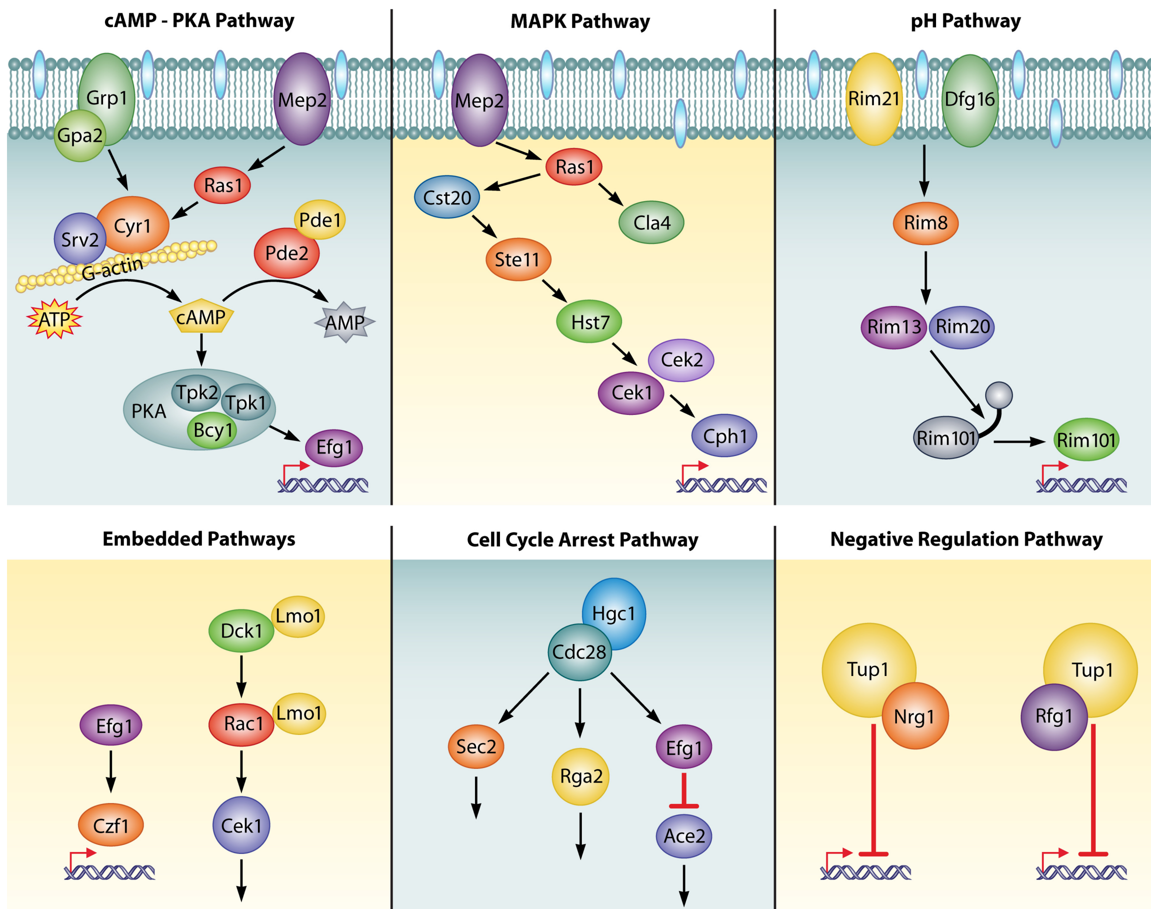

FIG. 4. Key cellular signaling cascades regulating morphogenesis in *C. albicans*. Numerous signaling pathways regulate *C. albicans* morphogenesis; the six most well-characterized pathways along with many of the key proteins involved in each pathway are depicted.

the GPCR Gpr1. Gpr1 along with its associated Gα protein Gpa2 are required for morphogenesis on many solid media (357, 386, 502). Gpr1 and Gpa2 have been shown to interact by yeast two-hybrid analysis, and epistasis analysis suggests that Gpr1 acts upstream of Gpa2, leading to the activation of the cAMP pathway (357, 386). Biochemical studies revealed that Gpr1 and Gpa2 are required for the glucose-dependent increase in intracellular cAMP levels (386), and they are important for amino-acid-induced morphogenesis in the presence of glucose (358). Although *gpr1* and *gpa2* homozygous deletion mutants are fully virulent in the mouse (386), the combined deletion of *GPR1* and *TPS2*, encoding the trehalose-6-phosphate phosphatase, results in avirulence in a mouse model of infection (356).

Ras1 is a guanine nucleotide binding protein which acts downstream of Mep2. Ras1 cycles between an active GTP-bound state and an inactive GDP-bound state. *ras1* homozygous deletion mutants are impaired in hyphal development under many different inducing conditions (179, 312). Strains harboring a dominant active *RAS1*^{V13} allele hyperfilament, while those harboring a dominant negative *RAS*^{A16} allele do not filament in response to most cues (179). The addition of cAMP restores the filamentation of the *ras1* mutant under all conditions tested, as does the overexpression of components of both the cAMP-PKA and MAPK pathways, positioning Ras1 upstream of both of these cascades (312). The deletion of *RAS1* causes reduced virulence in a mouse model of infection (312). Recently, Ras2 in *C. albicans* was characterized. Unlike the *ras1* mutant, the *ras2* mutant undergoes wild-type morphogenesis in response to most cues; however, the deletion of *RAS2* further aggravates the hyphal defect of a *ras1* mutant, suggesting that it may have a partial role in filamentous growth (656). Surprisingly, while the deletion of *RAS1* results in a reduction of the intracellular levels of cAMP, the deletion of *RAS2* restores these levels to ~30% of the wild-type level, suggesting complex and antagonistic roles between these two Ras proteins (656).

Ras1 is cycled between its GTP- and GDP-bound states by the GAP Cdc25 and the putative guanine exchange factor (GEF) Ira2. Biochemical assays have shown that *C. albicans* Cdc25 can activate the Ras/adenylyl cyclase pathway in *S. cerevisiae* (206), and *cdc25* homozygous deletion mutants are defective in filamentation in response to different cues (169, 527). Ira2 has not been well characterized for *C. albicans* but has been identified as the putative GEF for Ras1 based on homology to *S. cerevisiae* Ira2.

Ras1 and Gpa2 both function upstream of the adenylyl cyclase Cyr1, and the physical interaction between Ras1 and

Cyr1 is essential for increasing cellular cAMP levels (173, 490). Cyr1 is the single adenylyl cyclase in *C. albicans* and therefore is the sole source of cAMP production in the cell. *cyr1* homozygous deletion mutants have a slow-growth phenotype, are unable to form hyphae under numerous conditions, and are avirulent in a mouse model (490). The addition of cAMP can rescue the morphogenetic block of *cyr1* mutants (490). Interestingly, different morphogenetic cues have been shown to directly influence the activation of Cyr1. For instance, both CO_2 and peptidoglycans in serum directly stimulate Cyr1 to activate cAMP production and promote filamentous growth (276, 635). Recently, Cyr1 was found to copurify with the cyclase-associated protein Srv2 as well as with monomeric actin (G-actin), which together increase cAMP synthesis in response to hyphal signals (659). The formation of this tripartite protein complex is essential for hyphal induction in response to serum and peptidoglycans, and a disruption of the complex leads to a reduction in the levels of cAMP production (659). Considering that filamentous growth requires complex cytoskeletal restructuring, including the reorganization of actin filaments, this connection between G-actin, cAMP production, and filamentous growth provides an exciting and gratifying molecular link.

Aside from Cyr1, several other proteins influence the levels of cAMP production in the cell. As mentioned above, Srv2 is the adenylyl cyclase-associated protein that functions in cooperation with Cyr1. The homozygous deletion of *SRV2* causes defects in filamentation in response to different cues (32, 527), and the *srv2* mutant is avirulent in a mouse model of infection (32). As mentioned above, Srv2 functions in the tripartite protein complex with Cyr1 and G-actin, and *srv2* mutants lacking the specific G-actin binding site are impaired in cAMP synthesis and hyphal morphogenesis (659). Pde1 and Pde2 are low- and high-affinity phosphodiesterases, respectively, that convert cAMP into AMP and therefore negatively regulate filamentation (235). The homozygous deletion of *PDE2* causes hyperfilamentation under various conditions (31, 260). The hyperfilamentous *pde2* mutant has greatly reduced virulence in a mouse model of infection and has reduced adhesion and invasion capabilities *in vitro* (31, 631). The deletion of *PDE1* has much more modest effects than does the deletion of *PDE2*, although Pde1 plays a role in hyphal formation on certain solid media (630), and a *pde1 pde2* double-deletion mutant has even more reduced virulence than the *pde2* mutant alone (631).

The PKA complex itself consists of the catalytic subunits Tpk1 and Tpk2 and the regulatory subunit Bcy1, which together act downstream of Ras1 and cAMP signaling (65). Tpk1 and Tpk2 are both positive regulators of morphogenesis, and the overexpression of one can overcome the deletion of the other (65, 545). Interestingly, Tpk1 and Tpk2 have distinct roles in *C. albicans* morphogenesis: the deletion of *TPK1* renders cells unable to filament on solid media but not in liquid media, while the deletion of *TPK2* renders cells defective in filamentation in liquid media and only partially defective on solid media (65). Bcy1 is an essential negative regulator of the PKA complex, and strains that overexpress Bcy1 are defective in hyphal growth on solid media and in liquid media (549).

The PKA complex is responsible for the activation of transcription factors that regulate the expression of filament-specific transcripts. Efg1 is often considered the "master" transcriptional regulator of morphogenesis; therefore, we will focus first on Efg1 and then on other transcription factors downstream of PKA. Efg1 and Efh1 are members of the APSES family of transcription factors, which are unique to the fungal kingdom (155). Efg1 is phosphorylated directly by PKA at T206; the mutation of this residue to create an unphosphorylated inactive allele (T206A) blocks filamentation under different conditions, while the mutation of this residue to mimic a phosphoryl group (T206E) causes hyperfilamentation (64). Furthermore, the homozygous deletion of *EFG1* blocks filamentation under numerous conditions (167, 342, 566). The regulation of filamentation by Efg1 is complex. The overexpression of *EFG1* can promote pseudohyphal growth, and *EFG1* transcript levels are downregulated upon yeast-to-hypha morphogenesis (566). Moreover, *efg1* mutants show enhanced hyphal growth under embedded conditions (205), and other evidence suggests that Efg1 can act as both a transcriptional activator and a repressor (155, 499, 609).

Despite the central role of Efg1 in *C. albicans* morphogenesis, certain stimuli will induce morphogenesis independent of this transcription factor. For instance, *efg1* mutants retain the ability to filament on solid medium containing serum as well as upon ingestion by macrophages (342). Furthermore, although Efg1 has been shown to upregulate filament-specific transcripts *in vitro* (410), many of these transcripts are expressed independently of Efg1 in an *in vivo* intestinal tract model of colonization (622). Interestingly, a number of morphogenetic stimuli are dependent on upstream components of PKA activity but not on Efg1. For instance, the depletion of the polo-like kinase Cdc5 induces filamentation in a manner that is dependent on Cyr1 but not on Efg1 (Fig. 2) (20). The same holds true for filamentation induced by treatment with the DNA synthesis inhibitor hydroxyurea (HU) (20). Similarly, the depletion of the DNA damage checkpoint regulator Rad52 triggers filamentation that is Cyr1 dependent and Efg1 independent (12). Finally, the depletion of the molecular chaperone Hsp90 triggers filamentation that is dependent on all of the upstream components of the PKA pathway, including Ras1, Cyr1, and the PKA complex itself, but is not dependent on Efg1 (527). Taken together, this implicates other transcriptional regulators downstream of PKA in the regulation of *C. albicans* morphogenesis.

There are several transcription factors that have been proposed to act downstream of PKA in *C. albicans*. One such transcription factor is Flo8 (92). Flo8 is required for morphogenesis under many conditions, and the *flo8* homozygous deletion mutant has highly reduced virulence in a mouse model (92). Flo8 interacts with Efg1 in yeast and hyphal cells, and Flo8 regulates a subset of genes controlled by Efg1 (92). Recently, the transcriptional activator Mss11 was shown to interact with Flo8 and influence *C. albicans* morphogenesis (568). The overexpression of *MSS11* enhances filamentation, while the homozygous deletion of *MSS11* blocks filamentation under many conditions (568). Another transcription factor thought to act downstream of PKA is Sfl1. Sfl1 is a negative regulator of hyphal development, and the homozygous deletion of *SFL1* leads to hyperfilamentation and filament-specific gene expression in several media (45, 327). The homozygous deletion of *FLO8* and *SFL1* blocks hyphal development, suggesting that Sfl1 may antagonize Flo8 (327). Tec1 is part of the TEA/ATTS family of transcriptional regulators and is thought to act down-

stream of Efg1, as it is transcriptionally regulated by both Efg1 and Cph2 (304). *tec1* homozygous deletion mutants are blocked in hyphal development and show reduced virulence in the mouse (515). Finally, the transcription factor Bcr1 is downstream of Tec1 and activates the expression of hyphal adhesins involved in biofilm formation *in vitro* and *in vivo* (421, 423).

Recently, the Set3/Hos2 histone deacetylase complex (HDAC) was implicated as a regulator of *C. albicans* morphogenesis by acting upstream of Efg1 to repress cAMP-PKA signaling (226). The homozygous deletion of *HOS2* or *SET3* leads to extensive filamentation at 37°C on solid rich medium (226). Furthermore, *set3* or *hos2* mutants have elevated expression levels of Efg1-dependent target genes, including *HWP1* (226). Notably, *set3* mutants have attenuated virulence in a murine infection model (226).

Mitogen-activated protein kinase. There are several MAPK signaling cascades in *C. albicans*; this section will focus on the primary one involved in filamentation, while later sections will highlight other MAPK modules linked to morphogenesis (Fig. 4 and see Fig. 8). The principal MAPK pathway implicated in filamentation is generally defined based on the downstream transcription factor Cph1. It was the first pathway linked to *C. albicans* morphogenesis and was identified based on homology to the well-studied *S. cerevisiae* pheromone signaling pathway (388). Morphogenetic defects associated with the disruption of MAPK signaling through this pathway are generally less severe than those associated with a disruption of cAMP-PKA signaling and are limited to very specific growth conditions. For instance, homozygous deletion mutants of genes encoding components of the MAPK pathway are blocked in morphogenesis on solid Spider medium containing a poor carbon source but still filament in response to serum, altered pH, and other stimuli (311, 338). Therefore, the MAPK cascade is thought to be involved primarily in morphogenesis induced by nutrient limitation (311, 338).

Certain components of the MAPK pathway are shared with the cAMP-PKA cascade. As mentioned above, the ammonium permease Mep2 activates both cAMP-PKA and MAPK signaling under nitrogen starvation conditions in a Ras1-dependent manner (59). Furthermore, Ras1 has been shown to be upstream of both the cAMP-PKA and MAPK pathways (179, 342). Downstream of Ras1 in the MAPK module is the Rho-type GTPase Cdc42 and its GEF protein Cdc24, which are important for invasive hyphal growth as well as polarized growth in both yeast and hyphal cells (43, 595). Signaling from Cdc42 to its downstream effectors is required for morphogenesis under various conditions (311, 313, 569).

The two kinases downstream of Cdc42 are Cst20 and Cla4. Cst20 is a kinase from the Ste20/p65^{PAK} family of protein kinases. The *cst20* homozygous deletion mutant is defective in hyphal growth on solid medium in response to nutrient-limiting signals but still filaments in the presence of serum (127, 279, 311). The *cst20* mutant also has attenuated virulence in a mouse model of infection (311). Cla4 is part of the Ste20 family of serine/threonine kinases. Like Cst20, the *cla4* homozygous deletion mutant is defective in hyphal formation in response to different conditions and has attenuated virulence in a mouse model (314).

Ste11, Hst7, and Cek1/Cek2 represent the MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK upstream of the transcription factor Cph1, respectively. Similarly to Cst20, *hst7* and *cek1* homozygous deletion mutants both show defective filamentation on solid medium in response to nutrient limitation (127, 279, 311). The deletion of *CST20*, *HST7*, or *CEK1* does not block filamentation induced by serum and does not dramatically affect the transcriptional profile of the yeast-to-hypha transition (241). Recently, Msb2 has been identified as a regulator of Cek1 (496). Along with Sho1, Msb2 activates Cek1, and the deletion of *MSB2* leads to defects in hyphal invasion on solid media containing poor carbon sources or under conditions of hypoxia (496). Intriguingly, recent evidence suggests that Cek1 can be regulated directly by environmental cues. For instance, Cek1 activation is regulated by quorum sensing, as the proper phosphorylation of Cek1 is blocked by the quorum-sensing molecule farnesol, which also prevents filamentation (494). Furthermore, the coincubation of *C. albicans* with the commensal bacterium *Streptococcus gordonii* leads to the phosphorylation and activation of *C. albicans* Cek1 and promotes hyphal growth and biofilm formation by relieving the farnesol-mediated repression of filamentation (39).

Cek1 activates the MAPK transcription factor Cph1 (338). The *cph1* homozygous deletion mutant shows a delayed initiation of filamentous growth on solid Spider medium but wild-type filamentation in response to serum (338) and is still able to cause lethal infections in the mouse (342). Although no hypha-specific *C. albicans* genes whose expression is dependent on Cph1 have been identified (74, 528), the degree of activation of certain hypha-specific genes is reduced in *cph1* mutant cells (16, 318). Interestingly, the deletion of *CPH1* does not dramatically affect the transcriptional profile of the yeast-to-hypha transition, but the overexpression of *CPH1* under yeast conditions leads to a transcriptional profile similar to that of wild-type cells undergoing the yeast-to-hypha transition (241).

pH pathway. A specific signaling cascade is involved in pH-mediated *C. albicans* morphogenesis (Fig. 4). Commonly, the transcriptional regulator Rim101 and its upstream activators define this cascade. In general, the deletion of genes that activate Rim101 results in an altered or improper expression of the pH-regulated genes *PHR1* and *PHR2* as well as morphogenetic defects under several conditions, including alkaline pH, Lee's medium, or M199 medium (136, 326, 463, 482).

Rim101 binds to and induces the expression of genes expressed preferentially at an alkaline pH while repressing those expressed preferentially at an acidic pH (24, 481). In alkaline environments, Rim101 is activated upon the proteolytic cleavage of its C-terminal inhibitory domain, while under acidic conditions, although it still undergoes cleavage, it remains inactive (326). The proper proteolytic cleavage of Rim101 is thought to be accomplished by the protease Rim13 (326) and facilitated by Rim20 (291). Rim8 is also upstream of Rim101 and is required for its activation (136, 463). The sensors of environmental pH for the Rim101 pathway are proposed to be Rim21 and Dfg16, which are seven-transmembrane-domain-containing proteins (41, 134). Although little is known about Rim21, Dfg16 is located in the plasma membrane, and the transcriptional profile of the *dfg16* homozygous deletion mutant is consistent with a defect in pH sensing (577).

The various components of the Rim101 pathway have been

studied with diverse models of *C. albicans* infection and virulence. The *rim8* homozygous deletion mutant has reduced virulence in the mouse model of systemic infection, and the deletion of numerous components of the Rim101 pathway results in attenuated virulence in a mouse model of keratomycosis (135, 385, 642). Rim101 is required for damage to oral epithelial cells *in vitro* as well as for virulence in an oropharyngeal candidiasis model of infection (424).

As mentioned above, *PHR1* and *PHR2* are pH-regulated genes and were among the first genes implicated in pH-mediated morphogenesis. These genes were identified in genetic screens and encode pH-regulated glucosidases, which maintain the cell wall shape and structure (182, 398, 510). Phr1 is important for the later stages of hyphal maintenance in *C. albicans*, and the homozygous deletion of *PHR1* results in reduced adhesion and invasive growth as well as abnormal septum formation (87). *PHR1* and *PHR2* have opposite expression patterns, as *PHR1* is expressed at alkaline pH and *PHR2* is expressed under more acidic conditions, although the deletion of both *PHR* genes results in pH-independent growth and morphological defects (398, 510). Interestingly, the homozygous deletion of *PHR1* or *PHR2* causes inverse patterns of virulence: the *phr1* mutant has reduced virulence in a mouse model of systemic infection but causes normal vaginal infection, while the *phr2* mutant is avirulent in the vaginal infection model but remains virulent during systemic infection (138, 203). This may represent an interesting example of how *C. albicans* responds to morphogenetic stimuli in a niche-specific manner.

Embedded pathways. Filamentation can occur when *C. albicans* cells are embedded in soft agar, as described above, and specific factors are involved in this process (Fig. 4). The homozygous deletion of *EFG1* or *CPH1* does not block embedded morphogenesis (488), although it was suggested that Efg1 may in fact repress filamentation under embedded conditions (205). The first factor identified to play a role in morphogenesis in embedded pathways was Czf1, as the *czf1* homozygous deletion mutant is completely blocked in filamentation under embedded conditions (77). The regulation of Czf1 is complex, as Czf1 is autoregulated and positively regulated by Efg1 (604). It was also suggested that Czf1 promotes filamentous growth by relieving the Efg1-mediated repression of embedded morphogenesis (205).

Another factor involved in embedded filamentation is the G protein Rac1. The homozygous deletion of *RAC1* causes hyphal defects similar to those caused by the deletion of *CZF1* (42). Rac1 is activated by the putative GEF Dck1, which is also necessary for filamentous growth under embedded conditions (230). Rac1 and Dck1 interact with Lmo1, which also plays a role in embedded morphogenesis, and together, these proteins function upstream of Cek1 (231). Interestingly, epistasis analysis suggests that Rac1, Dck1, and Lmo1 do not function in the same pathway as Czf1, indicating that multiple pathways are involved in agar-embedded filamentation (230, 231).

Cell cycle arrest pathways. Conditions that disrupt proper cell cycle progression can result in polarized growth through diverse cell cycle pathways (Fig. 4) (20, 33, 219). Notably, depending on the phase of cell cycle arrest, the cells will have different filamentation phenotypes. It is unclear whether these filaments represent true hyphae or pseudohyphae, as they seem to possess characteristics of both morphologies (Fig. 2).

Treatment with pharmacological cell cycle inhibitors can often influence filamentation. For example, the treatment of *C. albicans* with hydroxyurea (HU), which blocks DNA replication and arrests cells in S phase, causes cells to filament (20). Similarly, the treatment of cells with nocodazole, which depolymerizes microtubules and arrests cells in M phase, also results in cell elongation that can be partially suppressed by the deletion of the spindle checkpoint factor Mad2 (33). The treatment of *C. albicans* with aphidicolin, which inhibits DNA polymerase α, results in constitutive filamentous growth similar to that with HU (533). Strikingly, DNA damage itself caused by treatment with UV radiation or the DNA-alkylating agent methyl methanesulfonate (MMS) induces yeast cells to undergo filamentous growth (533). Moreover, specific FHA1 domain mutations in the DNA damage checkpoint protein Rad53 can uncouple filamentation from cell cycle arrest (533). Specifically, these mutations block filamentous growth without blocking cell cycle arrest in response to UV or MMS treatment (533). This provides intriguing evidence that filamentous growth mediated through Rad53 may be a function of cell cycle checkpoints and is not necessarily simply the result of cell cycle arrest.

The depletion of proteins involved in cell cycle regulation can also influence morphogenesis in *C. albicans*. For instance, the depletion of the cell cycle regulatory polo-like kinase Cdc5 causes cells to filament (20). The depletion of Rad52 triggers the DNA damage checkpoint and cell cycle delays as well as filamentous growth and the expression of filament-specific transcripts (12). As discussed above, the yeast-to-filament transition triggered by the depletion of Cdc5 or Rad52 is Cyr1 dependent and Efg1 independent (12, 20). Clb2 and Clb4 are B-type cyclins that are cell cycle regulated and are differentially expressed between yeast and hyphal morphologies in *C. albicans* (47). The depletion of either Clb2 or Clb4 causes filamentation: Clb2-depleted cells are inviable and arrest with hyperelongated projections, while Clb4-depleted cells are viable and are constitutively pseudohyphal (47). The depletion of the G_1 cyclin Cln3 causes yeast cells to arrest in the G_1 phase and then develop into pseudohyphae and hyphae, which ultimately resume the cell cycle (21, 100). This yeast-to-filament transition is dependent on morphogenetic regulators such as Efg1, Cph1, and Ras1 (21).

The Hgc1-Cdc28 complex plays a critical role in the cell cycle and filamentation in *C. albicans* (Fig. 4). *HGC1* is the only known hypha-specific transcript that encodes a cyclin as opposed to a cell wall protein, adhesin, or secreted protease. Hgc1 is a cyclin of the cyclin-dependent kinase Cdc28 and has an important function in hyphal development, as an *hgc1* homozygous deletion mutant is defective in polarized growth and cell chain formation during filamentous growth (652). Hgc1 is also required for proper septa formation, hyphal extension, and agar invasion in filamentation induced by the constitutive expression of the transcriptional regulator *UME6* (94). Cdc28 is also involved in morphogenesis, as the depletion of Cdc28 leads to filamentous growth and the expression of hypha-specific transcripts, such as *HWP1* and *ECE1* (590). Furthermore, the phosphorylation of the vesicle-associated protein Sec2 by Cdc28 is required for proper localization to the Spitzenkörper and overall normal hyphal development (58). The Cdc28-Hgc1

complex is also required for the phosphorylation and activation of the GAP Rga2, which localizes to hyphal tips and has an important role in modulating cell polarity via Cdc42 (653).

Together, Cdc28 and Hgc1 play a role in regulating septin ring formation during hyphal growth (207), and it was recently found that Cdc28 and Hgc1 have an important role in hyphal chain formation (609). The Cdc28-Hgc1 complex phosphorylates Efg1, which acts as negative regulator of Ace2 target genes (609). Ace2 is involved in septum degradation and cell separation, and thus, the phosphorylation of Efg1 by Cdc28-Hgc1 downregulates genes involved in cell separation and leads to proper cell chain formation during hyphal growth (609). This suggests an exciting correlation between Efg1, a key transcriptional activator of morphogenesis, and cell cycle proteins that can directly influence the morphological transition between yeast and filamentous growth states.

Pathways negatively regulating filamentation. Most of the pathways discussed above have involved the positive regulation of morphogenesis, yet many negative regulatory pathways influence *C. albicans* filamentation as well (Fig. 4). Tup1 was among the first repressors of filamentous growth identified. *tup1* homozygous deletion mutants are locked as pseudohyphae, even under conditions that generally favor growth in the yeast morphology. Epistasis analyses suggest that Tup1 acts in a separate pathway from either Efg1 or Cph1 (69, 70), and microarrays have revealed that Tup1 represses genes normally induced during morphogenesis, including some virulence factors (262). Tup1 has also been implicated in the response of *C. albicans* to the quorum-sensing molecule farnesol. A *tup1* mutant produces more farnesol than wild-type cells yet also fails to respond to farnesol (269). Furthermore, farnesol treatment causes the upregulation of *TUP1* mRNA and protein levels, indicating a relationship between this quorum-sensing molecule and the negative regulator Tup1 (269).

Nrg1 was first identified as a DNA binding protein that helped mediate Tup1 repression of filamentation. The homozygous deletion of *NRG1* derepresses filamentous growth, while the overexpression of *NRG1* represses filamentation in a Tup1-dependent manner (71). Furthermore, *NRG1* itself is downregulated during the yeast-to-hypha transition (71). Transcriptional profiling suggests that Nrg1 is also involved in the negative regulation of filament-specific transcripts but only targets Tup1 to a subset of promoters in the *C. albicans* genome (199, 262, 406, 407). Nrg1 also has an important role in biofilm formation, where high levels of *NRG1* inhibit yeast-to-hypha morphogenesis yet still produce yeast cells that incorporate into biofilms. Furthermore, the overexpression of *NRG1* increases the ability of yeast cells to disperse from biofilms (593).

Similar to Nrg1, Rfg1 is a negative regulator of filamentous growth that is thought to act alongside Tup1 (263, 275). The *rfg1* homozygous deletion mutant hyperfilaments on solid media under nutrient starvation conditions and is avirulent in a mouse model of infection (263). Like Nrg1, Rfg1 appears to contribute to the transcriptional repression of filament-specific transcripts by recruiting Tup1 to promoter elements, although Nrg1 seems to have a greater role than Rfg1 in this process (262, 263). Interestingly, it was recently shown that the overexpression of *RFG1* does not inhibit filament formation but rather leads to the formation of a pseudohyphal growth program (105).

Other pathways. Various other signaling cascades are involved in the regulation of morphogenesis, including pathways known to play important roles in *C. albicans* virulence and drug resistance. The TOR signaling pathway functions in response to nutritional cues to regulate cellular growth. Recently, it was demonstrated that the specific TOR inhibitor rapamycin blocks *C. albicans* filamentation under a variety of conditions, including growth on M199 medium and Spider medium (44). Moreover, the Tor1 protein kinase plays a role in the expression of several hypha-specific genes, including those encoding adhesins and the transcriptional repressors of filamentous growth Nrg1 and Tup1. The Tor1-mediated expression of adhesins is dependent on the transcription factors Efg1 and Bcr1, leading to a model whereby Tor1 negatively regulates the expression of adhesins by repressing Efg1 and Bcr1 (44).

As mentioned above, aside from the Cph1-regulated MAPK cascade, other MAPK modules play a role in *C. albicans* morphogenesis. These include the protein kinase C (PKC) cell wall integrity pathway and the high-osmolarity glycerol (HOG) MAPK pathway. In the PKC cascade, the MAPK Mkc1 plays a role in invasive hyphal growth on solid media (292). Furthermore, an *mkc1* homozygous deletion mutant produces abnormal biofilms that have reduced filamentation compared to the wild type (292). Meanwhile, in the HOG pathway, the MAPK Hog1, the adaptor protein Sho1, and the response regulator protein Ssk1 all play a role in *C. albicans* morphogenesis. The *hog1* and *sho1* homozygous deletion mutants are both defective in filamentation under various environmental conditions (8, 66, 497). Similarly, the mutation of an important phosphorylation residue of Ssk1 renders strains defective in hyphal growth under several conditions (380). The inhibition of morphogenesis in this *ssk1* mutant is linked to the downregulation of both *EFG1* and *CPH1* transcript expressions (380).

At present, high-throughput analyses of known and uncharacterized transcriptional regulators are facilitating the discovery of many novel pathways and factors regulating morphogenetic transitions (228). This remains a dynamic area for future discovery and analysis.

CANDIDA ALBICANS DRUG RESISTANCE: THE AZOLES

In recent decades, the increasing frequency of life-threatening fungal infections has been accompanied by an increase in the use of azoles prophylactically for high-risk individuals due to concerns of developing fungal infections or to treat patients who have already acquired fungal disease. This widespread deployment of azoles coupled with the fungistatic nature of these drugs has led to the emergence of azole resistance in clinical isolates (13, 111, 120, 507, 624). Canonical mechanisms of azole resistance identified in clinical isolates and experimental populations include alterations of the drug target Erg11 and the overexpression of multidrug transporters. Recent work has also implicated numerous fungal stress response pathways in the evolution and maintenance of azole resistance in *C. albicans* (Fig. 5A) (91, 111, 120). Furthermore, multiple signaling pathways have been demonstrated to affect resistance acquired by diverse mechanisms. Multiple mechanisms also

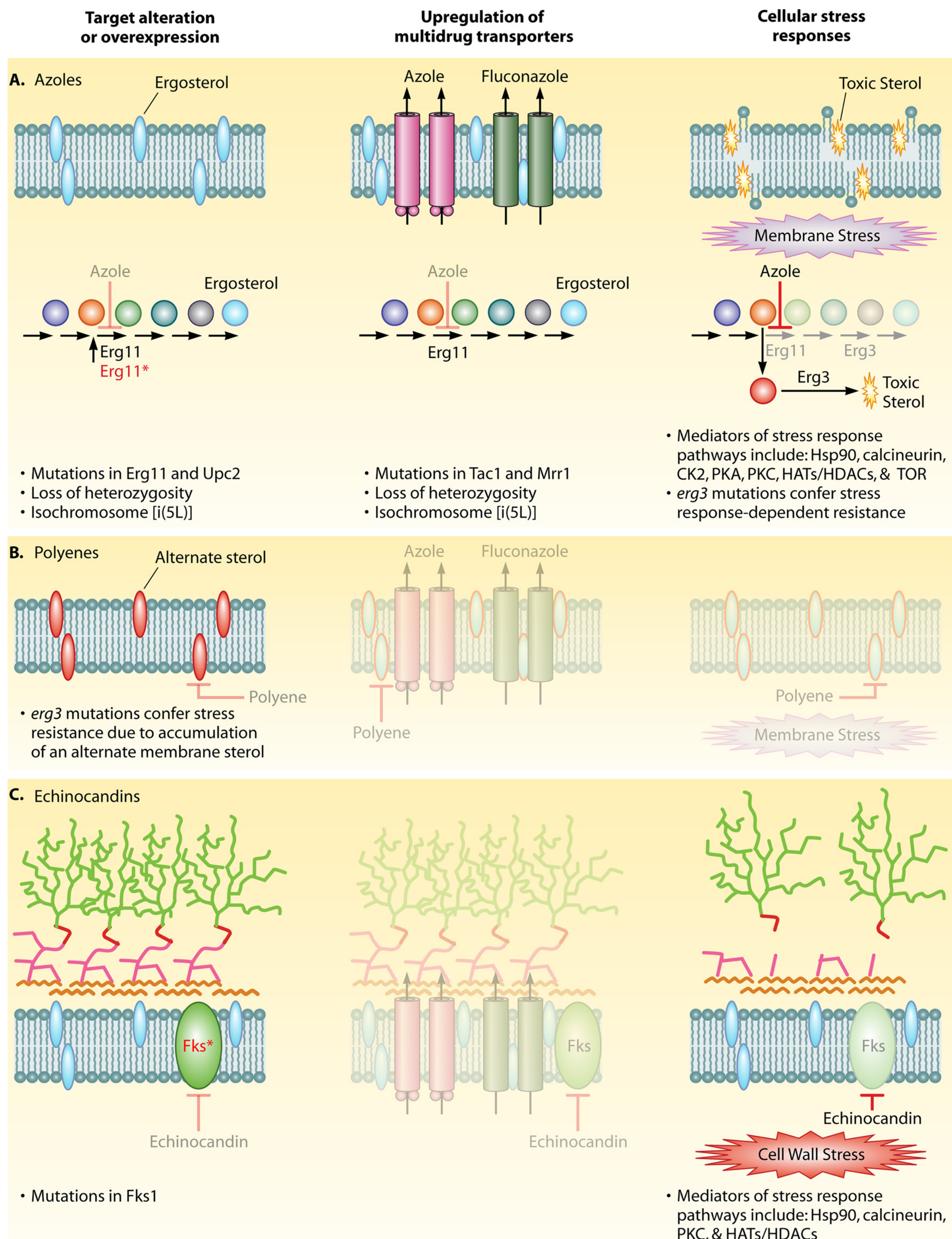

FIG. 5. *C. albicans* drug resistance mechanisms. (A) *C. albicans* can acquire resistance to the azoles through multiple mechanisms, including the upregulation or alteration of the drug target Erg11; the upregulation of the multidrug transporter Cdr1, Cdr2, or Mdr1 (fluconazole specific); or the induction of numerous cellular stress responses. (B) Although resistance to the polyenes is rare in *C. albicans*, resistance is acquired through loss-of-function mutations in *ERG3*, which block the production of ergosterol, inhibit the formation of the drug-lipid complex, and therefore prevent osmotic cellular lysis. The alteration of the drug transporters does not play a major role in polyene resistance, and cellular stress responses have not been implicated as major determinants of resistance. (C) Resistance to the echinocandins through mutations in two distinct hot-spot regions in *FKS1*, encoding the catalytic subunit of (1,3)-β-D-glucan synthase, has been widely found in *C. albicans* isolates. The upregulation of drug transporters does not play a major role in resistance; however, the induction of cellular stress responses is important for echinocandin resistance. The bullet points below each mechanism describe the manner in which resistance is acquired. Bright images represent those mechanisms important for that particular drug class, whereas dimmed images represent those mechanisms that do not play a key role. (Adapted from reference 111 with permission of Nature Publishing Group.)

contribute to the elevated azole resistance of *C. albicans* biofilms (142). The following sections will examine these mechanisms, including the effects of stress signaling pathways on azole resistance.

Alteration of the Drug Target

A mechanism of azole resistance that minimizes the impact of the drug on the cell is the alteration of the drug target, which can be achieved through mutation or overexpression. The three-dimensional structure of *C. albicans* Erg11 was modeled, and residues important for its interaction with the azoles were predicted (252). In addition, thus far, at least 12 different point mutations in Erg11, clustering into three distinct "hot-spot" regions, have been associated with azole resistance in clinical isolates (365). Biochemical analyses confirmed that mutations in Erg11 observed in the clinic decrease the affinity of azole binding *in vitro* (303). To determine the functional consequence of Erg11 mutations *in vivo* in the absence of other resistance mechanisms, *ERG11* alleles were cloned from a series of *C. albicans* clinical isolates and expressed in the model yeast *S. cerevisiae* (503). From this series, four Erg11 point mutations that increased azole resistance were identified (503), highlighting that individual mutations in Erg11 can confer azole resistance by decreasing the drug binding affinity. The loss of heterozygosity of *ERG11*, leading to the replacement of the wild-type allele with mutant alleles, can confer further increases in resistance (107). The overexpression of Erg11 has also been documented for *C. albicans* clinical isolates, although it is often accompanied by other alterations (623). In experimental populations of *C. albicans* evolved in the presence of fluconazole, resistance was acquired in distinct ways by distinct overexpression patterns of four genes important for fluconazole resistance, including *ERG11* (117). Although further work has shown that experimental increases in levels of *ERG11* alone can cause increased azole resistance (161), the clinical impact remains unclear.

ERG11 overexpression can be achieved through mutations in the transcription factor regulating its expression. The transcription factor Upc2 upregulates *ERG11* expression in response to azoles and thereby contributes to azole resistance (534). Upc2 binds to the azole-responsive enhancer element (ARE), a region localized to two distinct 7-bp sequences at positions −224 to −251 in the *ERG11* promoter (433). Both the ARE and Upc2 are necessary and sufficient for the azole induction of *ERG11* (433). Upc2 also binds to two distinct regions in its own promoter to autoregulate expression during azole exposure (229). Furthermore, G648D and G643A point mutations in Upc2 cause a hyperactivation of the transcription factor, resulting in the overexpression of ergosterol biosynthesis genes and increased fluconazole resistance (164, 223). These mutations have been identified in *C. albicans* clinical isolates (223).

Genomic alterations that lead to an increased gene dosage of *ERG11* provide an alternate route to target overexpression. Azole resistance in clinical isolates is also often associated with an isochromosome (two identical chromosome arms flanking a centromere) on the left arm of chromosome 5, [i(5L)], which harbors the *MTL* locus, *ERG11*, and a gene encoding a transcription factor that regulates multidrug transporters, *TAC1* (521). The predominant mechanism by which [i(5L)] increases azole resistance is through the amplification of *ERG11* and *TAC1* (522). The systematic deletion of *ERG11* and *TAC1* confirmed that these were the only open reading frames (ORFs) on [i(5L)] that affected azole resistance and that azole resistance increases exponentially with a linear increase in the number of *ERG11* and hyperactive *TAC1* alleles (522). The mechanism by which Tac1 regulates azole resistance will be discussed further in the following section on multidrug transporters. The isochromosome [i(5L)] also emerges as an azole resistance mechanism in experimental populations evolved in the presence of fluconazole, where it appears shortly after drug exposure in multiple populations (523). The isochromosome was associated with increased fitness in the presence and absence of drug and over time became fixed in independent populations (523). Genomic instability in *C. albicans* resulting in aneuploidies may be a common adaptive mechanism by which the pathogen responds to drug-induced stress in order to upregulate genes important for survival (521–523).

Upregulation of Multidrug Transporters

One of the two main classes of multidrug transporters that are upregulated in drug-resistant clinical isolates and experimentally evolved populations of *C. albicans* are the ATP binding cassette (ABC) transporter superfamily, including *CDR1* and *CDR2*. They contain two membrane-spanning domains and two nucleotide binding domains that utilize ATP to drive substrates across the membrane (90, 390). *CDR1* and *CDR2* were both identified based on their ability to complement a *PDR5* mutant in *S. cerevisiae* (466, 505). Further studies showed that a *C. albicans cdr1* homozygous deletion mutant was hypersensitive to azoles, whereas *cdr2* homozygous deletion mutants were not (505, 506). However, the combined deletion of both *CDR1* and *CDR2* resulted in an increased hypersensitivity compared to the deletion of *CDR1* alone, suggesting that *CDR2* contributes to azole resistance (505). Furthermore, many azole-resistant clinical isolates have up to a 10-fold increase in *CDR1* expression as well as an increase in *CDR2* expression (451, 507).

The expression of *CDR1* and *CDR2* is regulated by the transcription factor Tac1, which binds to a distinct *cis* sequence, termed the drug response element (DRE), found in their promoters (141). A *TAC1* hyperactive allele with an Asp977-to-Asn977 gain-of-function mutation has been identified in azole-resistant clinical isolates and confers the constitutive upregulation of Cdr1 and Cdr2 (108). Notably, this point mutation was coupled with a loss of heterozygosity, which is important since this zinc finger transcription factor functions as a homodimer, and the homozygosis of the hyperactive allele causes dramatic increases in fluconazole resistance (108). As mentioned above, *TAC1* is located on chromosome 5 along with *ERG11* and the *MTL* locus (109). Homozygosity at the *TAC1* locus is often associated with homozygosity at the *MTL* and *ERG11* loci (108). Recently, a study confirmed that mutations in *ERG11* and *TAC1* in a clinical isolate were solely responsible for its elevated fluconazole resistance (350). The deletion of *TAC1* followed by the sequential replacement of *ERG11* with wild-type alleles restored azole susceptibility to levels comparable to those of the parental isolate in both MIC

assays and mouse models of systemic candidiasis (350). In another recent study, five groups of related isolates containing azole-susceptible and azole-resistant counterparts were analyzed for mutations in *TAC1* and *ERG11* as well as for chromosome 5 alterations (107). It was proposed that many isolates acquired mutations conferring azole resistance in a predictable, sequential order: a gain-of-function mutation at *TAC1* along with mutations in *ERG11*, followed by a loss of heterozygosity of *TAC1* and *ERG11* and, finally, by the formation of [i(5L)], resulting in an increased copy number of azole resistance genes (107). Genome-wide studies were recently conducted to identify other *TAC1*-dependent genes in addition to *CDR1* and *CDR2*. Eight genes whose expression was modulated in a Tac1-dependent manner and whose promoters were bound by Tac1 were identified (341). Among these genes were *GPX1*, a putative glutathione peroxidase; *LCB4*, a putative sphingosine kinase; and *RTA3*, a putative phospholipid flippase (341). This suggests that the regulation of genes in other signaling pathways, such as oxidative stress responses and lipid metabolism, may play important roles in Tac1-mediated azole resistance.

The second main class of multidrug transporters important for azole resistance is the major facilitator (MF) class. MF drug pumps have no nucleotide binding domain but instead use the proton motive force of the membrane as an energy source (90, 390). *MDR1* is thus far the only MF gene cloned in medically important fungi, and it is involved specifically in resistance to fluconazole rather than other azoles (623). *MDR1* is overexpressed in fluconazole-resistant isolates (507), as documented for a series of 17 sequential isolates, where the overexpression of *MDR1* occurred early and correlated with major increases in resistance (624). The multidrug resistance regulator (Mrr1) is the transcription factor that controls the expression of *MDR1* (391) and is coordinately upregulated with *MDR1* in drug-resistant clinical isolates (391). In fact, in all clinical and *in vitro*-generated *C. albicans* strains that are fluconazole resistant due to increased levels of *MDR1* expression, gain-of-function mutations in *MRR1* are also present (163). Furthermore, most of these strains are homozygous for the *MRR1* allele, due predominantly to mitotic recombination on chromosome 3 (163). Much like Tac1, Mrr1 appears to have other targets besides drug efflux pumps, including oxidoreductases (391). Such targets may help prevent drug-induced cell damage that results from the generation of toxic molecules in response to azole exposure. This also suggests that multiple pathways are critical in order for the cell to survive the membrane stress associated with the azoles.

Thus far, we have illustrated several examples of the interconnectedness of resistance mechanisms that work together to help *C. albicans* survive in the presence of the azoles. Aneuploidies on chromosome 5 often lead to an increase in the level of expression of the drug target Erg11 as well as transcription factors that play important roles in drug efflux pump expression (522). Furthermore, these transcription factors regulate several other genes important for cellular stress response pathways in addition to drug transporters (341, 391). The remainder of this section will outline how different signaling pathways function to increase azole tolerance and resistance in *C. albicans*.

Cellular Stress Responses

The resistance mechanisms described above, including target alteration and the upregulation of drug transporters, are examples of how the cell is able to bypass the effect of the drug by blocking drug binding to the target or removing the drug from the cell. In addition to these mechanisms, *C. albicans* has evolved stress response pathways that enable the cell to cope with diverse stresses present in its environmental niche. The emerging paradigm is that these signal transduction pathways are critical for fungi to survive the stress induced by antifungal drugs; this applies both to the general tolerance of clinical isolates as well as to resistance acquired by specific mechanisms. One well-characterized mechanism that mitigates drug toxicity and confers resistance that is contingent upon stress responses involves a mutation in the Δ-5,6-desaturase encoded by *ERG3*. This blocks the production of the toxic sterol 14-α-methyl-3,6-diol, which would otherwise accumulate when Erg11 is inhibited; an alternate sterol, 14α-methyl fecosterol, becomes incorporated into the membrane, allowing the fungal cell to continue to grow and divide in the presence of the azoles. This mechanism of resistance has been detected in clinical isolates, although the prevalence and clinical significance remain unclear (273, 368).

Calcineurin. A key regulator of cellular stress responses in all eukaryotes is the Ca^{2+}-calmodulin-activated protein phosphatase calcineurin (189). Calcineurin functions in fungi to control a myriad of physiological processes, including cell cycle progression, cation homeostasis, morphogenesis, virulence, and antifungal drug responses (189). Calcineurin is not essential in *C. albicans*; however, it is critical for mediating cell survival during membrane stress exerted by fluconazole (see Fig. 9) (126). The inhibition of calcineurin pharmacologically with the well-characterized inhibitor tacrolimus (FK506) or cyclosporine acts synergistically with fluconazole in *C. albicans* (126). Furthermore, studies have shown that calcineurin inhibitors block *erg3*-mediated resistance in both *S. cerevisiae* and *C. albicans* (113, 115). In fact, calcineurin inhibition renders the normally fungistatic azoles fungicidal to several *Candida* species (437). It is important to note that FK506 and cyclosporine have other targets in the cell besides calcineurin, including multidrug transporters (483, 513). However, synergy between cyclosporine and fluconazole was confirmed *in vitro* and *in vivo* for strains lacking drug transporters implicated in azole resistance (364). In addition, the deletion of the regulatory subunit of calcineurin, *CNB1*, renders cells hypersensitive to the azoles, providing compelling evidence for calcineurin as a key regulator of azole resistance (126). Calcineurin function is essential for tolerance not only to the azoles but also to other antifungal agents, to several metabolic inhibitors, and to cell wall-perturbing agents, illustrating the importance of this protein in regulating responses to different cellular stresses (504, 536).

In *S. cerevisiae*, the best-characterized downstream effector of calcineurin is the zinc finger transcription factor Crz1. When calcineurin is activated, it dephosphorylates Crz1, resulting in Crz1 nuclear translocation (551, 552). Crz1 is the major effector of calcineurin-regulated gene expression in *S. cerevisiae*, activating genes involved in signaling pathways, small-molecule transport, cell wall integrity, and vesicular trafficking (641). The *C. albicans* homologue of Crz1 was identified by studies of

S. cerevisiae strains lacking *CRZ1* as a heterologous host (509). The *C. albicans* homologue was able to control the expression of calcineurin-responsive genes by acting on calcineurin-dependent response element (CDRE) sequences within their promoters (509). The deletion of *CRZ1* renders *C. albicans* hypersensitive to alkaline cations and membrane stress conditions, such as sodium dodecyl sulfate (SDS) and azoles, compared with a wild-type strain (438, 509). Notably, these phenotypes are not as pronounced, as a calcineurin mutant and *crz1* homozygous deletion mutants have no effect on virulence in a murine model of disseminated candidiasis (438). Moreover, in experiments with *S. cerevisiae*, the deletion of *CRZ1* only partially reduces *erg3*-mediated resistance, as opposed to the deletion of *CNB1*, which completely blocks resistance (113, 438). This suggests that Crz1 is not the sole downstream effector of calcineurin-mediated azole resistance.

Hph1 and Hph2 encode tail-anchored integral membrane proteins that colocalize to the ER. They serve redundant roles in *S. cerevisiae* by promoting survival during alkaline pH, high-salt, and cell wall stresses yet function independently of Crz1 (221). Calcineurin directly dephosphorylates Hph1, altering its distribution within the ER (221). Similar to Crz1, Hph1 and Hph2 serve partial roles in mediating azole resistance in *S. cerevisiae* (113). Furthermore, the deletion of these three calcineurin effector proteins still has only a partial effect on reducing *erg3*-mediated resistance, suggesting that other calcineurin targets are important for the response to azoles (113). No homologues of Hph1 or Hph2 have been identified in *C. albicans*, implicating other downstream effectors of calcineurin in antifungal responses.

Hsp90. Hsp90 is an essential molecular chaperone that regulates the form and function of diverse client proteins (450, 576). Hsp90 is more selective than general chaperones, as it preferentially interacts with a subset of the proteome, including proteins that function as key regulators of cellular signaling. As a consequence of its function in stabilizing key signal transducers, Hsp90 modulates the relationship between genotype and phenotype by acting as a capacitor for the storage and release of genetic variation (474, 501). Hsp90 potentiates the rapid emergence of azole resistance in *S. cerevisiae* (115). The Hsp90-dependent mechanism of resistance favored by this selection regimen is the loss of function of Erg3 (115). Notably, *S. cerevisiae* populations that gradually evolved azole resistance through the upregulation of the drug transporter Pdr5 maintain Hsp90-independent resistance (115). The role of Hsp90 in the evolution of azole resistance is conserved in the pathogenic yeast *C. albicans*, as inhibiting Hsp90 function blocked the emergence of azole resistance under a rapid-selection regimen (113, 115). In a *Galleria mellonella* model for *C. albicans* pathogenesis, there was a therapeutic benefit of combining clinically relevant Hsp90 inhibitors such as 17-AAG [17-(allylamino)-17-demethoxygeldanamycin] with fluconazole (118). Furthermore, in murine models of *C. albicans* disseminated disease, the genetic compromise of fungal *HSP90* expression enhanced the therapeutic efficacy of fluconazole (118). Thus, the targeting of fungal Hsp90 provides a powerful strategy for treating fungal disease by increasing the potency of existing antifungal agents.

Hsp90 regulates crucial responses to the membrane stress exerted by azoles via calcineurin (Fig. 6A). In diverse *S. cerevisiae* and *C. albicans* mutants tested, the inhibition of Hsp90 phenocopies the inhibition of calcineurin (115). In a series of clinical isolates recovered over time from an HIV-infected patient treated with fluconazole, the basal fluconazole resistance phenotypes of isolates recovered early during treatment were dependent on Hsp90 and calcineurin (Fig. 6B) (115). This dependence gradually evolved toward Hsp90 and calcineurin independence, corresponding with an upregulation of multidrug transporters (115). Calcineurin is an Hsp90 client protein in both *S. cerevisiae* and *C. albicans*; Hsp90 interacts with the catalytic subunit of calcineurin and is critical for maintaining its stability (247, 536). Genetic reporter assays revealed that azoles activate calcineurin-dependent stress responses in *C. albicans* and that the inhibition of Hsp90 blocks this activation (536), consistent with calcineurin being a key mediator of Hsp90-dependent azole resistance (113, 115). Hsp90 is one of the most highly connected hubs in cellular networks (376, 648). A high-throughput physical, genetic, and chemical-genetic study of *S. cerevisiae* suggested that approximately 10% of the yeast proteome interacts with Hsp90 (648), suggesting that Hsp90 may enable adaptation to diverse stresses in yeast through numerous distinct cellular regulators in addition to calcineurin.

Casein kinase 2. The casein kinase 2 (CK2) serine/threonine protein kinase plays vital roles in the cellular growth and proliferation of yeast. The kinase is composed of the catalytic subunits Cka1 and Cka2 and the regulatory subunits Ckb1 and Ckb2. In an insertional mutagenesis screen, mutations in *CKA2* were found to confer fluconazole resistance (78). The molecular basis for this increase in resistance was due to the overexpression of the drug pumps *CDR1* and *CDR2* in the *cka2* mutant (78). Although the *cka1* mutation had little effect on fluconazole resistance, the overexpression of *CKA1* suppressed the elevated fluconazole resistance of a *cka2* mutant, suggesting a role in resistance (78). Interestingly, the pharmacological inhibition of calcineurin with cyclosporine or the deletion of *CRZ1* reversed the fluconazole resistance of a *cka2* mutant, suggesting cross talk between these two signaling pathways (78). Furthermore, genome-wide chemical-genetic screens of *S. cerevisiae* revealed that subunits of the CK2 complex interact genetically with Hsp90 (376, 648). It will be interesting to further dissect the circuitry through which CK2, calcineurin, and Hsp90 interact to regulate fluconazole resistance.

cAMP-protein kinase A. The cAMP-dependent PKA is composed of the two catalytic subunits Tpk1 and Tpk2, along with the regulatory subunit Bcy1, and is critical for the regulation of stress responses (204). *tpk1* mutants have a lower tolerance to saline exposure, oxidative stress, and heat shock than do wild-type cells or *tpk2* mutants (204). As discussed above, Ras1 is an important component of the PKA signaling pathway. It is activated by the guanine exchange factor Cdc25 to stimulate the adenylyl cyclase activity of Cyr1, which works with the cyclase-associated protein Srv2 to produce cAMP. Elevated cAMP levels activate Tpk1 and Tpk2. Relative to a wild-type strain, *cyr1* and *srv2* mutants are hypersensitive to the azoles (248); this phenotype can be partially reversed by the addition of cAMP to the medium. Analyses of *CDR1* and *ERG11* transcript levels suggested that the basis of this phenotype is a defect in the azole-dependent upregulation of the *CDR1* drug pump (248). It would be interesting to explore how an impairment of PKA signaling affects other transcripts in order to evaluate if there are other factors affecting fluconazole resistance

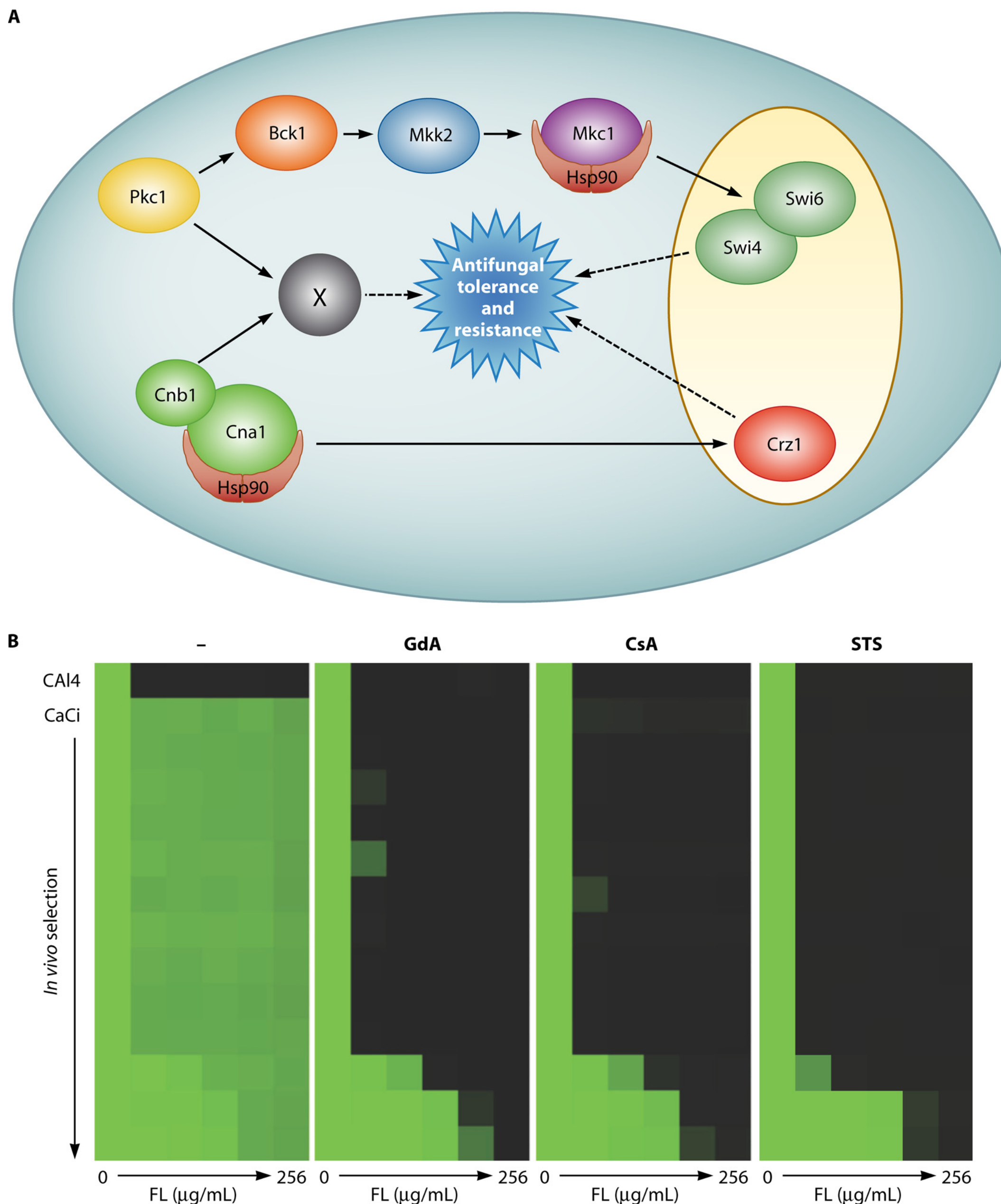

FIG. 6. Role of Hsp90 in antifungal drug resistance in *C. albicans*. (A) Simplified schematic of the mechanisms by which Hsp90 regulates responses to antifungals important for basal tolerance and resistance. Hsp90 interacts with and stabilizes the catalytic subunit of calcineurin (Cna1) to enable calcineurin-dependent stress responses through the effector protein Crz1 and through an additional target. Drug-induced stress also activates signaling through the Pkc1-regulated MAPK cascade, where the terminal kinase Mkc1 is an Hsp90 client protein. Notably, Pkc1 also signals through a distinct pathway in common with calcineurin to regulate antifungal drug resistance and tolerance. Hsp90, calcineurin, and PKC signaling regulate resistance to drugs that target the cell membrane and the cell wall. (B) A *C. albicans* laboratory strain (CAI4) and a series of clinical isolates (CaCi) obtained from an HIV-1-infected individual who was undergoing fluconazole (FL) treatment over the course of 2 years were evaluated for fluconazole resistance using an MIC assay. Clinical isolates at the top were recovered early in treatment, and those at the bottom were recovered late in treatment. Growth differences are color coded, with the brightest green representing maximal growth, light green representing intermediate growth, and black representing no growth. The laboratory strain was unable to grow at any concentration of fluconazole tested, while the clinical isolates displayed robust growth. The Hsp90 inhibitor geldanamycin (GdA), the calcineurin inhibitor cyclosporine (CsA), or the PKC inhibitor staurosporine (STS) reduced the resistance of all clinical isolates but had a greater effect on isolates recovered early in treatment than on those recovered late. (Left three panels of panel B adapted from reference 115 with permission of AAAS; right panel of panel B adapted from reference 301.)

besides *CDR1*. Recently, the key terminal morphogenetic regulator in the cAMP-PKA pathway, Efg1, was implicated in tolerance to azoles on solid medium (467). The *efg1* mutant exhibited a ~24% decrease in ergosterol production and showed an enhanced passive diffusion of radiolabeled fluconazole across the fungal membrane when grown on solid medium, which could potentially explain the increased azole susceptibility (467).

The PKA pathway also plays significant roles in the calcineu-

rin stress response pathway in *S. cerevisiae*. The activation of PKA decreases Crz1-dependent transcription through the phosphorylation of Crz1 at residues in or adjacent to the nuclear localization signal (264). The mutation of these residues to alanines results in the increased nuclear import of Crz1 and higher levels of both basal and calcium-induced Crz1 transcriptional activities (264). It seems somewhat contradictory that the PKA pathway blocks Crz1 activity, which is necessary for fluconazole resistance, while mutations in components of the PKA pathway confer hypersensitivity to the azoles. Future studies will be required to determine how the PKA and calcineurin pathways interact in *C. albicans* and how PKA signaling regulates fungal drug resistance.

Protein kinase C. Another key cellular stress response pathway implicated in mediating responses to antifungal drugs is the PKC cell wall integrity pathway. The sole PKC isoenzyme in *S. cerevisiae*, Pkc1, is essential under standard growth conditions and regulates the maintenance of cell wall integrity during growth and morphogenesis and in response to cell wall stress (195, 322, 650). Pkc1 signaling has been the focus of extensive study in *S. cerevisiae*, where it is known to regulate multiple targets, most notably the MAPK cascade composed of a linear series of protein kinases, including Bck1, Mkk1/2, and Slt2, that relays signals to the terminal transcription factors Rlm1 and Swi4/Swi6. While Pkc1 is not essential in *C. albicans*, the Pkc1-activated MAPK cascade is conserved in *C. albicans* with Bck1, Mkk1, and the Slt2 homologue Mkc1 (388). Recently, a drug screen of 1,280 pharmacologically active compounds identified molecules that abrogate the azole resistance of *C. albicans* and *S. cerevisiae* (301). Three out of the seven compounds identified in this screen were inhibitors of PKC. The deletion of *C. albicans PKC1* resulted in hypersensitivity to the azoles and rendered these normally fungistatic drugs fungicidal (301). Further genetic analyses confirmed that Pkc1 regulates responses to the azoles in part through the MAPK cascade, as the deletion of the downstream effector *BCK1* or *MKC1* enhanced sensitivity to the azoles (301). The deletion of *BCK1* or *MKC1* did not render the azoles fungicidal, implicating additional targets downstream of Pkc1 in azole tolerance. Strikingly, the pharmacological inhibition of Pkc1 phenocopied the inhibition of Hsp90 or calcineurin reducing the azole resistance of specific clinical isolates, suggesting a functional relationship between these regulators (Fig. 6B) (301). The genetic depletion of *C. albicans* Hsp90 resulted in the destabilization of Mkc1, thereby blocking PKC signaling via the MAPK cascade (301). This work reveals a new role for PKC signaling in tolerance to the cell membrane stress exerted by azoles and suggests that Hsp90 regulates responses to azoles via the novel client protein Mkc1 in addition to the established client protein calcineurin (Fig. 6A).

Histone deacetylases and histone acetyltransferases. Histone deacetylases (HDACs) and histone acetyltransferases (HATs) are most broadly recognized for their role in catalyzing the removal or addition of acetyl groups from the ε-amino group of lysines in core histones, resulting in changes in chromatin structure and gene expression (526, 640). With such central roles in the regulation of gene expression, it is not surprising that HDACs and HATs have important roles in regulating stress response pathways in yeast. One of the key HDAC complexes in yeast, the Rpd3 complex, is required for the proper expression of environmental stress response genes under multiple stress conditions in *S. cerevisiae* (6). Rpd3 is required for the binding and function of the stress-activated transcription factor Msn2, making it an important cofactor in the environmental stress response regulatory network (6). Moreover, Rpd3 suppresses both basal and induced *HSP82* expression in *S. cerevisiae* by interacting with its promoter region in response to heat shock (286). With regard to azoles, HDACs play a key role in maintaining tolerance and resistance. The broad-spectrum HDAC inhibitor trichostatin A (TSA) increases the sensitivity of *C. albicans* to azoles (540). One study has shown that TSA blocks the azole-induced upregulation of *ERG11*, *CDR1*, and *CDR2* (540). Finally, recent studies have implicated another HDAC, Hos2, in the response of *C. albicans* to the azoles. A specific Hos2 inhibitor, MGCD290, has synergistic activity with fluconazole against *C. albicans* clinical isolates (461).

HATs are also required for the maintenance of azole resistance. The Spt-Ada-Gcn5-acetyltransferase (SAGA) coactivator complex regulates numerous cellular processes through the coordination of histone posttranslational modifications (35). Recent work has shown that a *C. albicans ada2* homozygous deletion mutant is hypersensitive to fluconazole due to an impaired upregulation of *CDR1* and *MDR1* in response to azoles (518). Ada2 was found in the promoter regions of genes constitutively upregulated in azole-resistant clinical isolates, most probably through associations with Mrr1 (518). Moreover, Ada2 has roles in regulating Hsp90 by directly occupying its promoter region (518). In *C. albicans*, Hsp90 expression increases in response to heat during the unfolded protein response (UPR) (82). However, the upregulation of Hsp90 upon treatment with the UPR-inducing agent tunicamycin was abolished in an *ada2* mutant (518). Similar results have been found for *S. cerevisiae*, where the HAT enzyme Gcn5 is required for the basal and induced expression of *HSP82* through direct interactions with its promoter (286). The link between the SAGA coactivator complex and Hsp90 may help to further explain the importance of this complex in regulating azole resistance. A recent study showed that while the HAT enzyme Rtt109 has no impact on azole sensitivity, it is required for *C. albicans* pathogenesis in a mouse model of infection (344, 634). This is at least partially due to the increased susceptibility to macrophages and the altered profile of metabolic gene expression of the *rtt109* homozygous deletion mutant (344) as well as a weaker inflammatory response induced by the *rtt109* mutant (634). It is intriguing that different studies have implicated both HDAC and HAT complexes in regulating the elevated expressions of key drug resistance determinants in response to the azoles, although conventionally, these complexes have opposite effects, with HDAC complexes in general having repressive effects on gene expression.

In addition to HATs and HDACs, there are numerous other transcriptional regulators that act to orchestrate gene expression. Recently, a large-scale study examining the phenotypic consequences of deletion of 143 transcriptional regulators in *C. albicans* found that over 20% of the mutants had altered resistance to fluconazole or other ergosterol biosynthesis inhibitors (228). This supports the hypothesis that although antifungals may have a precise and focused mechanism of action,

a large number of transcriptional circuits regulate cellular responses required for the survival of drug-induced stress.

Other pathways. Tor protein kinases are global regulators of cellular growth in response to nutrient cues, including amino acids (512). In *S. cerevisiae*, the inhibition of Tor signaling has a multitude of effects, including the induction of autophagy, the inhibition of translation, the repression of ribosomal gene expression, as well as the induction of the expression of genes involved in the retrograde response, nitrogen catabolite response, and stress response (150, 512, 633). Tor protein kinases can be inhibited pharmacologically by the use of the immunosuppressant rapamycin. Recently, a study has shown that rapamycin decreases *erg3*-mediated azole resistance (489). This was confirmed to be due to an impairment of TOR signaling, as rapamycin had no effect on *C. albicans* TOR mutants that are rapamycin resistant (489). This was the first report of TOR signaling having a role in antifungal drug resistance. The molecular mechanism through which TOR signaling regulates responses to azoles remains an interesting area for further study.

Recently, studies have identified a critical role for Age3, an ADP ribosylation factor (ARF) GTPase-activating effector protein, in fluconazole resistance, morphogenesis, and virulence of *C. albicans* (170, 321). The deletion of *age3* abrogates resistance to a variety of azole drugs, and the pharmacological inhibition of ARF cycling with brefeldin A renders fluconazole fungicidal against a variety of azole-resistant mutants (170). The constitutive activation of calcineurin signaling renders wild-type cells resistant to the azoles; however, this effect is blocked in *age3* mutants (170). *age3* mutants abolish the resistance of *C. albicans* clinical isolates that constitutively overexpress Mdr1 and Erg11, suggesting that the effects of ARF signaling on azole resistance are distinct from the Hsp90-calcineurin stress response pathway (170). Furthermore, *age3* mutants have attenuated virulence in a wild-type mouse model of disseminated disease and an improved response to fluconazole in an immunocompromised mouse model (170), providing a genetic proof of principle for a new combinatorial therapy for *C. albicans* infections.

Biofilms

Biofilms are complex architectures of different cell types encompassed within an extracellular matrix that are initiated by the adherence of free-moving planktonic cells to surfaces such as catheters. These surface-attached communities have phenotypes distinct from those of their planktonic counterparts which have important implications for health and disease (60, 422). *C. albicans* biofilms are extremely resistant to most antifungals, including the azoles (60, 142, 422). This change in the resistance profile occurs in as little as 2 h after surface adherence (372). From a molecular perspective, there are several potential explanations for this increase in resistance. Ergosterol levels are depleted in intermediate and mature biofilms, and *CDR1* and *MDR1* are upregulated within 6 h of surface contact (399). In a *cdr1 cdr2 mdr1* homozygous deletion mutant, sensitivity to the azoles was observed in a biofilm grown for 6 h; however, resistance was fully restored after 48 h of growth (399). This suggests that the drug pumps contribute to azole resistance only at the early stages of biofilm formation. Calcineurin plays a key role in azole resistance in biofilms, and calcineurin inhibitors act synergistically with the azoles during biofilm growth *in vitro* and *in vivo* (592). The PKC pathway also has important functions in biofilm drug resistance. The terminal MAPK in the PKC pathway, Mkc1, is required for proper biofilm formation and for resistance to fluconazole (292). Finally, (1,3)-β-D-glucan levels in the fungal cell wall contribute to biofilm azole resistance (414). Biofilms are known to have increased (1,3)-β-D-glucan content, and the addition of glucanases increases the efficacy of fluconazole against *in vitro* and *in vivo* biofilms (355). With the clinical challenges of effectively treating *C. albicans* biofilms, it is critical to identify additional mechanisms that contribute to the azole resistance of these cellular communities.

CANDIDA ALBICANS DRUG RESISTANCE: THE POLYENES

Although the polyenes have been utilized in the clinic for decades, drug resistance is rare. This is in part due to the drugs' poor solubility and severe host toxicity, which limit their use over the long term. It is important to note that some *Candida* species, such as *C. lusitaniae* and *C. guillermondii*, display intrinsic resistance to amphotericin B (623). While the molecular basis for this primary resistance is not fully understood, these species do not often cause invasive infections and are therefore less of a concern in the clinic (623). Although the development of resistance is rare, case studies have documented amphotericin B-resistant clinical isolates of numerous *Candida* species in patients undergoing polyene treatment (561). Resistance to the polyenes has also been observed for individuals previously exposed to the azoles. *C. albicans* clinical isolates that were recovered from an AIDS patient undergoing long-term fluconazole therapy showed defective C-5,6-desaturase, leading to a block in the synthesis of ergosterol and the accumulation of an alternate sterol in the membrane (272, 273). Consequently, the clinical isolates showed resistance to fluconazole and cross-resistance to amphotericin B (Fig. 5B) (272). Similar situations have been documented for *C. glabrata*, in which mutations in the ergosterol biosynthesis gene *ERG6* led to the accumulation of late sterol intermediates and reduced susceptibility to the polyenes (598). Complementation with a wild-type copy of *ERG6* restored polyene susceptibility (598). Global gene expression studies have revealed that when *C. albicans* is exposed to amphotericin B, 256 genes are differentially expressed. These genes are involved primarily in protein synthesis, small-molecule transport, cell stress responses, and sterol metabolism (340). This suggests that there is a multitude of factors responsible for tolerance and resistance to polyenes besides target alteration.

A key regulator of cellular stress responses, the molecular chaperone Hsp90, has been implicated in responses to amphotericin B. A recombinant antibody against fungal Hsp90 (Mycograb) displays synergy with amphotericin B *in vitro* and in *in vivo* mouse models against a wide variety of *Candida* species (373). Combination therapy with Mycograb and amphotericin B has been shown to improve the outcome for patients with invasive candidiasis relative to treatment with either agent alone (442). The molecular mechanism by which this antibody enhances antifungal drug efficacy remains unclear. It is unlikely to be able to cross the fungal cell wall and affect the function

of cytosolic Hsp90, which is required for the regulation of crucial responses to drug-induced stress. Given the *in vitro* activity, it is unlikely to work solely by influencing the host immune response to the pathogen, although heat shock proteins are often immunodominant antigens for pathogen recognition and mediate both innate and adaptive immune responses (548, 565).

Similar to what has been observed for responses to the azoles, *C. albicans* biofilms generally display resistance to the polyenes (218, 477). The amphotericin B resistance of *Candida* biofilms has been attributed to the presence of persister cells within the population (302). A biphasic killing pattern was observed when cultures were exposed to amphotericin B (302). Furthermore, the reinoculation of surviving biofilm populations produced a new biofilm with novel subpopulations of persister cells (302). Finally, recent studies have revealed that HDAC inhibitors are able to increase the activity of amphotericin B against *C. albicans*, *C. krusei*, and *C. parapsilosis* biofilms (5), although the mechanism remains unclear. Notably, lipid formulations of amphotericin B have also been shown to improve the efficacy of this antifungal against *C. albicans* biofilms (290, 400).

CANDIDA ALBICANS DRUG RESISTANCE: THE ECHINOCANDINS

Although the echinocandins have been used in the clinic for only a relatively short time, cases of resistant isolates from patients treated with this antifungal have been documented (37, 224, 309, 447). The echinocandins are large lipopeptide molecules that act as noncompetitive inhibitors of (1,3)-β-D-glucan synthase, although the mechanistic details remain enigmatic (120, 143). The catalytic subunit is encoded by *FKS1* (157). The regulatory subunit encoded by *RHO1* is a GTP binding protein in the Rho/Rac subfamily of Ras-like GTPases and is a positive regulator of glucan synthase activity (471). The disruption of (1,3)-β-D-glucans causes a loss of cell wall integrity and severe cell wall stress on the fungal cell. Despite the fact that there are few long-term studies documenting echinocandin-resistant lineages, several mechanisms of resistance to these agents have been described, encompassing multiple stress response pathways. The section below outlines how *C. albicans* adapts to the cell wall stress exerted by the echinocandins.

Alteration of the Drug Target

Similar to the azoles and polyenes, a mechanism of resistance that bypasses the effect of the echinocandin on the cell is an alteration of the drug target (Fig. 5C). In fact, the best-characterized mechanism of resistance to the echinocandins is the mutation of the drug target Fks1. Laboratory studies of both *C. albicans* and *S. cerevisiae* have mapped two distinct hot-spot regions in the *FKS1* gene that are important for echinocandin resistance (455). The first hot-spot region encompasses amino acids 641 to 648 in *C. albicans FKS1*, with Ser645 playing a particularly important role in resistance (448). The mutation of this amino acid to Pro645, Phe645, or Tyr645 is the most prevalent mutation in *C. albicans* isolates and correlates with significantly higher levels of echinocandin resistance (37, 447). The second hot-spot region in *FKS1* corresponds to amino acids 1345 to 1365. Studies of *S. cerevisiae* identified a key mutation in this region, R1357S, which increases resistance to the echinocandins (447). Fks1 mutations have *in vivo* relevance, as they confer reduced drug efficacy in murine models of disseminated candidiasis (447). Furthermore, isolates of *C. albicans* that show increased resistance to the echinocandins in the clinic often contain mutations in the hot-spot regions described above (149, 447). Similar to the case for the azoles, *C. albicans* isolates collected from a single patient over the course of echinocandin treatment were used to identify the molecular basis of resistance. The progressive decline of a clinical response to micafungin therapy was associated with the acquisition of mutations in *FKS1* (309). Similar mutations in *FKS1* have been observed for multiple isolates of other *Candida* species with decreased susceptibility to the echinocandins (455). Recently, a series of *C. glabrata* bloodstream isolates that showed elevated echinocandin MICs were examined for mutations in the drug targets *FKS1* and *FKS2*, and all of these isolates contained mutations in previously identified hot-spot regions, implicating frequent drug target alterations in clinical echinocandin resistance (657).

Upregulation of Multidrug Transporters

Unlike the azoles, the upregulation of multidrug transporters plays a rather minor role in echinocandin resistance. Studies have monitored the caspofungin resistance of *C. albicans* clinical isolates that show increased levels of azole resistance. Regardless of the underlying azole resistance mechanism, the echinocandins displayed potent *in vitro* activity, suggesting that they are not substrates for the Cdr1, Cdr2, or Mdr1 drug transporters (22, 459). Experimental studies suggested that the Cdr drug pumps may play a minor role in echinocandin resistance. When Cdr2 was expressed in *S. cerevisiae*, an increase in resistance to caspofungin was observed (514). Likewise, Cdr2 increased caspofungin resistance when constitutively overexpressed in a drug-sensitive *C. albicans* strain (514). In contrast, it was observed that a *C. albicans* clinical isolate overexpressing both Cdr1 and Cdr2 exhibited reduced susceptibility to caspofungin and a slightly increased susceptibility to micafungin in agar plate assays (419). None of the strains showed significant resistance to micafungin or caspofungin in liquid microdilution susceptibility assays (419). Overall, these findings support the notion that the multidrug transporters play at most a minor role in echinocandin resistance, and this seems to be critically dependent on the drug and method used to assess antifungal susceptibility.

Cellular Stress Responses

Calcineurin. As discussed above, calcineurin is a key regulator of cellular stress responses crucial for resistance to the azoles in *C. albicans*. Historically, its role in echinocandin resistance has been less clear, although recent work confirms that it does indeed play an important role. The pharmacological inhibition of calcineurin with cyclosporine inhibits the growth of wild-type *C. albicans* strains at higher concentrations of caspofungin (628). However, whether calcineurin mediates basal tolerance to the echinocandins was unclear, since in one

study the deletion of the regulatory subunit of calcineurin encoded by *CNB1* had no effect on echinocandin susceptibility (126), while in another study the deletion of the catalytic subunit of calcineurin encoded by *CNA1* enhanced the caspofungin killing activity (504). A recent study validated the role of calcineurin in mediating resistance to the echinocandins. The pharmacological or genetic impairment of calcineurin function reduced the tolerance of *C. albicans* to the echinocandins, creating a fungicidal combination (536). The pharmacological inhibition of calcineurin with cyclosporine blocked the echinocandin-mediated upregulation of calcineurin-dependent stress responses, as measured by the ability of Crz1 to activate CDRE-regulated genes (536). The inhibition of calcineurin also had a synergistic effect with echinocandins against some but not all clinical isolates of *C. albicans* that evolved echinocandin resistance in the host by mutations in *FKS1* (536). Even isolates with the same Fks1 mutation had differential responses to calcineurin inhibition, suggesting that multiple mechanisms of resistance may be operating in some strains. Similar to the case for the azoles, the calcineurin downstream effector Crz1 plays a partial role in echinocandin tolerance (536). Furthermore, this transcription factor is required for the expression of several chitin synthases in response to stress conditions (405). This suggests that although Crz1 plays a role in echinocandin tolerance, other downstream effectors must also mediate echinocandin tolerance.

Hsp90. Given that calcineurin regulates echinocandin resistance and Hsp90 regulates calcineurin stability and function, it stands to reason that Hsp90 would have a key role in crucial responses to echinocandins. Indeed, the pharmacological or genetic compromise of Hsp90 abrogates echinocandin tolerance in *C. albicans* and results in a fungicidal combination under conditions where echinocandins alone are fungistatic (536). Moreover, the pharmacological inhibition of Hsp90 is synergistic with echinocandins against resistant clinical isolates, and the genetic compromise of *C. albicans* Hsp90 renders echinocandins more efficacious in a murine model of disseminated candidiasis (536). As a regulator of responses to azoles, polyenes, and echinocandins, Hsp90 holds much promise as a target for combinatorial antifungal therapies.

Protein kinase C. A key cellular stress response pathway that mediates resistance to the echinocandins is the PKC cell wall integrity pathway. Preliminary studies conducted with *S. cerevisiae* were the first to link this pathway to echinocandin tolerance. Genome-wide microarray analyses revealed that caspofungin treatment rapidly induced the expression of numerous genes from the PKC pathway (484). The transmembrane proteins of the Wsc family and Mid2 sense defects in cell wall integrity and signal through Rom2 to activate the GTPase Rho1. Rho1 positively regulates many downstream targets, including Pkc1 and the (1,3)-β-D-glucan synthase subunits Fks1 and Fks2 (322). Furthermore, the terminal MAPK in the cascade, Slt2, is activated by phosphorylation after exposure to echinocandins (484), and cells lacking *SLT2*, *BCK1*, and *PKC1* are hypersensitive to echinocandins, demonstrating that the PKC pathway is required for echinocandin tolerance (366, 484). Similar results have been reported for *C. albicans*. The terminal MAPK in *C. albicans*, encoded by *MKC1*, is required for echinocandin tolerance in wild-type strains, and its expression is upregulated in response to caspofungin (628).

The *C. albicans* PKC pathway has been implicated in upregulation of the expression of chitin synthase (CHS) genes in response to (1,3)-β-D-glucan synthase inhibition by the echinocandins. In *C. albicans* there are four members of the chitin synthase gene family: *CHS1*, *CHS2*, *CHS3*, and *CHS8*. In the presence of caspofungin, (1,3)-β-D-glucan and (1,6)-β-D-glucan levels decreased by 81% and 73%, respectively (562). However, levels of chitin increased by 898%, suggesting that the compensation of another polymer in the cell wall may provide a mechanism of echinocandin resistance (562). An additional MAPK cascade, the HOG pathway, has been implicated in regulating cell wall architecture in *C. albicans* (165). PKC, HOG, and calcineurin signaling coordinately controls chitin synthesis in *C. albicans* in response to a variety of cell wall and cell membrane stresses (405). Under normal conditions, Hog1 is required for basal levels of *CHS1* transcription; however, under stress conditions both Hog1 and Crz1 are required (405). Furthermore, the expressions of *CHS2* and *CHS8* are dependent on Crz1, Hog1, and Mkc1 under normal and stress conditions (405). Further work using echinocandins as the cell wall stressor confirmed that they stimulate chitin synthase gene expression, leading to an increase in CHS activity and a decrease in drug efficacy (607). The upregulation of the CHS genes in response to echinocandins is dependent on the PKC, HOG, and calcineurin pathways, and the pretreatment of cells with a cell wall stressor increases echinocandin resistance through the activation of these pathways (607). Intriguingly, the treatment of cells with echinocandins not only increases the chitin content but also induces the formation of novel structures, such as a salvage septum, that enable the cell to continue to grow and divide under otherwise lethal echinocandin concentrations (607). Finally, the PKC cell wall integrity pathway was found to operate through Rlm1 elements in the *CHS2* and *CHS8* promoters, although promoter regions recognized by the calcineurin and HOG pathways remain to be identified (317).

Histone deacetylases and histone acetyltransferases. Similar to what was observed with regard to azole resistance, HDACs and HATs influence echinocandin resistance in *C. albicans*. Recently, the HAT Rtt109 and the HDAC Hst3 were shown to regulate H3K56 acetylation in *C. albicans* (634). The deletion of *RTT109* conferred increased sensitivity to micafungin and caspofungin as well as to other genotoxic stresses such as hydroxyurea and methyl methanesulfonate (634). Furthermore, many clinical isolates of *C. albicans* were sensitive to nicotinamide, an inhibitor of NAD^+-dependent HDAC complexes such as Hst3, and when used in mouse models of infection, nicotinamide was shown to reduce the fungal kidney burden (634). Nicotinamide was active against other *Candida* species and against *A. fumigatus* and *Aspergillus nidulans*, suggesting that this compound may have broad antifungal properties worth further investigation (634).

Biofilms

As mentioned above, the biofilm cellular state confers dramatic increases in resistance to azoles; however, this does not seem to be the case with echinocandins (23, 142, 290). The paradoxical effect, or the ability of *C. albicans* to grow under

conditions with elevated concentrations of echinocandins, does seem to be pronounced and frequent when *C. albicans* clinical isolates are grown as biofilms compared to their planktonic counterparts (379). This resistance could be due to the fact that increased levels of (1,3)-β-D-glucans are found in the biofilm cell wall compared to the cell wall of their planktonic counterparts (414).

CONNECTIONS BETWEEN MORPHOGENESIS AND ANTIFUNGAL DRUG RESISTANCE IN *CANDIDA ALBICANS*

It has been appreciated for some time that there is a connection between antifungal drugs and morphological development in *C. albicans*. Several azole-derivative antifungals, including imidazole and triazole derivatives, affect hyphal development (427). These antifungals limit branch formation in hyphae and, at high concentrations, arrest hyphal development completely (427). Importantly, this antifungal-modulated morphogenesis phenotype does not affect the cellular growth rate, and the removal of the antifungals leads to a reversion to normal morphogenesis (427). One explanation for the relationship between azole treatment and morphogenetic defects comes from the finding that *C. albicans* cells treated with various different azoles produce elevated levels of farnesol, which blocks the morphogenetic transition from yeast to filamentous growth (233). Another explanation is that the blocking of sterol biosynthesis with azoles results in a loss of ergosterol polarization, which is needed for proper filamentous growth (369). In particular, ergosterol-enriched lipid rafts are present specifically during *C. albicans* filamentous growth and are thought to be important for the morphogenetic transition (369). Amphotericin B also inhibits the morphogenetic transition when used at subinhibitory concentrations (217).

Interestingly, there is also a relationship between morphogenesis and antifungal drug resistance in *C. albicans*. A positive correlation has been observed between the level of antifungal drug resistance and the ability to form hyphae in the presence of drugs (213). In the presence of hypha-inducing cues and azole drugs, azole-resistant *C. albicans* clinical isolates form hyphae, while susceptible isolates do not (213). This was observed to occur independent of the mechanism of resistance in the clinical isolates (213). Furthermore, gene expression profiling of azole-resistant and azole-sensitive *C. albicans* isolates from bone marrow transplant patients revealed the differential expression of genes involved in morphogenesis, such as *EFG1*, *CPH2*, and *TEC1*, in resistant compared to susceptible isolates (636).

As described in the previous sections of this review, numerous *C. albicans* signaling cascades are involved in regulating morphogenesis and drug resistance. Many of these pathways, including the cAMP-PKA, TOR, and MAPK signaling cascades, are involved in both drug resistance and morphogenesis. Specific proteins, such as Hsp90, are also crucial for both processes, emphasizing the important relationship between the regulatory control of morphogenetic transitions and resistance to antifungal agents. Several pathways and proteins that have not yet been subject to discussion also have overlapping roles in morphogenesis and antifungal resistance. In many cases, *C. albicans* mutants that are defective in filamentation display increased sensitivity to antifungal compounds. For instance, the family of protein *O*-mannosyltransferases (Pmt proteins), which regulate the O mannosylation of secretory proteins, is important for both morphogenesis and drug resistance. *pmt1*, *pmt2*, *pmt4*, and *pmt6* mutants are defective in hyphal formation under certain conditions and display hypersensitivity to various antifungal compounds (469, 580). Similarly, the deletion of the sphingolipid biosynthesis gene *IPT1* results in morphogenetic defects and hypersensitivity to numerous antifungals (468). Recently, it was shown that the transcriptional regulator Ndt80, which is a regulator of the *CDR1* drug efflux pump and which plays a role in sterol metabolism and drug resistance (101, 520), also plays a role in morphogenesis, the proper expression of filament-specific transcripts, and cell separation (519). Beyond signaling pathways, another way in which morphogenesis is linked to drug resistance is through biofilm formation. Morphogenesis is a critical part of biofilm formation (34, 60, 486), and biofilms are generally more resistant to antifungal drugs than are free-moving planktonic cells (34, 475, 479).

CRYPTOCOCCUS NEOFORMANS

C. neoformans is an important opportunistic pathogenic fungus of humans; the clinical prevalence has dramatically increased in the past 2 decades due to AIDS, cancer chemotherapy, and immunosuppression for organ transplantation. It is the etiological agent of a variety of diseases, including meningoencephalitis in immunocompromised individuals, and has mortality rates approaching 30% for AIDS patients (333, 452). Furthermore, this pathogen is estimated to cause 1 million cases of cryptococcal meningitis per year, with most incidents being reported in sub-Saharan Africa (446). *C. neoformans* is the most common fungal infection of the central nervous system (CNS) and the third most frequent neurological complication in AIDS patients (333). The key mediators of virulence in *C. neoformans* include growth at the mammalian host temperature, the production of a polysaccharide capsule, the deposition of laccase-synthesized melanin in the cell wall, the secretion of enzymes, and resistance to host defenses, such as oxidative and nitrosative killing (435, 454, 553, 606). Recently, a large-scale screen examined 1,201 gene deletion mutants in *C. neoformans* and discovered that mutants exhibiting virulence defects often displayed defects in growth at elevated temperatures, capsule formation, and/or melanization, confirming that these traits are strongly linked to virulence (339). Notably, this screen also identified 48 novel infectivity genes that affected proliferation in the mouse lung yet did not affect growth, capsule, or melanin, implicating additional molecular mechanisms governing *C. neoformans* virulence that remain to be discovered (339).

Initial infection with *C. neoformans* is acquired through the inhalation of small yeast cells or basidiospores from an environmental source, often pigeon guano (333). Primary infection is established in the lung, and when host immunity is compromised, the fungus is capable of disseminating throughout the body to other organs, including the CNS. It is postulated that the organism's predilection for the CNS is enhanced by the presence of neurotransmitters that can be scavenged as diphenolic precursors to synthesize the virulence factor melanin

(299, 333, 452). Several mechanisms are thought to contribute to brain invasion by *C. neoformans*, and defining the molecular mechanisms governing this process remains an important area of research. The treatment of cryptococcal disease has relied on the azoles, amphotericin, and 5-flucytosine, a drug that ultimately interferes with DNA and RNA synthesis (120).

C. neoformans is a basidiomycetous fungus that is classified into distinct varieties or serotypes. Traditionally, based on capsular agglutination reactions there are five classical serotypes: serotypes A, B, C, and D and an AD hybrid. More recently, molecular studies and genome sequence analyses have grouped *C. neoformans* into two distinct varieties, *C. neoformans* var. *grubii* (serotype A) and *C. neoformans* var. *neoformans* (serotype D), and a sibling species, *C. gattii*. Notably, this sibling species was originally defined as *C. neoformans* var. *gattii* (serotypes B and C) (333). Of the subtypes, *C. neoformans* var. *grubii* serotype A is the most common cause of human disease (333). In terms of global distribution, *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans* are found worldwide, corresponding to their prevalence in causing infection in immunocompromised hosts (333). In contrast, *C. gattii*, which is endemic primarily in tropical and subtropical regions, causes 70% to 80% of cryptococcal infections in immunocompetent hosts, mostly in Australia and Papua New Guinea (333) but also recently in Canada with the Vancouver Island outbreak (191). Strains of *C. gattii* were recently shown to be expanding from Vancouver throughout the Northwest United States, with recombination between strains contributing to the emergence of novel hypervirulent genotypes (85).

Several *C. neoformans* virulence factors contribute to its ability to evade immune responses (for a full review see reference 606). After phagocytosis into macrophages, *C. neoformans* is capable of proliferation within these infected cells and can eventually lead to host cell lysis (588). *C. neoformans* can also escape from macrophages by a nonlytic expulsive mechanism that may contribute to CNS invasion (11, 255, 348). Notably, this pathogen does not inhibit phagosome-lysosome fusion, nor does the yeast interfere with phagosome maturation or acidification, as is commonly achieved by other intracellular pathogens (606). Furthermore, the polysaccharide capsule inhibits phagocytosis by macrophages, dendritic cells, and neutrophils, in addition to its role in providing protection against reactive oxygen and nitrogen species within the immune cell (606). Finally, *C. neoformans* is able to undergo a process referred to as phenotypic switching, which alters the morphology of the colony between smooth and mucoid, with the latter showing increased survival in murine macrophages (192, 212).

Unlike the opportunistic pathogen *C. albicans*, *C. neoformans* has a fairly well-defined sexual cycle. It possesses a bipolar *MAT* structure with two distinct mating alleles, **a** and α. Its *MAT* locus is larger than those of many other fungi, spanning a 120-kb region that contains over 20 genes encoding homeodomain transcription factors, pheromones, pheromone receptors, and factors involved in the mating signaling cascade (246, 319). In many pathogenic fungi, including *C. neoformans*, sexual reproduction correlates with virulence. For example, the Ste3**a** and Ste3α pheromone receptors not only are required for pheromone sensing and mating but also have been implicated in pathogenicity (98, 104). Another intriguing facet of the *C. neoformans* mating cycle is that it is able to undergo same-sex mating. Fusion and meiosis occur between nonisogenic α strains, with recombination rates similar to those observed during **a**/α sporulation (334). This may explain how genetic exchange occurs in the natural environment where the α-mating type predominates (295). The following sections will focus on the role of morphogenesis and drug resistance in the life cycle and pathogenicity of *C. neoformans*.

INTRODUCTION TO *CRYPTOCOCCUS NEOFORMANS* MORPHOGENESIS

C. neoformans exists most commonly as a budding yeast in both clinical and environmental sources; however, it is capable of undergoing a dimorphic transition to filamentous growth by two main differentiation pathways: mating and monokaryotic fruiting (Fig. 7). There are key structural differences between filaments produced during mating and monokaryotic fruiting. Hyphal cells produced during fruiting are mononucleate with unfused clamp connections, whereas mating hyphal cells are dikaryotic and linked by fused clamps (333). This section will briefly introduce the two main differentiation pathways and summarize how *C. neoformans* morphogenesis is tightly linked to its virulence.

The mating pathway is initiated with the fusion of haploid cells of opposite mating types to produce dikaryotic filaments. This eventually leads to the formation of a basidium, where meiosis occurs to produce four chains of basidiospores (333). The environmental signals governing this process are either nutrient limitation or the presence of pheromones. Intriguingly, the *MAT*α mating type is highly predominant in environmental isolates, accounting for 98 to 99.9% of the population (295). Moreover, in a direct comparison of congenic strains, the *MAT*α mating type displays increased virulence in a murine model of systemic cryptococcal infection (298). A molecular mechanism explaining this mating-type-specific virulence attribute is the presence of a Ste12α homologue in the *MAT*α region, as the overexpression of *STE12* stimulates the production of important virulence factors such as melanin (627). This finding was corroborated by an *in vivo* study that showed that the overexpression of *STE12* alone increased virulence in animal models of infection (99). Notably, other groups have observed that Ste12α does not play a role in virulence in *C. neoformans* (643), although the discrepancies can conceivably be explained if the virulence attributes controlled by Ste12 are serotype dependent. Notably, *MAT*α strains preferentially disseminate to the CNS during coinfection, illustrating a potential evolutionary advantage and suggesting an explanation for why the majority of clinical isolates are of the *MAT*α mating type (417).

The second pathway regulating morphogenesis, monokaryotic fruiting, occurs when haploid spores produce filaments and basidiospores in response to severe nitrogen starvation or water deprivation in the absence of a mating partner. This phenomenon is common in other basidiomycetes, and it is thought to enable *C. neoformans* to scavenge for nutrients in the environment (333). Although the *MAT*α mating type is predominant in the natural environment, both *MAT***a** and *MAT*α cells are capable of undergoing this morphological transition (586). Furthermore, studies have shown that fruiting and filamentation are enhanced in *MAT*α cells upon contact with *MAT***a** cells (614). Thus, perhaps a normal function of haploid

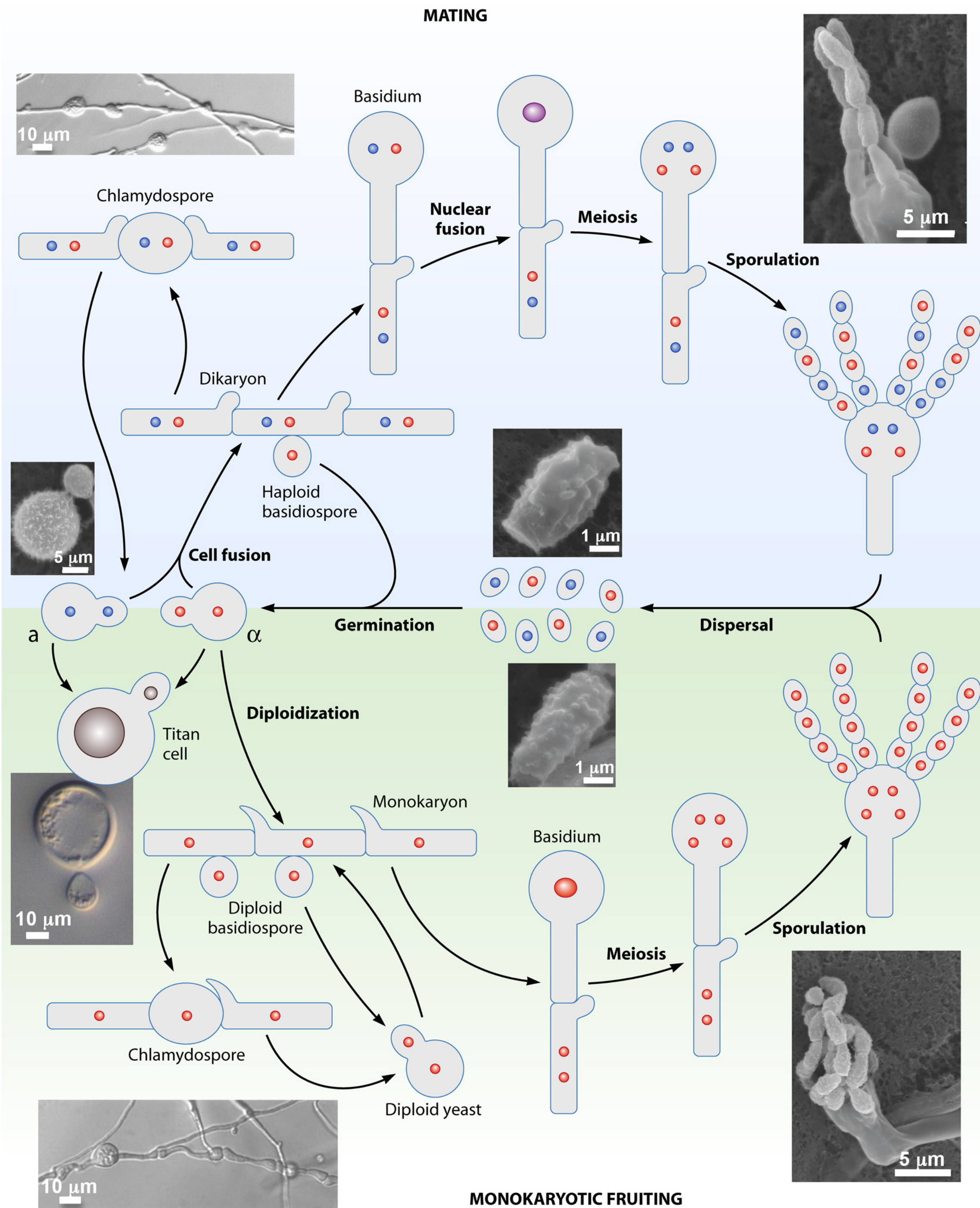

FIG. 7. The *C. neoformans* life cycle. *C. neoformans* exists most commonly as a budding yeast; however, it is capable of undergoing a dimorphic transition to filamentous growth by two main differentiation pathways: mating and monokaryotic fruiting. The mating pathway is initiated, under nutrient-limiting conditions, with the fusion of haploid cells of opposite mating types to produce dikaryotic filaments. During this time the two parental nuclei migrate coordinately in the hyphae, a septum forms to separate the cells, one nucleus is transferred to the penultimate hyphal cell via a clamp connection, and the clamp cell and hyphal cell fuse. During this hyphal growth, blastospores can bud from the hyphae and divide mitotically in the yeast form. Some hyphal cells can enlarge and form chlamydospores. As the basidium continues to develop, meiosis occurs, where eventually four chains of basidiospores are produced. The second pathway regulating morphogenesis, monokaryotic fruiting, occurs when haploid spores produce filaments and basidiospores in response to severe nitrogen starvation or water deprivation in the absence of a mating partner. Cells of one mating type form diploid monokaryotic hyphae, where rudimentary clamp connections are formed, which do not fuse to the preceding cell. Blastospores and chlamydospores can also form. At the stage of basidium development, meiosis occurs, and haploid basidiospores are produced in four chains. Finally, *C. neoformans* is capable of forming extremely large polyploid cells, referred to as "titan cells" or "giant cells," in a human host. Microscopy images of the various stages of the *C. neoformans* life cycle are included beside their cartoon representations. Red circles represent *MAT*α nuclei, blue circles represent *MAT***a** nuclei, purple circles represent diploid nuclei with **a**/α content, and gray circles represent either *MAT*α or *MAT***a** nuclei. (Figure adapted from reference 333 with permission from Annual Reviews; scanning electron microscopy images reprinted from reference 601 with permission; image of titan cell courtesy of K. Nielsen [University of Minnesota], reproduced with permission; images of chlamydospores reprinted from reference 332 with permission.)

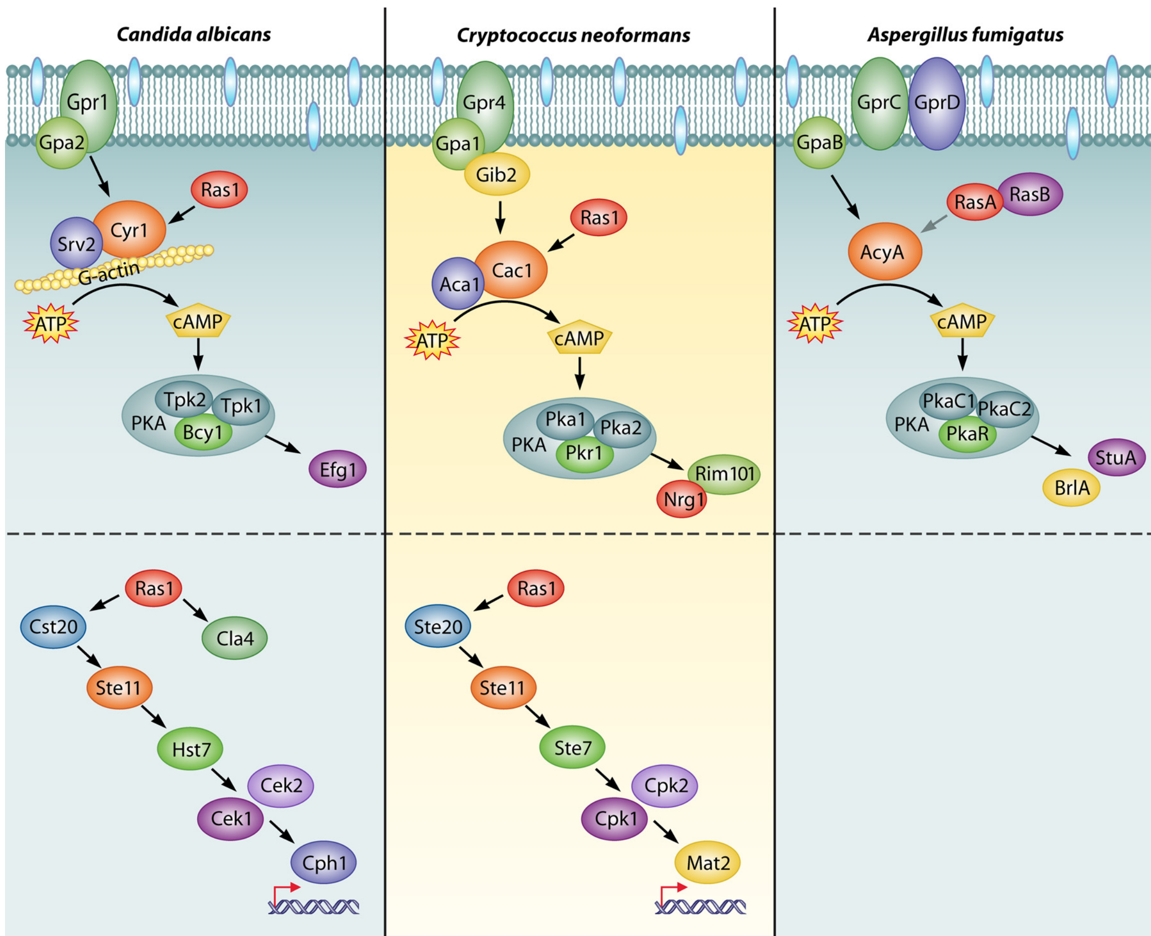

FIG. 8. Comparison of cAMP-PKA and MAPK signaling cascades in *C. albicans*, *C. neoformans*, and *A. fumigatus*. The cAMP-PKA signaling cascade and MAPK cascade represent two key morphogenetic signaling pathways conserved in these species. The MAPK signaling cascades modulating morphogenesis in *A. fumigatus* (the HOG-MAPK pathway and cell wall integrity MAPK pathway) are distinct from the MAPK module depicted in this figure. (Top) The cAMP-PKA pathway. (Bottom) The MAPK cascade. Corresponding colors between pathways indicate orthologous proteins between the species. Gray arrows indicate that links between certain factors in this pathway have not yet been established.

fruiting is to forage for *MAT***a** mating partners in nature, in addition to scavenging for nutrients.

Similar to other fungi, the ability of *C. neoformans* to transition to a filamentous state is dependent on numerous signaling pathways that respond to external stimuli and enable gene expression changes and cellular responses. These pathways will be described in detail below.

Major Morphogenetic Signaling Cascades

cAMP-protein kinase A. The cAMP-dependent PKA pathway in *C. neoformans* regulates important cellular processes, including capsule production, melanin formation, mating, and virulence (Fig. 8) (470). In *C. neoformans*, cAMP is synthesized from ATP by the adenylyl cyclase Cac1, which is regulated by the adenylyl cyclase-associated protein Aca1 and the Gα protein Gpa1. Once produced, cAMP activates the PKA complex, which in turn signals to downstream transcription factors to regulate the expression of genes important for morphogenesis, virulence, and mating.

Gpa1 is a G protein α subunit that is released from other subunits in the G protein complex in response to nutrient limitation in the environment (581). Its homologue in *S. cerevisiae*, Gpa2, senses nutrients and regulates pseudohyphal growth through an analogous signaling cascade (289). In *C. neoformans*, *gpa1* mutants fail to induce melanin and capsule production and are avirulent in rabbit models of cryptococcal meningitis (10). Gpa1 interacts with the Gβ-like protein Gib2, a positive regulator of cAMP signaling that is essential for growth (444). Gib2 also interacts with two Gγ subunit homologues, Gpg1 and Gpg2 (444). Notably, Gib2 also interacts with components of the PKC pathway, illustrating an element of cross talk between these signaling cascades (444). Moreover, previous work has identified an additional GPCR, Gpr4, that physically associates with Gpa1 and functions in sensing the amino acid methionine to activate cAMP-PKA signaling (637). Similarly to Gpa1, *gpr4* mutants exhibit capsule production and mating defects, although surprisingly, they show no defects in virulence in a murine inhalation model of systemic *C. neoformans* infection (637).

When Gpa1 is released from the trimeric G protein complex, it activates the adenylyl cyclase Cac1, a protein also reg-

ulated by Aca1. Aca1 physically interacts with Cac1 in order to regulate the induction of cAMP (27). Through cAMP production, Cac1 activates the PKA catalytic subunits Pka1 and Pka2. Intriguingly, research has shown that these two protein kinases possess distinct roles in strains of different serotypes. Pka1 plays a predominant role in cAMP signaling in serotype A strains, regulating melanin and capsule production and virulence (10), whereas Pka2 plays a predominant role in serotype D strains, regulating melanin and capsule production (225). Surprisingly, PKA is not required for virulence in serotype D strains, as measured with a murine model of cryptococcosis (225). Furthermore, in serotype A strains, the loss of the PKA regulatory subunit Pkr1 results in the overproduction of capsule, hypervirulence, and the suppression of phenotypes observed for a *gpa1* mutant (160). Recently, it was shown that serotype A *pka2* mutants are hypersensitive to osmotic shock under conditions of glucose starvation (353); hypersensitivity to osmotic shock is abolished by the additional disruption of *PKA1*, suggesting that Pka1 and Pka2 play opposite roles in the osmostress response (353).

One of the key transcription factors that functions downstream of Pka1 in *C. neoformans* is Nrg1 (121). Similar to other components of the cAMP-PKA signaling pathway, *nrg1* mutants exhibit reduced pathogenicity in mouse models of infection, and *MAT*α *nrg1* mutants form few hyphal structures (121). An additional transcription factor regulated by PKA, Rim101, is a highly conserved pH response regulator with important roles in the response to alkaline pH, increased salt concentrations, and iron limitation in addition to having a critical function in capsular production (436). Unlike other capsular mutants in *C. neoformans*, *rim101*Δ mutants are hypervirulent in a murine inhalation model of cryptococcosis (436). Intriguingly, Rim101 activation in other fungal species occurs exclusively through the conserved Rim pathway and not via the cAMP-PKA cascade, unveiling a novel mechanism of Rim101 activation in *C. neoformans* (436). The hypervirulence phenotype of the *rim101* mutant is striking in light of the fact that *rim101* mutants exhibit decreased virulence in *C. albicans* (424) as well as defects in melanization in *C. neoformans* (339), suggesting that this transcription factor mediates diverse responses upon activation by distinct signaling cascades.

Finally, transcriptional analysis has revealed links between PKA signaling and diverse biological functions, including ribosome biogenesis, stress responses, metabolic functions, and the regulation of secretory pathway components (239). The levels of approximately 20 stress response genes are elevated in a *pka1* mutant compared to the wild type, including numerous chaperones such as Hsp90 (239). Pka1 was also shown to regulate the expression of the phosphatidylethanolamine binding protein Ova1, a negative regulator of capsule and melanin formation (239). This genomic study highlighted that the links between PKA, ribosome biogenesis, stress response signaling, and metabolic functions found for *S. cerevisiae* and *C. albicans* are conserved in *C. neoformans* (214, 239, 256).

Mitogen-activated protein kinase. The Cpk1-MAPK cascade plays important roles in signaling during mating and monokaryotic fruiting and shares many features with the well-characterized pheromone response pathway in *S. cerevisiae* and *C. albicans* (Fig. 8). In *C. neoformans*, environmental factors activate a pheromone receptor, which stimulates the Gβ protein *GBP1* to activate a p21-activated protein kinase (PAK) homologue, Ste20 (612). The activation of Ste20 initiates the MAPK cascade through the phosphorylation of the MAPKKK Ste11, which phosphorylates the MAPKK Ste7, which activates the MAPK Cpk1/2 (612). The transcription factor homologue Ste12 is phosphorylated by the terminal MAPK to regulate gene expression changes related to mating, monokaryotic fruiting, and virulence. Notably, in contrast to *S. cerevisiae*, the disruption of *STE12* does not abolish pheromone sensing or mating (99, 643), implicating additional downstream effectors of Cpk1 in this cascade. In contrast to other fungi, *C. neoformans* homologues of several *S. cerevisiae* pheromone response MAPK cascade genes, such as *STE20*, *STE11*, and *STE12*, as well as three copies of the *MAT*α pheromone gene are found embedded in the *C. neoformans MAT* locus (268). Notably, the α and **a** alleles of the *MAT* locus encode divergent alleles of these genes.

When the pheromone receptor Ste3α comes into contact with pheromone, it activates the Gα proteins Gpa2 and Gpa3. Gpa2 and Gpa3 exhibit shared and distinct functions to collectively regulate pheromone responses, mating, and virulence (237, 325). Gpa2 normally couples to the Gβ protein Gbp1 and the Gγ subunit Gpg1 or Gpg2 as a heterotrimer G protein complex. The mechanism by which Gpa3 regulates pheromone production and conjugation tube formation remains unclear (325). Gbp1 mediates the response to pheromones in an manner analogous to that of the Ste4-Ste18 dimer in *S. cerevisiae*. Furthermore, *gbp1* mutants are sterile and exhibit severe defects in cell fusion, whereas the overexpression of *GPB1* stimulates conjugation germ tube formation (614). The induction of the MAPK homologue *CPK1* suppresses the mating defect of a *gbp1* mutant, although cAMP does not, supporting the exclusive role of Gbp1 in the Cpk1-MAPK pathway and not in cAMP-PKA signaling (614). In addition, *gpb1* mutant strains are defective in haploid fruiting, although this defect can be suppressed by the overexpression of *STE12* on galactose filament agar (614).

Gpa2 and Gpa3 are also regulated by two distinct regulators of G-protein signaling (RGS) proteins, Crg1 and Crg2, which cycle Gpa2 and Gpa3 from an active GTP-bound form to an inactive GDP-bound state. Crg1 negatively regulates Gpa2 and Gpa3, implicating it as a key regulator of mating (611). Crg2 also negatively regulates both Gpa2 and Gpa3, although it is also capable of acting as a GAP of Gpa1, downregulating the cAMP-PKA signaling cascade (529). Consequently, *crg2* mutants exhibit defects in filamentation and are highly attenuated in virulence (529, 626).

Recently, an additional GPCR was identified, which acts constitutively to engage the pheromone response pathway independent of pheromone ligand (236). Cells were shown to activate the pheromone response pathway in the absence of a stimulus through the Cpk1-MAPK module when *CPR2* was expressed. This resulted in a morphogenetic transition from yeastlike cells to polarized filaments (236). During filamentous growth, Cpr2 further serves to ensure the fidelity of nuclear transmission in the dikaryotic state (236).

As mentioned above, early on in MAPK signaling, Ste20 is activated by Gbp1. Studies have shown that mutations in the serotype A *STE20* gene result in a modest defect in mating, impaired growth at 39°C, and attenuated virulence (613). However, serotype D *ste20*α or *ste20***a** mutants grow comparably to

the wild type at elevated temperatures and are fully virulent (613). Although wild-type *C. neoformans* strains grow as budding yeast cells in rich medium at elevated temperatures, serotype A *ste20* mutants form elongated buds that fail to separate and often have abnormally wide mother-bud necks (613).

Downstream in the Cpk1-MAPK pathway, a disruption of the MAPKK *STE7* or the MAPK *CPK1* results in severe defects in mating, cell fusion, and haploid fruiting; however, these mutants are fully pathogenic in a murine tail vein injection model (133). Notably, the overexpression of the transcription factor *STE12* restores mating and fruiting in these mutants, placing it downstream of Cpk1 (133). However, the overexpression or activation of the upstream component Ste11α or Cpk1 restores signaling in an *ste12* mutant (133), suggesting that perhaps Ste12 regulates mating and haploid fruiting through a distinct pathway that functions in parallel with the MAPK cascade, or Ste12 may act together with another transcription factor(s) to transduce signals from Cpk1 during mating and fruiting. Recently, the HMG (high-mobility group) transcription factor Mat2 was identified in *C. neoformans* as a downstream effector of Cpk1 important for hyphal growth during mating (335).

Recently, the Ste50 homologue was identified and characterized in *C. neoformans* (259). In *S. cerevisiae*, Ste50 acts as an adaptor protein between Ste20 and Ste11 to regulate Ste11 autophosphorylation. It further controls HOG pathway activation for osmoregulation and interacts with Ras1 and Ras2 to regulate signaling through the Ras1-cAMP pathway (480). Hence, this adaptor protein has important roles in mating, filamentous growth, and stress responses in *S. cerevisiae*. In *C. neoformans*, *ste50* mutants displayed normal stress response phenotypes and normal capsule and melanin production and were not attenuated in virulence in a mouse model of infection, indicating that Ste50 is not involved in the regulation of the Hog1 or Ras signaling pathway (259). However, *STE50* was required for sexual reproduction through the Cpk1-MAPK pathway (259), highlighting the differences between *C. neoformans STE50* and other yeast species where *STE50* plays a role in numerous signaling cascades.

Ras. In *C. neoformans*, Ras1 has been implicated in the control of both the cAMP-PKA and Cpk1-MAPK cascades and mediates cross talk between the two. In the Cpk1-MAPK module, Ras1 functions upstream of Gbp1 in the mating process, whereas in the cAMP-PKA cascade, Ras1 regulates the activity of Cac1. To regulate thermotolerance and actin cytoskeleton dynamics, Ras signaling is mediated through the GEF protein Cdc24 and the Rho-like GTPase Cdc42 (416). *C. neoformans* also possesses a Ras2 protein. On their own, *ras2* mutants display no obvious phenotypes, although an overexpression of *RAS2* is able to partially suppress *ras1* mutant phenotypes (619). Additional studies have shown that *ras1* mutants are viable but are unable to grow at elevated temperatures, show defects in mating and agar adherence, and are avirulent in rabbit models of cryptococcal meningitis (9). Growth at elevated temperatures is not restored by exogenous cAMP or the overexpression of MAPK signaling elements, suggesting that there is at least one additional Ras-specific signaling cascade that plays a crucial role in tolerance to elevated temperatures (9).

Studies have since been conducted to identify components of the Ras1-dependent high-temperature-growth pathway. The overexpression of the G protein Rac was found to suppress multiple *ras1* mutant phenotypes, including growth at elevated temperatures (597). Additional analyses showed that Rac1 acts together with Ste20 to regulate high-temperature growth, as the overexpression of *STE20*α also suppressed *ras1* growth defects at 37°C (597). Furthermore, dominant active Rac1 (Rac1-G15V) and Ste20α physically interact in yeast two-hybrid assays, suggesting that Ste20α acts directly downstream of Rac1 and that these proteins likely interact only when Rac1 is activated by upstream signals (597). With regard to morphogenesis, *rac1* mutants produce few filaments in bilateral crosses, and the filaments that are generated tend to be shorter and thicker than those of the wild type and display marked defects in septum formation (597). Additional work has implicated the GEF Cdc24 as another downstream effector of Ras1 to regulate temperature-dependent growth (416). Genetic and two-hybrid analyses defined a signaling cascade comprised of Ras1, Cdc24, the Rho-like GTPase Cdc42, and Ste20, which function to mediate thermotolerance, polarized growth, and pathogenicity in *C. neoformans* (416). Notably, the overexpression of Rac1 does not suppress the *cdc24* high-temperature-growth defect, implicating at least two distinct signaling pathways controlling Ras1-mediated thermotolerance.

Posttranslational modifications also affect Ras-mediated signaling. The prenylation of Ras proteins was shown to be required for early membrane associations, and mutations that block prenylation resulted in the loss of all Ras functions (415). Furthermore, Ras1 palmitoylation was found to be a highly regulated and reversible process that allows Ras proteins to target different cellular membranes, thus affecting different downstream signaling pathways. Proper palmitoylation was required for normal morphogenesis, virulence, and survival at high temperatures, although it was not required for mating (415).

Recently, a comparative transcriptome analysis was conducted with *C. neoformans ras1*, *aca1*, *gpa1*, *cac1*, and *pka1 pka2* mutants to evaluate the functional connections between these signaling components in the cAMP-PKA cascade (353). That study identified numerous Ras1 and cAMP-dependent genes and illustrated that Ras1-mediated signaling was largely independent of the cAMP-PKA pathway. Ras1 was shown to be a regulator of the osmotic stress response and the oxidative stress response and was required for the maintenance of cell wall integrity (353). Ras1-dependent stress control was mediated primarily by Cdc24-dependent signaling, as *cdc24* mutants tightly phenocopied the stress response phenotypes exhibited by a *ras1* mutant (353). Furthermore, a significant proportion of Ras/cAMP-dependent genes were also controlled by the environmental stress response (353).

High-osmolarity glycerol pathway. The Hog1 MAPK pathway in *C. neoformans* is critical for adaptation to a wide variety of environmental stresses, including osmotic shock, UV irradiation, heat shock, oxidative damage, toxic metabolites, and antifungal drugs (25). Moreover, in *C. neoformans* this signaling pathway controls the production of the virulence factors capsule and melanin, and mutants in this pathway often display an enhanced production of pheromone during mating (25, 29). There are two main modules important for signaling: a two-component-system-like phosphorelay system and a MAPK module. In fungi, the MAPKKK Ssk2 is activated by a two-

component-like system sensor kinase hybrid protein, which consists of a histidine kinase domain and a response regulator domain fused in a single polypeptide. In *C. neoformans* there are seven protein homologues to a hybrid sensor kinase, *t*wo-*co*mponent-like protein 1 (Tco1) to Tco7. Tco1 and Tco2 have discrete and redundant roles in activating Hog signaling (28). Unusual phenotypes have been observed for *tco1* mutants, as strains showed increased melanin biosynthesis but attenuated virulence (28, 103). Furthermore, Tco2 promotes sensitivity to hydrogen peroxide; however, it has no function in relation to mating or melanin production (28). Presently, the functions of Tco3 to Tco7 remain enigmatic.

There are two response regulator proteins (Ssk1 and Skn7) that receive a phosphate group from the response regulator domain of the hybrid kinase through the essential phosphorelay protein Ypd1 (28). In *C. neoformans*, *ssk1* mutants display phenotypes similar to those of *hog1* mutants, implicating Ssk1 as the major upstream regulator of Hog1-MAPK signaling (28). Notably, unlike those of other fungi, Hog1 MAPKs are constitutively phosphorylated under unstressed conditions and become rapidly dephosphorylated in response to osmotic shock in a majority of *C. neoformans* strains, including serotype A strain H99 and serotype D strain B-3501 (29). In contrast, in some *C. neoformans* strains, including serotype D strain JEC21, Hog1 is not phosphorylated under normal conditions but is rapidly phosphorylated upon stress, similar to what occurs in other fungi (29). The MAPKKK Ssk2 is necessary and sufficient to control Hog1 activity and functions by phosphorylating the MAPKK Pbs2, which in turn phosphorylates Hog1 (26). *C. neoformans* strains that show higher levels of constitutive phosphorylation of Hog1 due to the activity of Ssk2 also display elevated-stress-resistance and elevated-virulence phenotypes (26). Ssk2 is the only component of the Hog1 MAPK cascade that is polymorphic between serotype D strain B-3501 and serotype D strain JEC21 (26). Furthermore, the allele exchange of *SSK2* completely interchanged the Hog1-controlled phenotypes and virulence levels of B-3501 and JEC21 (26). Although the evolutionary advantage that the constitutive phosphorylation of Hog1 may have for particular *C. neoformans* strains remains puzzling, it was hypothesized that the nuclear localization of Hog1 under unstressed conditions enables a more rapid response during times of stress (25).

Transcriptome analyses have been conducted with mutants of components of the HOG pathway in order to identify transcripts regulated by this signaling cascade. *SXI1*, a gene encoding a homeodomain-containing transcriptional regulator, and *GPA2*, the G protein α subunit in the MAPK pathway, are highly upregulated in *hog1* or *ssk1* mutants (277). This implicates elevated *GPA2* levels as a mechanism for increased pheromone production and sexual reproduction in these strains.

Calcineurin. The protein phosphatase calcineurin is essential for growth at an elevated temperature of 37°C and is a central regulator of virulence and morphogenesis in *C. neoformans* (430, 559). For example, studies have illustrated that calcineurin is essential for virulence in both rabbit models of cryptococcal meningitis and murine inhalation models of cryptococcal infection (430). Furthermore, the pharmacological or genetic inhibition of this phosphatase renders cells unable to mate, since it is required for elongation and survival of the dikaryon (125). Additional characterizations revealed that calcineurin is required for monokaryotic fruiting in *MAT*α cells in response to nitrogen limitation (125). Hence, this key regulator of cellular signaling is vital to many facets of *C. neoformans* biology (Fig. 9).

In *C. neoformans* the calcineurin inhibitor cyclosporine interacts with the related cyclophilin A proteins *CPA1* and *CPA2*. *cpa1 cpa2* double mutants show severe growth defects at 24°C to 37°C, are nonviable at 39°C, are avirulent, and are unable to undergo mating (610). In *C. neoformans*, the novel calcineurin binding protein Cbp1 functions as a targeting subunit to regulate events such as mating-dependent filamentation (208, 559). *cbp1* mutants show no temperature growth defects, minor pH or CO_2 sensitivities, no defects in monokaryotic fruiting, and weak attenuation for virulence; however, *CBP1* is essential for mating (208).

Novel Morphogenetic State

Recently, *C. neoformans* var. *grubii* was observed to undergo cellular enlargement in an *in vivo* mouse model of infection (431, 645). These giant cells were reported previously in the literature (124, 346, 412); however, they had never been isolated or characterized. These enlarged cells, sometimes referred to as titan cells, represent a distinct morphogenetic state, as they display a decreased tendency to be phagocytosed by host mononuclear cells, increased resistance to oxidative and nitrosative stresses, reduced dissemination throughout the host, and elevated DNA content (431, 645). Furthermore, cell enlargement is regulated by the MAPK pathway involved in pheromone sensing, as *ste3***a** mutants are impaired in titan cell formation (431). Cell enlargement is also dependent on cAMP accumulation, as *cac1* mutants are unable to form giant cells during murine infection (645). These studies provide novel insights into a morphogenetic state of *C. neoformans* with a profound impact on pathogenicity.

CRYPTOCOCCUS NEOFORMANS DRUG RESISTANCE: THE AZOLES

The treatment of cryptococcal disease often relies on the azoles, which target the ergosterol biosynthetic enzyme Erg11. The development of azole resistance in *C. neoformans* is rare, as observed by a global study of the antifungal susceptibilities of 1,811 clinical isolates obtained between 1990 and 2004 (460). *In vitro* resistance to the azoles was uncommon and did not increase during the period of study (460). Another study of 70 *C. neoformans* isolates obtained in Spain from 1994 to 2005 found that itraconazole and voriconazole resistance levels did not change significantly, while fluconazole resistance levels actually decreased (7). Despite the rarity, azole-resistant *C. neoformans* strains have been detected among 80 isolates from the cerebrospinal fluids of hospitalized patients with cryptococcal meningitis in Kenya (57). In total, 11.2% of the strains were resistant to fluconazole, and 65% were susceptible-dose dependent (57). Furthermore, the emergence of azole resistance has been documented for AIDS patients undergoing long-term fluconazole therapy (17, 449). Recent epidemiological studies observed that isolates collected from different geographic regions have altered susceptibilities to fluconazole, as emerging resistance was documented among isolates from the Asia-Pa-

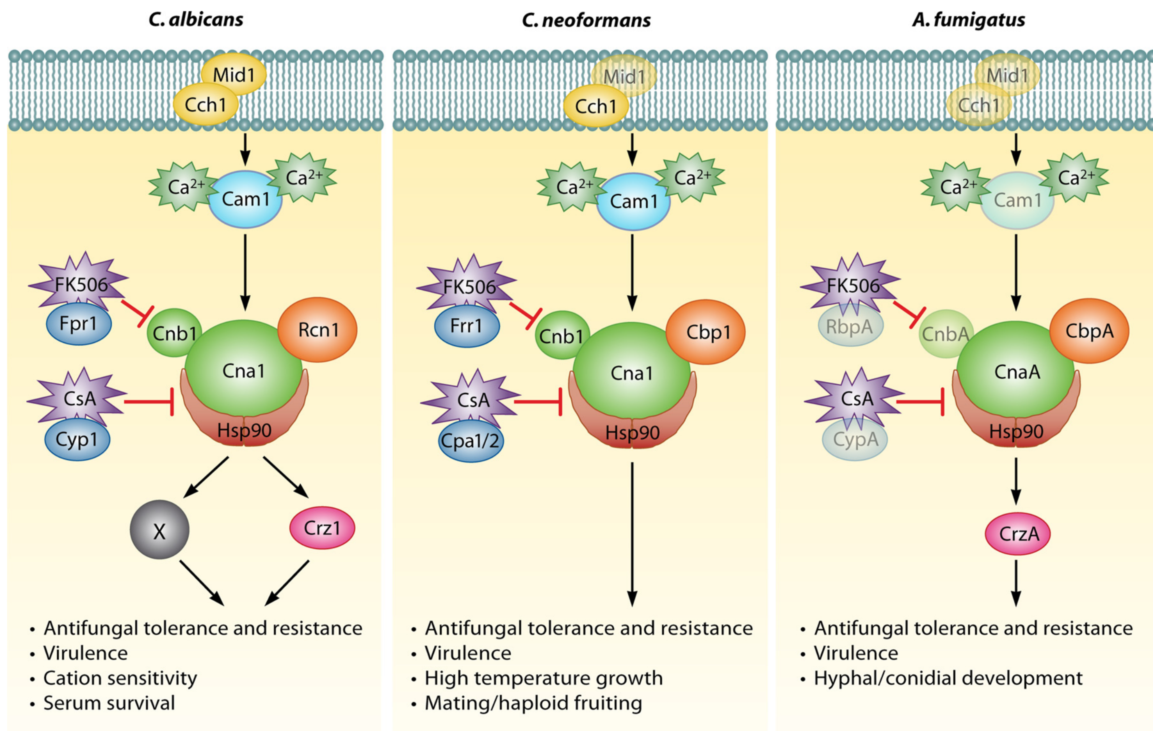

FIG. 9. The calcineurin signaling pathway in *C. albicans*, *C. neoformans*, and *A. fumigatus*. Shown is a simplified schematic of how calcineurin regulates a myriad of responses in *C. albicans*, *C. neoformans*, and *A. fumigatus*. In *C. albicans*, the activation of the Cch1-Mid1 channel leads to the accumulation of intracellular Ca^{2+}, which is bound by calmodulin (encoded by *CAM1*), leading to the activation of calcineurin. The molecular chaperone Hsp90 physically interacts with the catalytic subunit of calcineurin, Cna1, keeping it poised for activation. Once activated, calcineurin dephosphorylates the transcription factor Crz1 as well as other unknown effectors to regulate a myriad of cellular responses. In *C. neoformans* and *A. fumigatus*, homologues of this signaling pathway are depicted in identical colors. Components that have been identified only based on sequence homology are dimmed. Drugs and signaling molecules are depicted as stars, whereas proteins are depicted as circles. The cellular responses mediated by calcineurin are listed below the pathway. (Adapted from reference 559 with permission of Macmillan Publishers Ltd.)

cific, Africa/Middle East, and Latin America regions but not among isolates from Europe or North America (458). Furthermore, the molecular genotype of cryptococcal species influences fluconazole susceptibility, with genotype VGII of *C. gattii* and genotype VNI of *C. neoformans* displaying elevated fluconazole MICs compared to those of other genotypes within their respective species (102). Finally, it was also found that after initial treatment with fluconazole, relapses of cryptococcal meningitis tended to be associated with elevated levels of fluconazole resistance (55). Similar to the case for other pathogenic fungi, the ability of *C. neoformans* to evolve azole resistance is dependent on several mechanisms, including drug target alterations, the overexpression of efflux pumps, and the modulation of stress signaling pathways, as discussed in the following sections (Fig. 10).

Alteration of the Drug Target

In *C. neoformans*, the alteration of the azole target Erg11 has been documented for numerous clinical isolates (492, 602). Recently, a three-dimensional structure of *C. neoformans* Erg11 was elucidated, and residues important for its normal enzymatic function as well as those that interact with fluconazole were identified (531). For example, the authors of that study found that although Gly484 does not interact with fluconazole directly, the position is likely important for the proper conformation of the heme environment in order to achieve optimal enzymatic activity (531). Furthermore, it is expected that changing Gly484 to another residue would decrease the flexibility required for substrate or inhibitor binding, providing a potential structural explanation for how this mutation in Erg11 could lead to azole resistance (531). *C. neoformans* isolates with Gly484 mutations have been recovered in the clinic. For a series of isolates recovered from an AIDS patient with recurrent meningitis, elevated levels of fluconazole resistance were observed for a later isolate that acquired a G484S substitution in Erg11 (492), providing clinical relevance for the structural data. In an additional study, 3 out of 11 clinical isolates with low levels of resistance exhibited an altered affinity of Erg11 for fluconazole (602). Notably, 4 out of 11 isolates with high levels of resistance also had a decreased intracellular accumulation of fluconazole (602), suggesting a possible role for multidrug transporters.

Upregulation of Multidrug Transporters

Similar to the case for *C. albicans*, a major mechanism of drug resistance in *C. neoformans* is the overexpression of multidrug transporters, which leads to a decreased cellular accumulation of the azoles. The most well-characterized drug transporter in *C. neoformans* is the ABC transporter *AFR1*. Fluconazole-resistant mutants generated *in vitro* through the

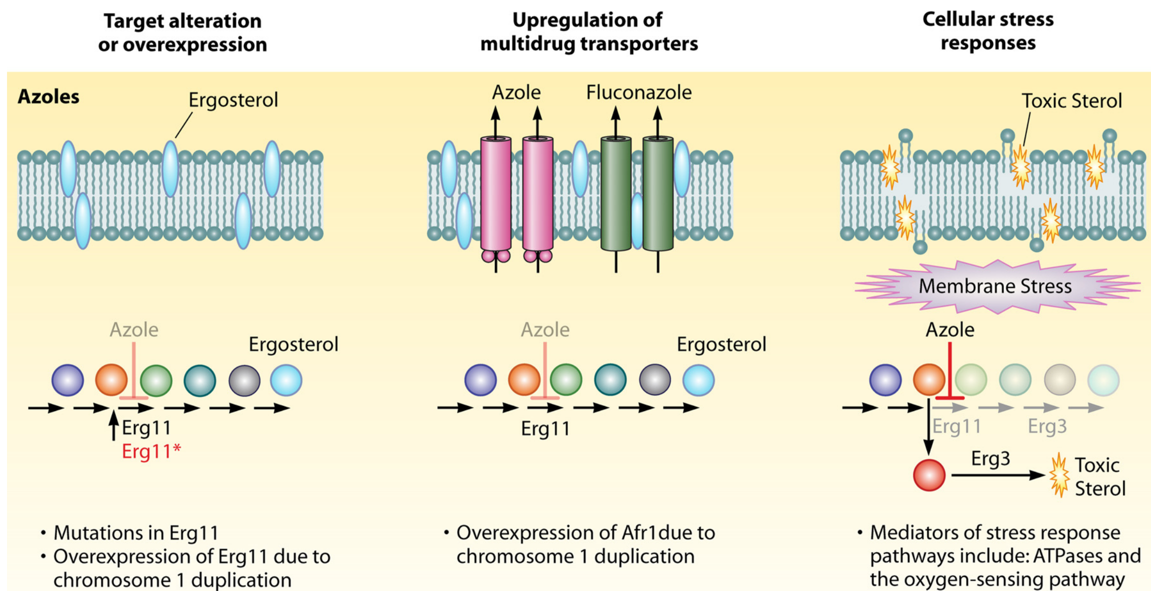

FIG. 10. *C. neoformans* drug resistance mechanisms. *C. neoformans* can acquire resistance to the azoles through multiple mechanisms, including the upregulation or alteration of the drug target Erg11, the upregulation of the multidrug transporter Afr1, or the induction of numerous cellular stress responses. The bullet points below each mechanism describe the manner in which resistance is acquired. Notably, in *C. neoformans*, resistance to the polyenes is extremely rare. Furthermore, *C. neoformans* displays intrinsic resistance to the echinocandins. (Adapted from reference 111 with permission of Nature Publishing Group.)

exposure of an initially sensitive clinical isolate to fluconazole were found to overexpress a cDNA that encodes *AFR1* (465). The disruption of *AFR1* enhanced susceptibility to fluconazole, and the complementation of the gene restored resistance (465). *In vivo* data from a mouse model of infection support the hypothesis that *AFR1* is important for virulence and drug resistance (508). A strain of *C. neoformans* overexpressing *AFR1* had significantly increased virulence, and an *afr1* mutant exhibited increased sensitivity to fluconazole (508). Finally, *AFR1* also seems to have important roles in modulating the host immune response. *AFR1*-overexpressing fluconazole-resistant mutants are resistant to microglia-mediated anticryptococcal activity compared to fluconazole-susceptible isogenic strains (439). These strains were phagocytosed to similar extents; however, in the strain overexpressing *AFR1*, there was reduced acidification and delayed maturation of the phagosomes (439). This is intriguing, as it linked the Afr1 ABC transporter to both fluconazole resistance and virulence. In addition to encoding AFR1, *C. neoformans* encodes a protein related to eukaryotic multidrug-resistant proteins, Mdr1 (579). This gene was identified, cloned, and characterized from a clinical isolate of *C. neoformans*; however, its role in antifungal drug resistance remains elusive (579).

Cellular Stress Responses

Heteroresistance. Similar to the other pathogenic fungi examined thus far, cellular stress responses are crucial for cells to survive drug-induced stress. In *C. neoformans*, an intriguing pattern of cellular responses to the azoles has been reported and termed heteroresistance. This phenomenon occurs when a single cell gives rise to progeny with heterogeneous resistance phenotypes, with a small subset of progeny that are highly resistant to azoles (537). The resistant subpopulations can adapt to increasing concentrations of the azoles in a stepwise manner; however, the original susceptibility is restored after passage in the absence of an antifungal. This mechanism of resistance has been observed among clinical isolates and is unrelated to prior drug exposure and unaffected by pH or osmolarity (387, 639). Notably, resistance is affected by temperature, as heteroresistance phenotypes can be suppressed at 35°C and are abolished at 40°C (387, 639). Most recently, a study analyzing 130 *C. neoformans* strains isolated from clinical and environmental sources before 1979, prior to azole use, and 16 fluconazole-resistant strains isolated from AIDS patients undergoing fluconazole therapy from 1990 to 2000 found that all the strains manifested heteroresistance, confirming that this phenomenon is universal and unrelated to prior drug exposure (537). Furthermore, repeated transfer on drug-free medium caused the highly resistant subpopulations to revert to the original levels of heteroresistance (537). Finally, the fluconazole transporter *AFR1* was shown to be unrelated to the heteroresistance phenotype, as *afr1*Δ mutants exposed to increasing concentrations of the drug in a stepwise manner were able to acquire an elevated level of fluconazole resistance, which was subsequently lost upon growth under drug-free conditions (537). Clinical and environmental isolates of *C. gattii* also display heteroresistance phenotypes that are associated with increased virulence. Notably, a considerably higher proportion of the *C. gattii* strains surveyed (86%) than *C. neoformans* strains (46%) showed signs of heteroresistance to fluconazole above 16 μg/ml (600).

Recently, a genomic approach was used to elucidate the molecular mechanism by which *C. neoformans* acquires high levels of resistance to the azoles. In that study it was discovered that adaptive resistance to the azoles was achieved by the

duplication of multiple chromosomes in response to fluconazole (538). A duplication of chromosome 1 was common, resulting in an increased copy number of the gene encoding the azole target Erg11, which resides on chromosome 1 (538). Furthermore, the duplication of chromosome 1 was infrequent in an *erg11* or *afr1* mutant, implicating these genes as the targets of selection for chromosome 1 duplication during times of stress (538). Similar findings with *C. albicans* involve the duplication of the left arm of chromosome 5, leading to elevated levels of azole resistance due to the amplification of the resistance determinants *ERG11* and *TAC1* and increased fitness in the absence of antifungal stress (521–523). Intriguingly, with the repeated transfer of *C. neoformans* in drug-free medium, duplicated chromosomes were lost, and cells returned to their original level of drug tolerance (538). This is the first description of aneuploidies in *C. neoformans* as an adaptive mechanism to survive azole stress.

Calcineurin. Inhibitors of the protein phosphatase calcineurin have a profound impact on *C. neoformans* azole resistance, although the mechanism is independent of calcineurin function. The calcineurin inhibitor FK506 exhibits synergistic activity with fluconazole. Specifically, the combined use of these agents results in a 30-fold reduction in the MIC of FK506 and a 4-fold reduction in the MIC of fluconazole (140). Strikingly, the synergistic activity of FK506 with fluconazole does not depend on the presence of calcineurin or FKBP12, suggesting that FK506 enhances the fluconazole action by a calcineurin-independent mechanism, perhaps through the capacity of FK506 to inhibit multidrug resistance (MDR) pumps. In clinical studies, patients receiving the immunosuppressant and calcineurin inhibitor FK506 have fewer life-threatening infections of the CNS, although they tend to acquire more infections of the skin, where the temperature is lower (535). In addition, isolates hypersensitive to the calcineurin inhibitor *in vitro* are less likely to infect the CNS (61). Since patients receiving calcineurin inhibitors still present with invasive *C. neoformans* infections, future drug design efforts will have to focus on increasing the antifungal activity of these inhibitors while minimizing immunosuppressive effects on the host, potentially by identifying fungus-specific targets in the calcineurin signaling cascade (559).

ATPases. The sarcoplasmic/endoplasmic Ca^{2+}-ATPases (SERCAs) are a family of ER Ca^{2+} pumps that are highly conserved in eukaryotes. SERCAs function by transporting Ca^{2+} from the cytosol to the ER and thereby play critical roles in maintaining Ca^{2+} homeostasis in the cell. In *C. neoformans*, *ECA1* encodes the SERCA ATPase. *eca1* mutants are virulent or hypervirulent at permissive growth temperatures in a murine macrophage model, in the wax moth *G. mellonella*, and in the nematode *Caenorhabditis elegans* but are attenuated in virulence at 37°C (172). Furthermore, *eca1* mutants exhibit hypersensitivity to calcineurin inhibition and to osmotic stresses, with *eca1 cna1* double mutants showing an elevated sensitivity to high temperatures and ER stresses compared to either single mutant. Thus, it is proposed that *ECA1* contributes to stress tolerance and virulence by acting in parallel with calcineurin signaling.

P-type ATPases are a large family of multi-transmembrane-domain, ATP-dependent transporters, which include aminophospholipid translocases (APTs). APTs function to maintain the asymmetrical distribution of aminophospholipids in membranes by translocating phosphatidylserine and/or phosphatidylethanolamine from one leaflet of the bilayer to the other. In *C. neoformans*, *APT1* encodes an integral membrane P-type ATPase belonging to the APT family. *apt1* mutants display an altered actin distribution, increased sensitivity to oxidative stress, and hypersensitivity to the antifungals fluconazole and amphotericin B (238).

Oxygen-sensing pathway. *C. neoformans* causes severe infections of the brain, a low-oxygen environment, and hence, it has evolved mechanisms to survive in this harsh host niche. When cellular cholesterol or ergosterol levels decrease, the sterol regulatory element binding protein (SREBP) is escorted by an SREBP cleavage-activating protein (SCAP) to the Golgi apparatus, where SREBP undergoes two sequential proteolytic cleavage events catalyzed by site 1 and site 2 proteases. *SRE1*, a homologue of mammalian SREBP, functions in the oxygen-sensing pathway by stimulating ergosterol production when oxygen-dependent ergosterol synthesis is limited by hypoxia (96). *SRE1* is required for virulence in a tail vein injection mouse model of infection. Furthermore, *SRE1* functions under low-oxygen conditions as a transcriptional activator of numerous genes involved in ergosterol biosynthesis and the transport of iron, copper, and other molecules (96). In addition, the *C. neoformans* orthologue of the mammalian site 2 protease, Stp1, is required for both Sre1-dependent and Sre1-independent gene transcription (56). Studies have also shown that *sre1* and *stp1* mutants cause itraconazole to act in a fungicidal manner (56). Recently, six additional genes that play a role in the SREBP pathway in *C. neoformans* were identified: *SFB2*, *STP1*, *SCP1*, *KAP123*, *GSK3*, and *DAM1* (97). Interestingly, *SFB2*, *KAP123*, and *GSK3* were not known to be involved in the SREBP pathway in other fungi, suggesting a divergence of this pathway in *C. neoformans*. Furthermore, all novel mutants discovered in that study exhibited hypersensitivity to fluconazole, and all but *KAP123* showed defects in mouse virulence models (97).

CRYPTOCOCCUS NEOFORMANS DRUG RESISTANCE: THE POLYENES

C. neoformans resistance to amphotericin B remains extremely rare. In a global survey of the antifungal susceptibilities of 1,811 clinical *C. neoformans* isolates, only ~1% displayed increases in polyene resistance (460). Despite the infrequent prevalence, resistance has still been documented both for clinical isolates and in response to *in vitro* selection. Amphotericin B-resistant isolates from an AIDS patient were found to have defective Δ8-7 isomerase activity (274). Furthermore, isolates selected *in vitro* that were cross-resistant to amphotericin B and fluconazole showed a reduced cellular accumulation of the antifungals, suggesting a possible role for a common multidrug transporter (258). Another means by which *C. neoformans* can acquire resistance to amphotericin B is through the formation of biofilms, which display elevated levels of resistance to the polyenes and azoles in comparison to their planktonic counterparts (370).

Recently, a study investigated the downstream signaling of the HOG pathway by the performance of a comparative transcriptome analysis of the wild type and *hog1*, *ssk1*, and *skn7*

mutants in response to multiple stresses (277). Genes required for antiphagocytic polysaccharide capsule production and melanin biosynthesis were upregulated in these mutants (277). Furthermore, a majority of ergosterol biosynthesis genes were upregulated in *hog1* and *ssk1* mutants relative to the wild type, resulting in an increase in ergosterol levels. Additional genetic analyses concluded that *ssk1*, *ssk2*, *pbs2*, and *hog1* mutants all showed dramatic hypersensitivity to amphotericin B and increased resistance to fluconazole and ketoconazole (277), indicating that the HOG pathway regulates tolerance to these distinct classes of antifungals in opposing directions. In another comparative transcriptome analysis, connections between drug resistance and the Ras1/cAMP signaling cascades were identified. The inhibition of components of the cAMP-PKA signaling pathway or Ras1 increased polyene sensitivity without altering ergosterol biosynthesis (353), implicating Ras1/cAMP signaling in polyene sensitivity.

CRYPTOCOCCUS NEOFORMANS DRUG RESISTANCE: THE ECHINOCANDINS

C. neoformans is intrinsically resistant to the echinocandins both *in vitro* and *in vivo* (1, 287). This inherent resistance is surprising, since the *C. neoformans* gene encoding Fks1 is essential for growth and (1,3)-β-D-glucans are found in the cell wall of *C. neoformans* (578). A possible explanation for this resistance would be if the target (1,3)-β-D-glucan synthase enzyme was resistant to the echinocandins in *C. neoformans*. This possibility was ruled out by conducting an *in vitro* (1,3)-β-D-glucan synthase assay, which demonstrated that the *C. neoformans* (1,3)-β-D-glucan synthase is indeed sensitive to echinocandins, with an apparent caspofungin K_i of 0.17 ± 0.02 (mean ± standard deviation) (359). Furthermore, caspofungin reduces (1,3)-β and (1,6)-β glucan linkages within the *C. neoformans* cell wall (177). The molecular explanation for the intrinsic echinocandin resistance of *C. neoformans* remains enigmatic, although it is postulated that resistance could be due to rapid efflux from the cell or the degradation of the drug intracellularly or extracellularly.

Since calcineurin inhibitors have a profound impact on azole resistance, studies have examined their impact on echinocandin resistance. The calcineurin inhibitor FK506 has been shown to act synergistically with the pneumocandin caspofungin acetate, and this was mediated through the FKBP12-dependent inhibition of calcineurin (140). Notably, caspofungin was also shown to display synergy with the azoles and polyenes against *C. neoformans in vitro* (190).

An additional cellular stress response pathway that regulates echinocandin resistance in *C. neoformans* is the PKC cell wall integrity pathway, which is essential for the defense against oxidative and nitrosative stresses. In *C. neoformans*, the MAPK Mpk1 is activated by phosphorylation in response to caspofungin as well as the chitin synthase inhibitor nikkomycin Z (285). Furthermore, *mpk1* mutants show enhanced susceptibility to caspofungin, are unviable at 37°C, and display attenuated virulence (285). Interestingly, FK506 activates Mpk1, which results in the induction of *FKS1* expression. In the absence of calcineurin, Mpk1 is able to partially protect cells from caspofungin-induced cell wall stress (285). Further studies have also shown that *pkc1* mutants exhibit osmotic instability, alterations in capsule and melanin production, as well as sensitivity to temperature and cell wall-inhibiting agents (202).

CRYPTOCOCCUS NEOFORMANS DRUG RESISTANCE: FLUDIOXONIL

A final antifungal that activates numerous stress response pathways in *C. neoformans* is fludioxonil. This compound is used as an agricultural fungicide to control plant-associated pathogenic fungi; however, studies have examined its effect on *C. neoformans*. Specifically, three distinct signaling pathways have been shown to contribute to the tolerance of *C. neoformans* to fludioxonil. This antifungal has been shown to activate the Hog1 MAPK pathway, and *hog1* mutants are resistant to fludioxonil (280). The two-component sensor kinases Tco1 and Tco2 play distinct and redundant roles in fludioxonil sensitivity through the Hog1 MAPK signaling cascade (28). Moreover, fludioxonil exerts fungicidal activity against calcineurin mutants and acts synergistically with FK506 (280). Finally, *mpk1* mutants from the PKC cell wall integrity pathway exhibit fludioxonil sensitivity (280).

ASPERGILLUS FUMIGATUS

The genus *Aspergillus* is comprised of a diversity of species, including the model fungus *A. nidulans* and species commonly used in the industry, including *A. oryzae*, *A. niger*, and *A. terreus*. Although hundreds of *Aspergillus* species have been described, only a few are known to cause invasive aspergillosis, including *A. fumigatus*, *A. flavus*, *A. niger*, *A. terreus*, *A. versicolor*, and *A. nidulans* (457). *A. fumigatus* reigns as the most common cause of invasive aspergillosis. It is a saprophytic filamentous fungus that inhabits dead or decaying organic debris in the soil (307, 404). It is also one of the most ubiquitous fungi with airborne conidia, which, at 2 to 3 μm in size, are readily inhaled into human lungs (307, 404). In fact, it is estimated that humans inhale several hundred *A. fumigatus* conidia daily, although inhalation by immunocompetent individuals rarely results in severe disease (144, 307). On the other hand, in patients with pulmonary disorders such as asthma or cystic fibrosis, *A. fumigatus* infection can cause allergic bronchopulmonary aspergillosis, while in high-risk immunocompromised individuals, infection can lead to invasive aspergillosis, with a 40 to 90% mortality rate (331). The immunocompromised populations that are most susceptible to invasive aspergillosis include patients with leukemia or genetic immunodeficiency diseases and patients undergoing bone marrow, hematopoietic stem cell, or solid-organ transplants (130, 367, 443, 464). *A. fumigatus* can also form a spheroid mass of hyphae called an aspergilloma. These tend to form in preexisting pulmonary cavities, which can be caused by diseases such as tuberculosis.

The life cycle of *A. fumigatus* involves the formation of both conidia and hyphae (Fig. 11). Like other filamentous fungi, *A. fumigatus* produces conidiophore stalks, which undergo conidiation, a process of asexual development following nutrient limitation, which produces clonal spores known as conidia (130, 307). Conidia are generally metabolically quiescent and resilient to environmental stresses. Airborne conidia represent the infectious particles of *A. fumigatus* that are readily inhaled into human bronchioles or alveoli (130, 307). The innate im-

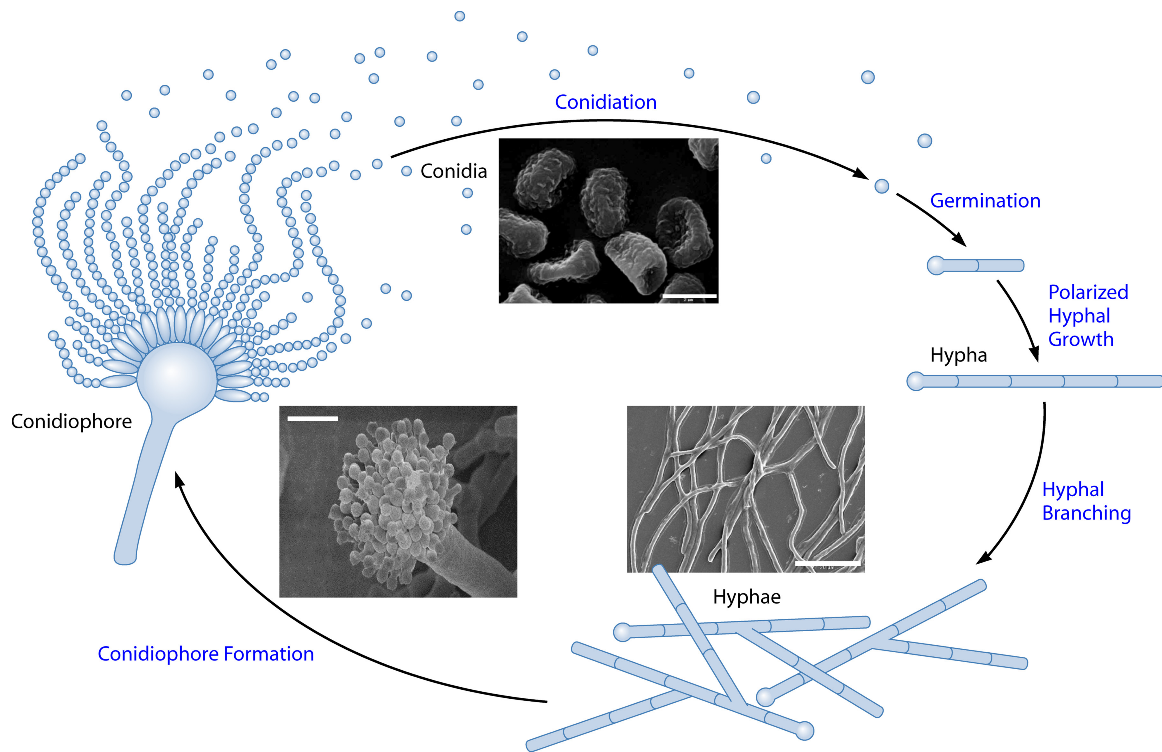

FIG. 11. The life cycle and distinct morphological states of *A. fumigatus*. Like other filamentous fungi, *A. fumigatus* produces conidiophore structures (scale bar, 10 μm), which produce conidial spores (SEM images) (scale bar, 2 μm) through the process of conidiation. Under certain environmental conditions, conidia can germinate, develop, and begin to undergo polarized growth, ultimately becoming hyphal cells (scale bar, 20 μm). Hyphal cells will continue to grow, elongate, and branch and can eventually go on to form conidiophores. (Image of spores reprinted from reference 122 with permission; image of hyphae courtesy of W. J. Steinbach [Duke University Medical Center], reproduced with permission; image of conidiophore courtesy of A. Beauvais and J. P. Latgé [Institut Pasteur, France], reproduced with permission.)

mune response, including that mediated by alveolar macrophages, is responsible for eliminating these spores from the lungs of healthy individuals (38, 266, 267). However, conditions in the human lung favor conidial germination, and conidia that are able to evade macrophage killing will break their metabolic dormancy and undergo germination (18, 130, 307). After several nuclear divisions, each conidium will establish an axis of polarity and develop into a hypha. Unlike the case with conidia, it is polymorphonuclear neutrophils that target *A. fumigatus* hyphae (38, 151, 323) and produce neutrophil extracellular traps (NETs) in response to *A. fumigatus* infection (79). Growing hyphal cells can eventually damage the lung epithelia, allowing *A. fumigatus* to penetrate endothelial cells, enter the vascular system, and migrate to distal organs (18). The deletion of genes involved in this morphogenetic process can reduce germination and growth rates as well as impair virulence in murine models of invasive aspergillosis (123, 188, 649).

Other traits of *A. fumigatus* that enable its pathogenic prowess include its capacity to withstand stress, its small conidial size, and its ability to produce damaging enzymes and toxins. *A. fumigatus* has the capacity to withstand harsh environmental stresses, including high temperatures of up to 70°C (18, 52). The heat shock response of this fungus has been the subject of in-depth investigations (2, 154, 418). It was suggested that factors that convey thermotolerance may also contribute to virulence (52); for instance, the ribosome biogenesis protein CgrA has important roles in the survival at elevated temperatures, growth, and virulence of *A. fumigatus* (54). Another important pathogenic trait of *A. fumigatus* is the small size of its conidia. While other *Aspergillus* species such as *A. flavus* and *A. niger* have larger conidia that are more readily cleared by the upper respiratory tract, *A. fumigatus* readily infiltrates into human alveoli (130). *A. fumigatus* can also produce and secrete numerous secondary metabolites into its environment during mycelial growth (270). These secreted products are important for the pathogenic capability of *A. fumigatus* and include enzymes such as proteases, catalases, and phospholipases as well as toxins, such as the potent immunosuppressive gliotoxin (270, 402, 403).

INTRODUCTION TO *ASPERGILLUS FUMIGATUS* MORPHOGENESIS

A. fumigatus undergoes several distinct morphogenetic transitions. These transitions include the formation of conidial spores through conidiation, the process of conidial germination, and polarized hyphal growth (Fig. 11). These morphogenetic processes in *A. fumigatus* are regulated by diverse signaling cascades and are correlated with virulence, the host immune response, and biofilm formation. This section will report what is known regarding the stimulation of morphogenesis by diverse cues, the link between morphogenesis and vir-

ulence, and, finally, the major signaling networks involved in regulating morphogenetic transitions.

Stimulation of Morphogenesis

Although standard laboratory conditions that are usually used to initiate germination involve defined medium at 37°C, multiple studies have examined how various external factors can influence the conidiation or germination of *A. fumigatus*. Among the first environmental factors found to influence morphogenesis were factors involved in nutrient sensing. Glucose in particular is an important signal that regulates the conidial swelling that precedes germination, with higher concentrations of glucose leading to more efficient germination (89). *A. fumigatus* morphogenesis is also correlated with conditions that the fungus encounters during human infection. For instance, germination rates of *A. fumigatus* increase with increasing temperatures, with the highest rate of germination being observed at 41°C (15). This differs from *A. flavus* or *A. niger*, whose germination rates peak between 30°C and 37°C and then decrease at higher temperatures (15). Furthermore, human albumin promotes the germination of *A. fumigatus* but not of *A. flavus* or *A. niger* (493). Another interesting factor found to regulate germination is conidial density. At higher inoculum concentrations of conidia, germination is greatly inhibited (15).

Morphogenesis and Virulence

A significant relationship exists between *A. fumigatus* morphogenesis and pathogenicity. The deletion of numerous genes that regulate germination results in morphogenetic defects as well as decreased virulence. For instance, the deletion of key components of the cAMP-PKA pathway results in altered germination as well as decreased virulence in murine models (330, 649). Furthermore, in the *G. mellonella* model of *A. fumigatus* infection, conidial germination is tightly coupled with virulence (485). In this model, conidia that are nongerminating or just commencing germination are avirulent or virulent only at very high concentrations of inoculation (485). However, conidia that are in the outgrowth phase of germination and that are beginning to form hyphal germ tubes are highly virulent (485).

Another mechanism through which *A. fumigatus* morphogenesis is linked with virulence is the production of biofilms. *A. fumigatus* biofilm-like structures have been observed for some time, as cultures grown under aerial static conditions show different structures of mycelial networks from those grown under submerged liquid conditions (46, 395, 396). Specifically, colonies grown under aerial static conditions have increased mycelial growth and exhibit an extracellular hydrophobic matrix composed of galactomannan, (1,3)-α glucans, monosaccharides, melanin, and various proteins (46). Recently, a similar extracellular matrix was observed for *in vivo A. fumigatus* biofilms during invasion of host lung tissue (345). These biofilm-like aerial static colonies are more resistant to polyenes, azoles, and echinocandins than colonies grown in submerged liquid cultures (46, 395). Furthermore, secondary metabolite biosynthesis genes, including the gliotoxin secondary metabolite gene cluster, are significantly upregulated during biofilm production (80).

As with biofilms produced by other fungal species, *A. fumigatus* adheres to and develops biofilms on polystyrene, human bronchial epithelial cells, and, especially, bronchial epithelial cells of cystic fibrosis patients (517). The ability of antifungal-resistant biofilms to form on human bronchial epithelial cells demonstrates the important correlation between biofilms and *A. fumigatus* virulence. Recently, factors involved in biofilm formation have been uncovered for *A. fumigatus* (209). The developmentally regulated protein MedA is involved in biofilm formation and virulence. An *A. fumigatus* Δ*medA* strain is impaired in adherence to both plastic and pulmonary epithelial cells and is impaired in biofilm maturation (209). The Δ*medA* strain also has reduced virulence in *G. mellonella* and murine models of invasive aspergillosis as well as a reduced capacity to damage pulmonary epithelial cells and stimulate a cytokine response (80, 209).

A. fumigatus also influences virulence through its interactions with human immune cells. As mentioned above, different morphogenetic forms of *A. fumigatus* interact differently with the immune system. For instance, alveolar macrophages target *A. fumigatus* conidia (38, 266, 267), while neutrophils recognize hyphae (38, 151, 323). There is evidence to suggest that exposed β-glucan on *A. fumigatus* germinating conidia activates Dectin-1 on macrophages to produce factors required to recruit neutrophils (310). In accordance with this, the gene expression profiles of conidia and hyphae exposed to neutrophils are dramatically different, with many stress response transcripts being upregulated in conidia but not hyphae (572). This may indicate a specialized conidium-specific response to neutrophils. It was also shown that early and rapid neutrophil recruitment is an essential component of preventing the germination of *A. fumigatus* conidia in the lungs in a murine model of infection (67). Mice depleted of neutrophils prior to, or shortly after, *A. fumigatus* infection showed high mortality rates (384). This was not the case for mice depleted of alveolar macrophages or depleted of neutrophils at later time points (384).

Major Morphogenetic Signaling Cascades

cAMP-protein kinase A. The cAMP-PKA pathway is among the earliest- and best-characterized signaling cascades involved in regulating *A. fumigatus* morphogenesis. The key components of this pathway share homology and function with *C. albicans* factors (Fig. 8). In brief, the *A. fumigatus* PKA complex is composed of PkaR, a type II regulatory subunit, and PkaC1 and PkaC2, the two catalytic subunits (196, 432). Based on homology to other fungi, components that regulate PKA activity have been identified, including the adenylyl cyclase AcyA, which produces cAMP, as well as the Gα protein GpaB, the two GPCRs GprC and GprD, and the two Ras homologues RasA and RasB (187, 201, 329). However, unlike many other fungi, in *A. fumigatus* the connection between Ras and PKA remains unclear, and therefore, Ras signaling will be discussed separately.

All of these components of cAMP-PKA signaling are involved in the regulation of *A. fumigatus* morphogenesis. For instance, Δ*acyA* mutant strains produce fewer hyphae than does the wild type and are extremely deficient in conidiation (329). The addition of dibutyryl-cAMP (db-cAMP) can rescue the phenotype of a Δ*acyA* mutant (329). The deletion of *grpC*

and *gprD* results in defects in hyphal extension and branching as well as reduced germination (201). Δ*gpaB* mutant strains show a phenotype similar to, although less severe than, that of the Δ*acyA* mutant (329).

The PKA complex itself also plays an important role in morphogenesis. A Δ*pkaR* mutant strain has reduced germination rates, reduced conidiation, morphological abnormalities of conidiophores, and reduced virulence in an intranasal mouse model of infection (649). Similarly, the simultaneous deletion of both PkaC subunits resulted in delayed germination and reduced virulence (196). Interestingly, the Δ*pkaR* mutant produces an increased abundance of hyphal nuclei and shows a downregulation of several cell cycle transcripts compared to the wild type (197), indicating that PKA is important for regulating the cell cycle as well as for morphogenesis in *A. fumigatus*.

Downstream effectors of PKA are also involved in *A. fumigatus* morphogenesis. StuA was identified in *A. fumigatus*, based on homology to the well-characterized *C. albicans* PKA downstream effector protein Efg1. Δ*stuA* mutant strains are impaired in their ability to undergo asexual reproduction and conidiation (532). This mutant also displays abnormal conidiophore morphology as well as precocious conidial germination (532). Transcriptional analyses revealed a number of StuA-dependent transcripts, including secondary metabolite biosynthesis genes, and genes encoding proteins involved in morphogenesis (532). The transcription factor BrlA was also suggested to function downstream of PKA signaling in *A. fumigatus* (589). BrlA and StuA govern overlapping, though distinct, transcriptional responses in *A. fumigatus*, and the Δ*brlA* mutant strain has an abnormal conidiophore morphology (589).

Ras. RasA and RasB are also both involved in conidial germination and asexual development in *A. fumigatus* (187). The deletion of *rasB* causes decreased germination, and Δ*rasB* mutant strains have an irregular hyphal morphology and exhibit increased hyphal branching. The Δ*rasB* mutant also has diminished virulence in a mouse model of invasive aspergillosis (188). Similarly, the deletion of *rasA* causes delayed germination, poor conidiation, and defects in radial growth (184). Δ*rasA* mutants also exhibit a mutant hyphal morphology, including wider hyphal diameters and abnormal nuclear distributions, and display changes in axis polarity during hyphal growth (184). Furthermore, Δ*rasA* mutants have decreased virulence in an immunosuppressed mouse model of intranasal infection (184).

Protein modification pathways. The cell wall of *A. fumigatus* is composed primarily of glucans, chitin, and galactomannan, coated with glycoproteins containing mannose and galactose. These glycoproteins are derived from protein modification events, such as glycosylation (181, 308), or the addition of a glycosylphosphatidylinositol (GPI) anchor. In *A. fumigatus*, protein modifications play an important role in morphogenetic transitions. These transitions include O glycosylation (in fungi, generally referred to as O mannosylation) as well as N glycosylation and the attachment of GPI anchors.

(i) O mannosylation. Protein *O*-mannosyltransferases (PMTs) are responsible for O mannosylation at serine or threonine residues of secreted proteins. This process mediates the import of secretory proteins into the ER and is highly conserved across eukaryotic organisms. PMTs have been well characterized for many fungal species and are involved in many important cellular processes, including the morphogenetic transitions of both *C. albicans* (469, 580) and *C. neoformans* (434). In *A. fumigatus*, Pmt1 acts as an *O*-mannosyltransferase, and a Δ*pmt1* mutant strain has defects in cell wall integrity as well as cellular morphology (654). The Δ*pmt1* mutant displays impaired conidium formation as well as a decreased rate of conidial germination (654), indicating the important role of Pmt1 in morphogenesis. Interestingly, although severely impaired in morphogenetic transitions, the Δ*pmt1* mutant does not have a significant defect in virulence in a murine model of *A. fumigatus* infection (654).

The other *A. fumigatus* PMT proteins, Pmt2 and Pmt4, also play an important role in *A. fumigatus* morphogenesis. A disruption of *pmt4* results in abnormal mycelial growth as well as reduced conidiation (393). Similarly, the depletion of *pmt2* leads to reduced conidiation as well as a delay in both conidial germination and hyphal growth (174). Furthermore, the genetic depletion of *pmt2* causes improper actin rearrangements and polarization in the growing hyphal cell. In this mutant, actin patches fail to localize to the growing tip of the germ tube in a polarized manner and instead are randomly distributed throughout the cell (174).

(ii) N glycosylation. Another important protein modification involved in *A. fumigatus* morphogenesis is N glycosylation. α-Mannosidases have an important function in the processing of N-glycans, and the 1,2-α-mannosidase MsdS in *A. fumigatus* plays an important role in morphogenesis. The deletion of *msdS* results in defective N-glycan processing, and a Δ*msdS* mutant displays abnormal germination, hyphal growth, and conidiation caused by defects in polarity and septation (328). Similarly, the α-glucosidase Cwh41, which is also involved in *A. fumigatus* N-glycan processing, is required for proper polar growth, elongation, and septation during hyphal growth (646).

GDP-mannose is an activated form of mannose and is a substrate for glycosyltransferases or mannosyltransferases. GDP-mannose pyrophosphorylase (GMPP) catalyzes the synthesis of GDP-mannose and therefore plays a key role in protein glycosylation. In *A. fumigatus*, the GMPP Srb1 is involved in morphogenesis. The depletion of the *srb1* gene results in reduced conidiation as well as rapid and precocious germination (253). Furthermore, the corresponding mutant is defective in polarity maintenance and branching-site selection and produces hyperbranched hyphal cells (253). Other proteins involved in the processing of mannose, including the phosphomannose isomerase (PMI) protein Pmi1, have also been implicated in *A. fumigatus* morphogenesis (175).

(iii) GPI modifications. The GPI anchor is a conserved protein modification in eukaryotes, which allows many cell surface proteins to become anchored to the cell membrane. In other fungi, such as *S. cerevisiae* and *C. albicans*, many GPI-anchored proteins have important roles in cell wall organization and morphogenesis. Similarly, GPI-anchored proteins, as well as GPI anchor biosynthesis proteins, have important roles in *A. fumigatus* cell wall formation and morphogenesis.

Several GPI-anchored proteins are involved in *A. fumigatus* morphogenesis. Gel2 is a GPI-anchored β(1-3)glucanosyltransferase, and the deletion of *gel2* causes irregular morphogenesis, including an abnormal conidiophore morphology

(394). Similarly, the disruption of the GPI-anchored cell wall integrity protein Ecm33 results in abnormal morphogenesis, including the rapid germination of conidia (498).

In addition to the importance of GPI-anchored proteins, GPI anchor biosynthesis plays a critical role in morphogenesis. In *A. fumigatus*, the GPI–*N*-acetylglucosaminyltransferase complex catalyzes GPI biosynthesis via the PigA complex (324). The deletion of *pigA* leads to an inhibition of GPI anchor synthesis and causes severe cell wall defects as well as abnormal hyphal growth and abnormal conidiation (324).

Calcineurin. The protein phosphatase calcineurin is a key component for mediating cellular stress response pathways in diverse fungi. The highly conserved calcineurin protein is typically composed of a catalytic A subunit and a regulatory B subunit, although in *A. fumigatus* only the catalytic subunit CnaA/CalA has been subject to investigation. Calcineurin is activated by Ca^{2+}-calmodulin, and in turn, calcineurin regulates downstream effectors, such as *A. fumigatus* CrzA. Aside from its important roles in the stress response, the calcineurin pathway is involved in the morphogenetic transitions of *A. fumigatus* (Fig. 9).

Compromising calcineurin function with specific pharmacological inhibitors impairs filamentation, resulting in delayed hypha production (560). Furthermore, Δ*cnaA* mutants lacking the calcineurin catalytic subunit display defective hyphal morphology, including improper polarized growth and overall decreased filamentation (132, 557). These mutants have altered sporulation and abnormal conidial morphology as well as decreased virulence in several independent models of *A. fumigatus* infection (132, 557). The key role for CnaA in regulating *A. fumigatus* morphogenesis is reinforced by the fact that green fluorescent protein (GFP)-tagged CnaA localizes to actively growing hyphal tips and to hyphal septa (261). The deletion of *cnaA* or the inhibition of calcineurin pharmacologically causes abnormal septation and a loss of conidiophore formation (261), which may explain the morphogenetic defect.

Other factors involved in calcineurin signaling are similarly involved in regulating morphogenesis. For instance, CrzA, the transcription factor downstream of calcineurin, plays an important role in germination and hyphal growth. A Δ*crzA* mutant strain displays decreased conidiation, delayed and reduced germination rates, as well as defects in hyphal morphology and polarized hyphal growth (123, 546). This mutant also has highly reduced virulence in a mouse model of invasive aspergillosis (123, 546). Another factor involved in calcineurin signaling is the calcipressin CbpA, which belongs to a conserved class of calcineurin binding proteins that negatively regulate calcineurin function. The deletion of *cbpA* reduces hyphal growth but not as severely as does the deletion of *cnaA* or *crzA* (462).

Mitogen-activated protein kinase. In fungi, MAPK pathways play an integral role in regulating cellular functions in response to environmental changes and perturbations. This includes maintaining cell wall integrity, controlling osmoregulation, responding to nutrients, and regulating filamentation in different fungal species, including *C. albicans* and *S. cerevisiae* (195, 495). MAPKs are the terminal kinases in the cascades of three kinases, which activate each other in series. *A. fumigatus* has four MAPKs: SakA, which is closely related to the HOG MAPKs of other fungi; MpkC, which is similar to SakA; MpkB, which is most similar to MAPKs involved in pheromone signaling; and MpkA, which is most similar to MAPKs involved in cell wall integrity (375). SakA and MpkA are both involved in *A. fumigatus* morphogenesis.

The HOG-MAPK pathway is responsible for regulating cellular responses to osmotic stress conditions and also plays a role in morphogenesis. The deletion of the HOG pathway MAPK *sakA* results in abnormal germination and hyphal growth under different environmental conditions, including increased germination rates on reduced-nitrogen medium (638). Similarly, the Sho1 adaptor protein, which is an important component of upstream sensing in the HOG-MAPK pathway, is important for conidial germination (349). A Δ*sho1* mutant strain has reduced germination rates and produces irregular hyphae (349).

The cell wall integrity MAPK module is also involved in *A. fumigatus* morphogenesis. The deletion of the MAPK *mpkA* results in defective hyphal formation, including shorter, more branched hyphal cells (596). A similar mutant hyphal phenotype can be observed upon the deletion of *mkk1* or *bck1*, the upstream MAPKK and MAPKKK, respectively (596), indicating the important role for this signaling cascade in maintaining proper hyphal growth.

Other pathways. Numerous other factors and pathways have been shown to influence *A. fumigatus* morphogenesis. Many of these factors are related to ones influencing the morphogenetic transitions of other fungal organisms. For instance, the transcription factor Ace2, which has an important role in mediating the *C. albicans* morphogenetic program (271, 401, 609), also functions in *A. fumigatus* morphogenesis. The *A. fumigatus* Δ*ace2* mutant strain displays abnormal conidiation as well as accelerated germination (166). Furthermore, this mutant is hypervirulent is a mouse model of invasive aspergillosis (166). Interestingly, Ace2 plays distinct roles in virulence in different fungal species: mutants of *C. albicans* or *S. cerevisiae* that lack Ace2 have attenuated virulence in mouse models of infection, while, similarly to *A. fumigatus*, mutants of *C. glabrata* lacking Ace2 are hypervirulent (265, 271, 351). Another link between *C. albicans* and *A. fumigatus* morphogenesis is the contribution of HDACs. In *A. fumigatus*, the HDAC HdaA as well as the ribosome biogenesis protein CgrA both play a role in wild-type germination and radial growth of *A. fumigatus* (53, 54, 315).

ASPERGILLUS FUMIGATUS DRUG RESISTANCE: THE AZOLES

The azoles selectively target a cytochrome P-450 ergosterol biosynthesis enzyme, encoded by *cyp51A* and *cyp51B*, thereby disrupting the production of ergosterol and causing the accumulation of toxic sterol intermediates. *A. fumigatus* is intrinsically resistant to the commonly used azole fluconazole (148), and it was suggested that the specific azole binding properties of *A. fumigatus cyp51A* and *cyp51B* may result in the intrinsic fluconazole resistance of this species (618). In 1990, itraconazole became the first azole introduced for the treatment of aspergillosis (146). Subsequently, voriconazole and posaconazole both became available as treatment options as well. The first documented case of *A. fumigatus* resistance to an azole was itraconazole resistance, described in 1997 (147). Since then, many azole-resistant strains of *A. fumigatus* have been isolated and characterized. The resistance mechanisms of

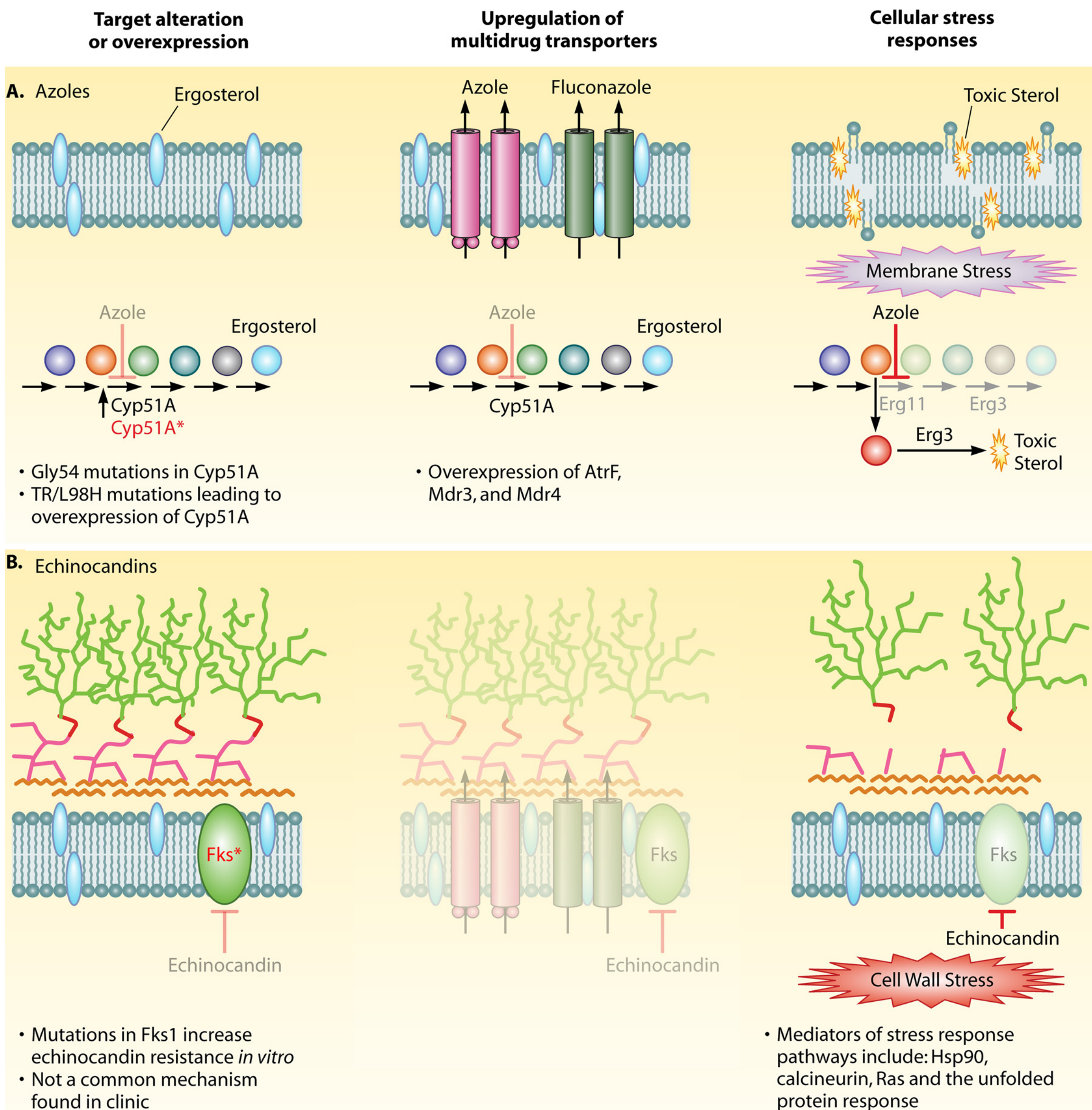

FIG. 12. *A. fumigatus* drug resistance mechanisms. (A) *A. fumigatus* can acquire resistance to the azoles through multiple mechanisms, including the upregulation or alteration of the drug target Cyp51A or the upregulation of the multidrug transporters AtrF, Mdr3, and Mdr4. (B) Resistance to the echinocandins through mutations in *fks1*, encoding the catalytic subunit of (1,3)-β-D-glucan synthase, has been discovered in experimentally evolved populations but has not yet been found in the clinic. Numerous stress response pathways are also important for the basal tolerance and resistance of *A. fumigatus* to echinocandins. The bullet points below each mechanism describe the manner in which resistance is acquired. Bright images represent those mechanisms important for that particular drug class, whereas dimmed images represent those mechanisms that do not play a key role. (Adapted from reference 111 with permission of Nature Publishing Group.)

these isolates, which will be described here, include an alteration of the drug target, the upregulation of drug efflux pumps, or alterations in stress response pathways (Fig. 12A).

Alteration of the Drug Target

The alteration of the drug target is the primary mechanism of resistance among *A. fumigatus* isolates. The first studies of azole-resistant *A. fumigatus* strains hypothesized a role for target alteration (147, 360), although the first direct evidence was established for *A. nidulans*, where the overexpression of cytochrome P-450 lanosterol 14α-demethylase increased the MIC of itraconazole 36-fold (440). Since then, mutations in *cyp51A* have become well established as a significant cause of azole resistance.

The first mutation identified to have a role in *A. fumigatus* azole resistance was the *cyp51A* glycine 54 (G54) point mutation, which was detected in both laboratory-evolved posaconazole-resistant strains as well as clinical isolates with reduced posaconazole susceptibility (362). This same mutation was also found to contribute to itraconazole resistance in clinical isolates (152). A high-throughput multiplex reverse transcription (RT)-PCR developed to identify mutations in *A. fumigatus*

cyp51A revealed that nearly 50% of clinical isolates or laboratory-derived mutants with reduced susceptibility to itraconazole had a G54 mutation (36). Other less commonly identified mutations that are involved in *A. fumigatus* azole resistance include methionine 220 (M220) and glycine 138 (G138) mutations (234, 377).

More recently, alternative mutations have emerged as a prevalent mechanism of azole resistance in *A. fumigatus*. In a study analyzing *A. fumigatus* clinical isolates with reduced susceptibility to azoles, all 14 isolates investigated had a *cyp51A* leucine 98-for-histidine (L98H) mutation as well as two copies of a 34-bp sequence in tandem (TR) in the *cyp51A* promoter (378). Further analyses demonstrated that this genotype (TR/L98H) conferred up to an 8-fold increase in the level of expression of *cyp51A* compared to that of an azole-susceptible strain (378). Interestingly, it was found that the transformation of the azole-resistant *cyp51A* open reading frame or promoter into a susceptible strain could confer moderate increases in resistance, but only transformants with both the tandem repeats and the L98H substitution exhibited full resistance (378), establishing the importance of the combination of both alterations in azole resistance. Independent large-scale studies have confirmed that the TR/L98H genotype is the most prevalent resistance mechanism observed for azole-resistant *A. fumigatus* isolates (392, 543).

Intriguingly, the TR/L98H genotype is not restricted to clinical isolates. Itraconazole-resistant *A. fumigatus* isolates were sampled from a wide range of environmental locations, including indoor hospital environments, soil, and compost (392, 541). Many of these environmental isolates displayed cross-resistance to other azoles, including the azole fungicides metconazole and tebuconazole, and the majority of environmental isolates possessed the TR/L98H genotype (392, 541). This, along with evidence that resistant environmental and clinical isolates genetically cluster together and apart from susceptible isolates (541), suggests the possibility that patients with azole-resistant *A. fumigatus* infections may have been colonized with resistant isolates from the environment. This raises the interesting possibility that this mechanism of azole resistance in *A. fumigatus* may be partially a consequence of environmental fungicide use (603).

Upregulation of Drug Pumps

Early studies attempting to discover azole resistance mechanisms of *A. fumigatus* found resistant isolates that had a reduced intracellular accumulation of itraconazole, suggesting a role for drug efflux pumps in mediating resistance (147, 361). The efflux pumps described for *A. fumigatus* include the ABC transporter AtrF and four Mdr-like pumps, Mdr1 to Mdr4.

An analysis of *atrF* mRNA from susceptible isolates and from the earliest-identified itraconazole-resistant isolates revealed that resistance correlated with increased levels of *atrF* (539). The MDR pumps are also involved in *A. fumigatus* azole resistance. In itraconazole-resistant mutants selected *in vitro*, *mdr3*, *mdr4*, and *atrF* showed pronounced changes in expression in many evolved mutants, with either constitutively high levels of expression of these transcripts or an induction of expression upon exposure to itraconazole (132, 411). Some resistant mutants also showed increased expression levels of *mdr1* or *mdr2*, although this expression change is very uncommon (132).

Stress Response

Very little is known regarding stress response pathways that may regulate azole resistance in *A. fumigatus*; however, a limited number of stress response regulators have been implicated in azole resistance.

SrbA is a sterol-regulatory element binding protein, which is crucial for mediating stress responses under hypoxic conditions. The deletion of *srbA* results in an inability of *A. fumigatus* to grow in hypoxic environments and an inability to cause disease in a mouse model of invasive aspergillosis (629). SrbA also plays a key role in ergosterol biosynthesis and mediates antifungal resistance (629). SrbA is specifically required for resistance to the azole class of antifungals, including fluconazole and voriconazole, although not for resistance to polyenes or echinocandins (629).

Other stress response factors have also been implicated in azole resistance, including mediators of oxidative and cell wall stresses. Yap1 is involved in regulating the oxidative stress response in *A. fumigatus* (320, 472). A truncated mutant allele of *yap1* lacking its C-terminal cysteine-rich domain is hyperactive and confers increased resistance to oxidative stress *in vitro* (473). This mutant allele also confers attenuated susceptibility to voriconazole *in vitro* (473), demonstrating that the oxidative stress response pathway may have a role in antifungal resistance. Similarly, Mkk2 is involved in the *A. fumigatus* cell wall integrity pathway, and Δ*mkk2* mutants have increased sensitivity to both posaconazole and voriconazole (153), indicating a relationship between cell wall integrity and azole resistance in *A. fumigatus*.

Hsp90 is a highly conserved molecular chaperone, which plays a central role in the emergence and maintenance of fungal drug resistance (115). Hsp90 mediates resistance to the azoles in *C. albicans* as well as resistance to the echinocandins in *Aspergillus terreus* (115). In *A. fumigatus*, the pharmacological inhibition of Hsp90 enhances the efficacy of voriconazole against a clinical isolate under certain environmental conditions (118), suggesting a potential role for Hsp90 in the azole resistance of *A. fumigatus*.

ASPERGILLUS FUMIGATUS DRUG RESISTANCE: THE POLYENES

The polyene amphotericin B associates with ergosterol and forms a transmembrane channel that leads to ion leakage and, often, cellular death. Although there are conflicting data on the correlation between *in vitro* susceptibility to amphotericin B and clinical outcomes in *A. fumigatus* infection (254, 306, 336, 428), resistance to amphotericin B is well established for many *Aspergillus* species.

Interestingly, amphotericin B resistance varies considerably across *Aspergillus* species. For instance, *A. terreus* is frequently found to be resistant to amphotericin B *in vitro* as well as *in vivo* in animal models, even at high concentrations (210, 556, 574, 608). Similarly, *A. nidulans* clinical isolates are frequently found to be resistant to amphotericin B *in vitro* (281). On the other hand, evidence suggests that *A. fumigatus* amphotericin B resistance is unlikely to emerge during treatment for invasive aspergillosis, as amphotericin B MICs remain similar between isolates recovered from patients before and those recovered

after amphotericin B treatment (131, 389). Amphotericin B susceptibility testing of hundreds of environmental and clinical *Aspergillus* isolates suggests intrinsic reduced susceptibilities of both *A. terreus* and *A. flavus* and increased susceptibilities of *A. fumigatus* and *A. glaucus* (14). It was proposed that the basis for the amphotericin B resistance of *A. terreus* may be the high level of catalase production compared with that of *A. fumigatus*, although this remains unconfirmed (63).

ASPERGILLUS FUMIGATUS DRUG RESISTANCE: THE ECHINOCANDINS

The echinocandins block the synthesis of glucan found in the cell wall via the inhibition of (1,3)-β-D-glucan synthase. The echinocandins are the newest class of antifungal agents, and recent studies have found favorable results for the use of echinocandins such as micafungin, caspofungin, or aminocandin for the treatment of invasive aspergillosis (145, 283, 616, 617). Indeed, most *Aspergillus* species tested, with the exception of *A. flavus* and certain *A. nidulans* isolates, are susceptible to micafungin (128). The echinocandins act in a fungistatic manner against *A. fumigatus*, and recently, resistance to the echinocandins has been identified for numerous *Aspergillus* species. The first case of *A. fumigatus* with *in vitro* resistance to caspofungin was described in 2008 for an isolate from a patient with invasive aspergillosis (171). Unlike azole resistance in *A. fumigatus*, the molecular mechanisms involved in echinocandin resistance involve mostly cellular stress response pathways, although there are also examples of target alterations regulating resistance (Fig. 12B).

Alteration of the Drug Target

In other fungi, echinocandins such as caspofungin have been shown to function by inhibiting Fks1, the catalytic subunit of (1,3)-β-D-glucan synthase (157). Caspofungin-resistant isolates of *C. albicans* and *S. cerevisiae* have been reported to contain mutations within the *FKS1* gene, specifically at two distinct hot-spot regions (157, 294, 447, 455). In *A. fumigatus* isolates, the introduction of a site-directed mutation within *fks1* confers increased resistance to caspofungin (200). Similarly, an *A. fumigatus* Ser678Pro *fks1* mutation, equivalent to a mutation known to confer echinocandin resistance in *Candida*, confers high levels of cross-resistance to three echinocandins in *A. fumigatus* (491). However, mutations in *fks1* are not commonly identified in clinical isolates with reduced echinocandin susceptibilities.

Stress Response

Common mechanisms of echinocandin resistance in *A. fumigatus* appear to be independent of Fks1. An analysis of a class of spontaneously generated *A. fumigatus* mutants with reduced susceptibility to the echinocandins found no mutations in *fks1* and no changes in its expression (200), suggesting that other mechanisms of resistance must be involved. In accordance with this, well-characterized stress response pathways have been implicated in *A. fumigatus* echinocandin resistance and will be discussed here.

In *A. fumigatus*, different calcineurin inhibitors enhance the activity of caspofungin and lead to attenuated growth *in vitro* (282, 560). The deletion of *cnaA*, which encodes the catalytic subunit of calcineurin, similarly enhances the efficacy of caspofungin (558). Furthermore, the calcineurin inhibitor FK506 renders the fungistatic activity of caspofungin fungicidal against *A. fumigatus* (560).

Interestingly, the deletion of either *cnaA* or its downstream effector, *crzA*, leads to decreased amounts of (1,3)-β-D-glucan in the cell wall of *A. fumigatus*, suggesting that the inhibition of calcineurin signaling disrupts cell wall biosynthesis (123, 186, 558). When these Δ*cnaA* or Δ*crzA* mutants are treated with caspofungin, they show even further decreased (1,3)-β-D-glucan content (123, 186). Intriguingly, while wild-type cells compensate for caspofungin-induced (1,3)-β-D-glucan depletion by increasing chitin synthase gene expression and cell wall chitin contents, the Δ*cnaA* and Δ*crzA* deletion strains fail to do so (185, 186), indicating that calcineurin may regulate compensatory chitin synthesis in the presence of echinocandin stress.

Calcineurin activity is dependent on the stabilization of the catalytic subunit by the molecular chaperone Hsp90, which also plays a role in *A. fumigatus* echinocandin resistance. Treatment with a pharmacological inhibitor of Hsp90 *in vitro* reduces the caspofungin resistance of *A. fumigatus* as well as *A. terreus* (115, 118). Furthermore, in the *G. mellonella* model of infection, combination therapy with an Hsp90 inhibitor and caspofungin drastically improves the survival of *G. mellonella* with *A. fumigatus* infections, while treatment with either agent alone fails to improve the outcome of the lethal infection (118).

Other cell wall stress response factors are also involved in mediating echinocandin resistance. Ecm33 is a GPI-anchored protein with important roles in *A. fumigatus* morphogenesis and the cell wall stress response (498). A disruption of *ecm33* leads to increased resistance to caspofungin in *A. fumigatus* (498). Similarly, RasA, which has important roles in the cell wall integrity of *A. fumigatus* (184), is also involved in echinocandin resistance. Δ*rasA* mutant strains have decreased cell wall (1,3)-β-D-glucan content yet have increased resistance to echinocandins (186), suggesting that the deletion of *rasA* may promote compensatory mechanisms in response to cell wall stress and thereby enable echinocandin resistance.

The unfolded protein response (UPR) also has a function in mediating resistance to the echinocandins. HacA is the major regulator of the UPR in *A. fumigatus*, and a Δ*hacA* mutant is hypersensitive to ER stress as well as thermal stress (487). Interestingly, the Δ*hacA* mutant also has heightened susceptibility to all antifungals tested, including caspofungin, amphotericin B, itraconazole, and fluconazole (487). In the case of caspofungin, not only is the Δ*hacA* mutant hypersensitive, but no viable mutant cells could be recovered after treatment, indicating that caspofungin was fungicidal against this UPR response mutant (487).

CONNECTIONS BETWEEN MORPHOGENESIS AND ANTIFUNGAL DRUG RESISTANCE IN *ASPERGILLUS FUMIGATUS*

The morphological stage of *A. fumigatus* can impact its response and resistance to antifungal agents. In the case of the polyenes, ungerminated conidia are insensitive to low concen-

trations of amphotericin B but become sensitive during the early stages of hyphal germination (500). At higher drug concentrations, amphotericin B inhibits ungerminated conidia, germinated conidia, and hyphae (360, 599), although a much higher dose of the drug is required to inhibit the activity of hyphae in hyphal clumps (599).

CONCLUSION

C. albicans, *C. neoformans*, and *A. fumigatus*, though evolutionarily disparate pathogenic fungi, exhibit both divergent and highly conserved cellular circuitries. As evidenced here, complex signaling networks govern the development, morphogenetic transitions, and evolution of antifungal drug resistance in these species. Intriguingly, the morphogenetic programs of these organisms are very distinct, in that *C. albicans* yeast-to-filament morphogenesis is an environmentally regulated transition, while morphogenesis in *C. neoformans* and *A. fumigatus* represents discrete phases of the fungal life cycle. Despite these unique contexts governing morphogenesis, the underlying cellular signaling networks remain highly conserved between the species. Signaling pathways such as the cAMP-PKA and MAPK cascades are important components of morphogenetic signaling in these as well as other fungal species. Furthermore, in spite of the fact that different antifungal agents have different efficacies against the pathogenic fungi discussed here, many mechanisms of resistance to antifungal drugs, including target alteration, drug transporter overexpression, and stress responses, are conserved in these species.

Although the three human-pathogenic fungi highlighted in this review are those most frequently encountered in the clinic today, they represent a fraction of the diversity of pathogenic fungi worldwide. Pathogenic fungi are not limited to humans but possess a diverse host range that encompasses other mammalian species, amphibians, insects, and plants. The complex interplay between these diverse hosts and pathogens is governed by environmental change, which has lead to altered distributions of pathogenic fungi in response to selective pressures. This is supported by the recent emergence of *C. gattii*, a species previously associated with tropical and subtropical climates (296, 297), as a primary pathogen of otherwise healthy hosts in northwestern North America (40, 84–86, 191, 352, 594). Other recent examples of changing fungus-host interactions include the sudden extinctions of frog populations caused by the chytrid fungus *Batrachochytrium dendrobatidis* (49, 250, 337, 567) as well the dramatic collapse of bee colonies associated with the microsporidian *Nosema ceranae* (73, 575). It is likely that such pathogenic fungi sense and respond to their changing environment through many of the same cellular signaling networks discussed in this review. The conservation of such signaling pathways, coupled with both current genomic resources developed for the well-studied species discussed here and the availability of newly identified fungal genome sequences, will facilitate experimental analysis and promote in-depth inquiries into a broader range of pathogenic fungi.

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REFERENCES

1. **Abruzzo, G. K., et al.** 1997. Evaluation of the echinocandin antifungal MK-0991 (L-743,872): efficacies in mouse models of disseminated aspergillosis, candidiasis, and cryptococcosis. Antimicrob. Agents Chemother. **41:** 2333–2338.
2. **Albrecht, D., R. Guthke, A. A. Brakhage, and O. Kniemeyer.** 2010. Integrative analysis of the heat shock response in Aspergillus fumigatus. BMC Genomics **11:**32.
3. **Alby, K., and R. J. Bennett.** 2009. Stress-induced phenotypic switching in Candida albicans. Mol. Biol. Cell **20:**3178–3191.
4. **Alby, K., D. Schaefer, and R. J. Bennett.** 2009. Homothallic and heterothallic mating in the opportunistic pathogen Candida albicans. Nature **460:**890–893.
5. **Al-Dhaheri, R. S., and L. J. Douglas.** 2010. Apoptosis in Candida biofilms exposed to amphotericin B. J. Med. Microbiol. **59:**149–157.
6. **Alejandro-Osorio, A. L., et al.** 2009. The histone deacetylase Rpd3p is required for transient changes in genomic expression in response to stress. Genome Biol. **10:**R57.
7. **Aller, A. I., et al.** 2007. Antifungal susceptibility of Cryptococcus neoformans isolates in HIV-infected patients to fluconazole, itraconazole and voriconazole in Spain: 1994-1996 and 1997-2005. Chemotherapy **53:**300–305.
8. **Alonso-Monge, R., et al.** 1999. Role of the mitogen-activated protein kinase Hog1p in morphogenesis and virulence of Candida albicans. J. Bacteriol. **181:**3058–3068.
9. **Alspaugh, J. A., L. M. Cavallo, J. R. Perfect, and J. Heitman.** 2000. RAS1 regulates filamentation, mating and growth at high temperature of Cryptococcus neoformans. Mol. Microbiol. **36:**352–365.
10. **Alspaugh, J. A., J. R. Perfect, and J. Heitman.** 1997. Cryptococcus neoformans mating and virulence are regulated by the G-protein alpha subunit GPA1 and cAMP. Genes Dev. **11:**3206–3217.
11. **Alvarez, M., and A. Casadevall.** 2006. Phagosome extrusion and host-cell survival after Cryptococcus neoformans phagocytosis by macrophages. Curr. Biol. **16:**2161–2165.
12. **Andaluz, E., T. Ciudad, J. Gomez-Raja, R. Calderone, and G. Larriba.** 2006. Rad52 depletion in Candida albicans triggers both the DNA-damage checkpoint and filamentation accompanied by but independent of expression of hypha-specific genes. Mol. Microbiol. **59:**1452–1472.
13. **Anderson, J. B.** 2005. Evolution of antifungal-drug resistance: mechanisms and pathogen fitness. Nat. Rev. Microbiol. **3:**547–556.
14. **Araujo, R., C. Pina-Vaz, and A. Goncalves Rodrigues.** 2007. Susceptibility of environmental versus clinical strains of pathogenic *Aspergillus*. Int. J. Antimicrob. Agents **29:**108–111.
15. **Araujo, R., and A. G. Rodrigues.** 2004. Variability of germinative potential among pathogenic species of Aspergillus. J. Clin. Microbiol. **42:**4335–4337.
16. **Argimon, S., et al.** 2007. Developmental regulation of an adhesin gene during cellular morphogenesis in the fungal pathogen Candida albicans. Eukaryot. Cell **6:**682–692.
17. **Armengou, A., C. Porcar, J. Mascaro, and F. Garcia-Bragado.** 1996. Possible development of resistance to fluconazole during suppressive therapy for AIDS-associated cryptococcal meningitis. Clin. Infect. Dis. **23:**1337–1338.
18. **Askew, D. S.** 2008. Aspergillus fumigatus: virulence genes in a street-smart mold. Curr. Opin. Microbiol. **11:**331–337.
19. **Bachewich, C., A. Nantel, and M. Whiteway.** 2005. Cell cycle arrest during S or M phase generates polarized growth via distinct signals in Candida albicans. Mol. Microbiol. **57:**942–959.
20. **Bachewich, C., D. Y. Thomas, and M. Whiteway.** 2003. Depletion of a polo-like kinase in Candida albicans activates cyclase-dependent hyphal-like growth. Mol. Biol. Cell **14:**2163–2180.
21. **Bachewich, C., and M. Whiteway.** 2005. Cyclin Cln3p links G1 progression to hyphal and pseudohyphal development in Candida albicans. Eukaryot. Cell **4:**95–102.
22. **Bachmann, S. P., T. F. Patterson, and J. L. Lopez-Ribot.** 2002. In vitro activity of caspofungin (MK-0991) against Candida albicans clinical isolates displaying different mechanisms of azole resistance. J. Clin. Microbiol. **40:**2228–2230.
23. **Bachmann, S. P., et al.** 2002. In vitro activity of caspofungin against Candida albicans biofilms. Antimicrob. Agents Chemother. **46:**3591–3596.
24. **Baek, Y. U., S. J. Martin, and D. A. Davis.** 2006. Evidence for novel

pH-dependent regulation of Candida albicans Rim101, a direct transcriptional repressor of the cell wall beta-glycosidase Phr2. Eukaryot. Cell **5:**1550–1559.

25. **Bahn, Y. S.** 2008. Master and commander in fungal pathogens: the two-component system and the HOG signaling pathway. Eukaryot. Cell **7:**2017–2036.
26. **Bahn, Y. S., S. Geunes-Boyer, and J. Heitman.** 2007. Ssk2 mitogen-activated protein kinase kinase kinase governs divergent patterns of the stress-activated Hog1 signaling pathway in Cryptococcus neoformans. Eukaryot. Cell **6:**2278–2289.
27. **Bahn, Y. S., J. K. Hicks, S. S. Giles, G. M. Cox, and J. Heitman.** 2004. Adenylyl cyclase-associated protein AcaI regulates virulence and differentiation of Cryptococcus neoformans via the cyclic AMP-protein kinase A cascade. Eukaryot. Cell **3:**1476–1491.
28. **Bahn, Y. S., K. Kojima, G. M. Cox, and J. Heitman.** 2006. A unique fungal two-component system regulates stress responses, drug sensitivity, sexual development, and virulence of Cryptococcus neoformans. Mol. Biol. Cell **17:**3122–3135.
29. **Bahn, Y. S., K. Kojima, G. M. Cox, and J. Heitman.** 2005. Specialization of the HOG pathway and its impact on differentiation and virulence of Cryptococcus neoformans. Mol. Biol. Cell **16:**2285–2300.
30. **Bahn, Y. S., and F. A. Muhlschlegel.** 2006. CO2 sensing in fungi and beyond. Curr. Opin. Microbiol. **9:**572–578.
31. **Bahn, Y. S., J. Staab, and P. Sundstrom.** 2003. Increased high-affinity phosphodiesterase PDE2 gene expression in germ tubes counteracts CAP1-dependent synthesis of cyclic AMP, limits hypha production and promotes virulence of Candida albicans. Mol. Microbiol. **50:**391–409.
32. **Bahn, Y. S., and P. Sundstrom.** 2001. CAP1, an adenylate cyclase-associated protein gene, regulates bud-hypha transitions, filamentous growth, and cyclic AMP levels and is required for virulence of Candida albicans. J. Bacteriol. **183:**3211–3223.
33. **Bai, C., N. Ramanan, Y. M. Wang, and Y. Wang.** 2002. Spindle assembly checkpoint component CaMad2p is indispensable for Candida albicans survival and virulence in mice. Mol. Microbiol. **45:**31–44.
34. **Baillie, G. S., and L. J. Douglas.** 1998. Effect of growth rate on resistance of Candida albicans biofilms to antifungal agents. Antimicrob. Agents Chemother. **42:**1900–1905.
35. **Baker, S. P., and P. A. Grant.** 2007. The SAGA continues: expanding the cellular role of a transcriptional co-activator complex. Oncogene **26:**5329–5340.
36. **Balashov, S. V., R. Gardiner, S. Park, and D. S. Perlin.** 2005. Rapid, high-throughput, multiplex, real-time PCR for identification of mutations in the cyp51A gene of *Aspergillus fumigatus* that confer resistance to itraconazole. J. Clin. Microbiol. **43:**214–222.
37. **Balashov, S. V., S. Park, and D. S. Perlin.** 2006. Assessing resistance to the echinocandin antifungal drug caspofungin in Candida albicans by profiling mutations in FKS1. Antimicrob. Agents Chemother. **50:**2058–2063.
38. **Balloy, V., and M. Chignard.** 2009. The innate immune response to Aspergillus fumigatus. Microbes Infect. **11:**919–927.
39. **Bamford, C. V., et al.** 2009. Streptococcus gordonii modulates Candida albicans biofilm formation through intergeneric communication. Infect. Immun. **77:**3696–3704.
40. **Bartlett, K. H., S. E. Kidd, and J. W. Kronstad.** 2008. The emergence of Cryptococcus gattii in British Columbia and the Pacific Northwest. Curr. Infect. Dis. Rep. **10:**58–65.
41. **Barwell, K. J., J. H. Boysen, W. Xu, and A. P. Mitchell.** 2005. Relationship of DFG16 to the Rim101p pH response pathway in Saccharomyces cerevisiae and Candida albicans. Eukaryot. Cell **4:**890–899.
42. **Bassilana, M., and R. A. Arkowitz.** 2006. Rac1 and Cdc42 have different roles in Candida albicans development. Eukaryot. Cell **5:**321–329.
43. **Bassilana, M., J. Blyth, and R. A. Arkowitz.** 2003. Cdc24, the GDP-GTP exchange factor for Cdc42, is required for invasive hyphal growth of Candida albicans. Eukaryot. Cell **2:**9–18.
44. **Bastidas, R. J., J. Heitman, and M. E. Cardenas.** 2009. The protein kinase Tor1 regulates adhesin gene expression in Candida albicans. PLoS Pathog. **5:**e1000294.
45. **Bauer, J., and J. Wendland.** 2007. Candida albicans Sfl1 suppresses flocculation and filamentation. Eukaryot. Cell **6:**1736–1744.
46. **Beauvais, A., et al.** 2007. An extracellular matrix glues together the aerial-grown hyphae of Aspergillus fumigatus. Cell. Microbiol. **9:**1588–1600.
47. **Bensen, E. S., A. Clemente-Blanco, K. R. Finley, J. Correa-Bordes, and J. Berman.** 2005. The mitotic cyclins Clb2p and Clb4p affect morphogenesis in Candida albicans. Mol. Biol. Cell **16:**3387–3400.
48. **Berbee, M. L., D. A. Carmean, and K. Winka.** 2000. Ribosomal DNA and resolution of branching order among the ascomycota: how many nucleotides are enough? Mol. Phylogenet. Evol. **17:**337–344.
49. **Berger, L., et al.** 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc. Natl. Acad. Sci. U. S. A. **95:**9031–9036.
50. **Berman, J.** 2006. Morphogenesis and cell cycle progression in Candida albicans. Curr. Opin. Microbiol. **9:**595–601.
51. **Berman, J., and P. E. Sudbery.** 2002. Candida albicans: a molecular revolution built on lessons from budding yeast. Nat. Rev. Genet. **3:**918–930.
52. **Bhabhra, R., and D. S. Askew.** 2005. Thermotolerance and virulence of Aspergillus fumigatus: role of the fungal nucleolus. Med. Mycol. **43**(Suppl. 1)**:**S87–S93.
53. **Bhabhra, R., et al.** 2004. Disruption of the Aspergillus fumigatus gene encoding nucleolar protein CgrA impairs thermotolerant growth and reduces virulence. Infect. Immun. **72:**4731–4740.
54. **Bhabhra, R., et al.** 2008. Impaired ribosome biogenesis disrupts the integration between morphogenesis and nuclear duplication during the germination of Aspergillus fumigatus. Eukaryot. Cell **7:**575–583.
55. **Bicanic, T., T. Harrison, A. Niepieklo, N. Dyakopu, and G. Meintjes.** 2006. Symptomatic relapse of HIV-associated cryptococcal meningitis after initial fluconazole monotherapy: the role of fluconazole resistance and immune reconstitution. Clin. Infect. Dis. **43:**1069–1073.
56. **Bien, C. M., Y. C. Chang, W. D. Nes, K. J. Kwon-Chung, and P. J. Espenshade.** 2009. Cryptococcus neoformans site-2 protease is required for virulence and survival in the presence of azole drugs. Mol. Microbiol. **74:**672–690.
57. **Bii, C. C., et al.** 2007. Antifungal drug susceptibility of Cryptococcus neoformans from clinical sources in Nairobi, Kenya. Mycoses **50:**25–30.
58. **Bishop, A., et al.** 2010. Hyphal growth in Candida albicans requires the phosphorylation of Sec2 by the Cdc28-Ccn1/Hgc1 kinase. EMBO J. **29:**2930–2942.
59. **Biswas, K., and J. Morschhauser.** 2005. The Mep2p ammonium permease controls nitrogen starvation-induced filamentous growth in Candida albicans. Mol. Microbiol. **56:**649–669.
60. **Blankenship, J. R., and A. P. Mitchell.** 2006. How to build a biofilm: a fungal perspective. Curr. Opin. Microbiol. **9:**588–594.
61. **Blankenship, J. R., N. Singh, B. D. Alexander, and J. Heitman.** 2005. Cryptococcus neoformans isolates from transplant recipients are not selected for resistance to calcineurin inhibitors by current immunosuppressive regimens. J. Clin. Microbiol. **43:**464–467.
62. **Blasi, E., et al.** 1995. Differential susceptibility of yeast and hyphal forms of Candida albicans to proteolytic activity of macrophages. Infect. Immun. **63:**1253–1257.
63. **Blum, G., et al.** 2008. Potential basis for amphotericin B resistance in Aspergillus terreus. Antimicrob. Agents Chemother. **52:**1553–1555.
64. **Bockmuhl, D. P., and J. F. Ernst.** 2001. A potential phosphorylation site for an A-type kinase in the Efg1 regulator protein contributes to hyphal morphogenesis of Candida albicans. Genetics **157:**1523–1530.
65. **Bockmuhl, D. P., S. Krishnamurthy, M. Gerads, A. Sonneborn, and J. F. Ernst.** 2001. Distinct and redundant roles of the two protein kinase A isoforms Tpk1p and Tpk2p in morphogenesis and growth of Candida albicans. Mol. Microbiol. **42:**1243–1257.
66. **Boisnard, S., et al.** 2008. Role of Sho1p adaptor in the pseudohyphal development, drugs sensitivity, osmotolerance and oxidant stress adaptation in the opportunistic yeast Candida lusitaniae. Yeast **25:**849–859.
67. **Bonnett, C. R., E. J. Cornish, A. G. Harmsen, and J. B. Burritt.** 2006. Early neutrophil recruitment and aggregation in the murine lung inhibit germination of Aspergillus fumigatus conidia. Infect. Immun. **74:**6528–6539.
68. **Braun, B. R., W. S. Head, M. X. Wang, and A. D. Johnson.** 2000. Identification and characterization of TUP1-regulated genes in Candida albicans. Genetics **156:**31–44.
69. **Braun, B. R., and A. D. Johnson.** 1997. Control of filament formation in Candida albicans by the transcriptional repressor TUP1. Science **277:**105–109.
70. **Braun, B. R., and A. D. Johnson.** 2000. TUP1, CPH1 and EFG1 make independent contributions to filamentation in Candida albicans. Genetics **155:**57–67.
71. **Braun, B. R., D. Kadosh, and A. D. Johnson.** 2001. NRG1, a repressor of filamentous growth in C. albicans, is down-regulated during filament induction. EMBO J. **20:**4753–4761.
72. **Brega, E., R. Zufferey, and C. B. Mamoun.** 2004. Candida albicans Csy1p is a nutrient sensor important for activation of amino acid uptake and hyphal morphogenesis. Eukaryot. Cell **3:**135–143.
73. **Bromenshenk, J. J., et al.** 2010. Iridovirus and microsporidian linked to honey bee colony decline. PLoS One **5:**e13181.
74. **Brown, A. J., et al.** 2000. Gene regulation during morphogenesis in Candida albicans. Contrib. Microbiol. **5:**112–125.
75. **Brown, A. J., and N. A. Gow.** 1999. Regulatory networks controlling Candida albicans morphogenesis. Trends Microbiol. **7:**333–338.
76. **Brown, A. J., F. C. Odds, and N. A. Gow.** 2007. Infection-related gene expression in Candida albicans. Curr. Opin. Microbiol. **10:**307–313.
77. **Brown, D. H., Jr., A. D. Giusani, X. Chen, and C. A. Kumamoto.** 1999. Filamentous growth of Candida albicans in response to physical environmental cues and its regulation by the unique CZF1 gene. Mol. Microbiol. **34:**651–662.
78. **Bruno, V. M., and A. P. Mitchell.** 2005. Regulation of azole drug susceptibility by Candida albicans protein kinase CK2. Mol. Microbiol. **56:**559–573.
79. **Bruns, S., et al.** 2010. Production of extracellular traps against Aspergillus

fumigatus in vitro and in infected lung tissue is dependent on invading neutrophils and influenced by hydrophobin RodA. PLoS Pathog. **6:**e1000873.
80. **Bruns, S., et al.** 2010. Functional genomic profiling of Aspergillus fumigatus biofilm reveals enhanced production of the mycotoxin gliotoxin. Proteomics **10:**3097–3107.
81. **Buffo, J., M. A. Herman, and D. R. Soll.** 1984. A characterization of pH-regulated dimorphism in Candida albicans. Mycopathologia **85:**21–30.
82. **Burt, E. T., et al.** 2003. Isolation and partial characterization of Hsp90 from Candida albicans. Ann. Clin. Lab. Sci. **33:**86–93.
83. **Butler, G., et al.** 2009. Evolution of pathogenicity and sexual reproduction in eight Candida genomes. Nature **459:**657–662.
84. **Byrnes, E. J., III, et al.** 2009. Molecular evidence that the range of the Vancouver Island outbreak of Cryptococcus gattii infection has expanded into the Pacific Northwest in the United States. J. Infect. Dis. **199:**1081–1086.
85. **Byrnes, E. J., III, et al.** 2010. Emergence and pathogenicity of highly virulent Cryptococcus gattii genotypes in the northwest United States. PLoS Pathog. **6:**e1000850.
86. **Byrnes, E. J., III, and J. Heitman.** 2009. Cryptococcus gattii outbreak expands into the Northwestern United States with fatal consequences. F1000 Biol. Rep. **1:**62.
87. **Calderon, J., et al.** 2010. PHR1, a pH-regulated gene of Candida albicans encoding a glucan-remodelling enzyme, is required for adhesion and invasion. Microbiology **156:**2484–2494.
88. **Calderone, R.** 2002. Candida and candidiasis. ASM Press, Washington, DC.
89. **Campbell, J.** 1971. Fine structure and physiology of conidial germination in Aspergillus fumigatus. Trans. Br. Mycol. Soc. **57:**393–402.
90. **Cannon, R. D., et al.** 2009. Efflux-mediated antifungal drug resistance. Clin. Microbiol. Rev. **22:**291–321.
91. **Cannon, R. D., et al.** 2007. Candida albicans drug resistance another way to cope with stress. Microbiology **153:**3211–3217.
92. **Cao, F., et al.** 2006. The Flo8 transcription factor is essential for hyphal development and virulence in Candida albicans. Mol. Biol. Cell **17:**295–307.
93. **Carlisle, P. L., et al.** 2009. Expression levels of a filament-specific transcriptional regulator are sufficient to determine Candida albicans morphology and virulence. Proc. Natl. Acad. Sci. U. S. A. **106:**599–604.
94. **Carlisle, P. L., and D. Kadosh.** 2010. Candida albicans Ume6, a filament-specific transcriptional regulator, directs hyphal growth via a pathway involving Hgc1 cyclin-related protein. Eukaryot. Cell **9:**1320–1328.
95. **Casadevall, A., and L. A. Pirofski.** 2003. The damage-response framework of microbial pathogenesis. Nat. Rev. Microbiol. **1:**17–24.
96. **Chang, Y. C., C. M. Bien, H. Lee, P. J. Espenshade, and K. J. Kwon-Chung.** 2007. Sre1p, a regulator of oxygen sensing and sterol homeostasis, is required for virulence in Cryptococcus neoformans. Mol. Microbiol. **64:**614–629.
97. **Chang, Y. C., S. S. Ingavale, C. Bien, P. Espenshade, and K. J. Kwon-Chung.** 2009. Conservation of the sterol regulatory element-binding protein pathway and its pathobiological importance in Cryptococcus neoformans. Eukaryot. Cell **8:**1770–1779.
98. **Chang, Y. C., G. F. Miller, and K. J. Kwon-Chung.** 2003. Importance of a developmentally regulated pheromone receptor of Cryptococcus neoformans for virulence. Infect. Immun. **71:**4953–4960.
99. **Chang, Y. C., B. L. Wickes, G. F. Miller, L. A. Penoyer, and K. J. Kwon-Chung.** 2000. Cryptococcus neoformans STE12alpha regulates virulence but is not essential for mating. J. Exp. Med. **191:**871–882.
100. **Chapa y Lazo, B., S. Bates, and P. Sudbery.** 2005. The G1 cyclin Cln3 regulates morphogenesis in Candida albicans. Eukaryot. Cell **4:**90–94.
101. **Chen, C. G., Y. L. Yang, H. I. Shih, C. L. Su, and H. J. Lo.** 2004. CaNdt80 is involved in drug resistance in Candida albicans by regulating CDR1. Antimicrob. Agents Chemother. **48:**4505–4512.
102. **Chong, H. S., R. Dagg, R. Malik, S. Chen, and D. Carter.** 2010. In vitro susceptibility of the yeast pathogen Cryptococcus to fluconazole and other azoles varies with molecular genotype. J. Clin. Microbiol. **48:**4115–4120.
103. **Chun, C. D., O. W. Liu, and H. D. Madhani.** 2007. A link between virulence and homeostatic responses to hypoxia during infection by the human fungal pathogen Cryptococcus neoformans. PLoS Pathog. **3:**e22.
104. **Chung, S., et al.** 2002. Molecular analysis of CPRalpha, a MATalpha-specific pheromone receptor gene of Cryptococcus neoformans. Eukaryot. Cell **1:**432–439.
105. **Cleary, I. A., et al.** 2010. Pseudohyphal regulation by the transcription factor Rfg1p in Candida albicans. Eukaryot. Cell **9:**1363–1373.
106. **Clinical and Laboratory Standards Institute.** 2008. Reference method for broth dilution antifungal susceptibility testing of yeasts, 3rd ed. Approved standard M27-A3. Clinical and Laboratory Standards Institute, Wayne, PA.
107. **Coste, A., et al.** 2007. Genotypic evolution of azole resistance mechanisms in sequential Candida albicans isolates. Eukaryot. Cell **6:**1889–1904.
108. **Coste, A., et al.** 2006. A mutation in Tac1p, a transcription factor regulating CDR1 and CDR2, is coupled with loss of heterozygosity at chromosome 5 to mediate antifungal resistance in Candida albicans. Genetics **172:**2139–2156.
109. **Coste, A. T., M. Karababa, F. Ischer, J. Bille, and D. Sanglard.** 2004. TAC1, transcriptional activator of CDR genes, is a new transcription factor involved in the regulation of Candida albicans ABC transporters CDR1 and CDR2. Eukaryot. Cell **3:**1639–1652.
110. **Cowen, L. E.** 2001. Predicting the emergence of resistance to antifungal drugs. FEMS Microbiol. Lett. **204:**1–7.
111. **Cowen, L. E.** 2008. The evolution of fungal drug resistance: modulating the trajectory from genotype to phenotype. Nat. Rev. Microbiol. **6:**187–198.
112. **Cowen, L. E., J. B. Anderson, and L. M. Kohn.** 2002. Evolution of drug resistance in Candida albicans. Annu. Rev. Microbiol. **56:**139–165.
113. **Cowen, L. E., A. E. Carpenter, O. Matangkasombut, G. R. Fink, and S. Lindquist.** 2006. Genetic architecture of Hsp90-dependent drug resistance. Eukaryot. Cell **5:**2184–2188.
114. **Cowen, L. E., L. M. Kohn, and J. B. Anderson.** 2001. Divergence in fitness and evolution of drug resistance in experimental populations of Candida albicans. J. Bacteriol. **183:**2971–2978.
115. **Cowen, L. E., and S. Lindquist.** 2005. Hsp90 potentiates the rapid evolution of new traits: drug resistance in diverse fungi. Science **309:**2185–2189.
116. **Cowen, L. E., et al.** 2002. Population genomics of drug resistance in Candida albicans. Proc. Natl. Acad. Sci. U. S. A. **99:**9284–9289.
117. **Cowen, L. E., et al.** 2000. Evolution of drug resistance in experimental populations of Candida albicans. J. Bacteriol. **182:**1515–1522.
118. **Cowen, L. E., et al.** 2009. Harnessing Hsp90 function as a powerful, broadly effective therapeutic strategy for fungal infectious disease. Proc. Natl. Acad. Sci. U. S. A. **106:**2818–2823.
119. Reference deleted.
120. **Cowen, L. E., and W. J. Steinbach.** 2008. Stress, drugs, and evolution: the role of cellular signaling in fungal drug resistance. Eukaryot. Cell **7:**747–764.
121. **Cramer, K. L., Q. D. Gerrald, C. B. Nichols, M. S. Price, and J. A. Alspaugh.** 2006. Transcription factor Nrg1 mediates capsule formation, stress response, and pathogenesis in Cryptococcus neoformans. Eukaryot. Cell **5:**1147–1156.
122. **Cramer, R. A., Jr., et al.** 2006. Disruption of a nonribosomal peptide synthetase in Aspergillus fumigatus eliminates gliotoxin production. Eukaryot. Cell **5:**972–980.
123. **Cramer, R. A., Jr., et al.** 2008. Calcineurin target CrzA regulates conidial germination, hyphal growth, and pathogenesis of Aspergillus fumigatus. Eukaryot. Cell **7:**1085–1097.
124. **Cruickshank, J. G., R. Cavill, and M. Jelbert.** 1973. Cryptococcus neoformans of unusual morphology. Appl. Microbiol. **25:**309–312.
125. **Cruz, M. C., D. S. Fox, and J. Heitman.** 2001. Calcineurin is required for hyphal elongation during mating and haploid fruiting in Cryptococcus neoformans. EMBO J. **20:**1020–1032.
126. **Cruz, M. C., et al.** 2002. Calcineurin is essential for survival during membrane stress in Candida albicans. EMBO J. **21:**546–559.
127. **Csank, C., et al.** 1998. Roles of the Candida albicans mitogen-activated protein kinase homolog, Cek1p, in hyphal development and systemic candidiasis. Infect. Immun. **66:**2713–2721.
128. **Cuenca-Estrella, M., et al.** 2009. Activity profile in vitro of micafungin against Spanish clinical isolates of common and emerging species of yeasts and molds. Antimicrob. Agents Chemother. **53:**2192–2195.
129. **Dabas, N., S. Schneider, and J. Morschhauser.** 2009. Mutational analysis of the Candida albicans ammonium permease Mep2p reveals residues required for ammonium transport and signaling. Eukaryot. Cell **8:**147–160.
130. **Dagenais, T. R. T., and N. P. Keller.** 2009. Pathogenesis of Aspergillus fumigatus in invasive aspergillosis. Clin. Microbiol. Rev. **22:**447–465.
131. **Dannaoui, E., et al.** 2004. Susceptibility testing of sequential isolates of *Aspergillus fumigatus* recovered from treated patients. J. Med. Microbiol. **53:**129–134.
132. **da Silva Ferreira, et al.** 2004. In vitro evolution of itraconazole resistance in Aspergillus fumigatus involves multiple mechanisms of resistance. Antimicrob. Agents Chemother. **48:**4405–4413.
133. **Davidson, R. C., C. B. Nichols, G. M. Cox, J. R. Perfect, and J. Heitman.** 2003. A MAP kinase cascade composed of cell type specific and non-specific elements controls mating and differentiation of the fungal pathogen Cryptococcus neoformans. Mol. Microbiol. **49:**469–485.
134. **Davis, D.** 2003. Adaptation to environmental pH in Candida albicans and its relation to pathogenesis. Curr. Genet. **44:**1–7.
135. **Davis, D., J. E. Edwards, Jr., A. P. Mitchell, and A. S. Ibrahim.** 2000. Candida albicans RIM101 pH response pathway is required for host-pathogen interactions. Infect. Immun. **68:**5953–5959.
136. **Davis, D., R. B. Wilson, and A. P. Mitchell.** 2000. RIM101-dependent and-independent pathways govern pH responses in Candida albicans. Mol. Cell. Biol. **20:**971–978.
137. **Davis-Hanna, A., A. E. Piispanen, L. I. Stateva, and D. A. Hogan.** 2008. Farnesol and dodecanol effects on the Candida albicans Ras1-cAMP signalling pathway and the regulation of morphogenesis. Mol. Microbiol. **67:**47–62.
138. **De Bernardis, F., F. A. Muhlschlegel, A. Cassone, and W. A. Fonzi.** 1998. The pH of the host niche controls gene expression in and virulence of Candida albicans. Infect. Immun. **66:**3317–3325.
139. **Delmas, G., et al.** 2002. Efficacy of orally delivered cochleates containing

amphotericin B in a murine model of aspergillosis. Antimicrob. Agents Chemother. **46:**2704–2707.
140. **Del Poeta, M., M. C. Cruz, M. E. Cardenas, J. R. Perfect, and J. Heitman.** 2000. Synergistic antifungal activities of bafilomycin A(1), fluconazole, and the pneumocandin MK-0991/caspofungin acetate (L-743,873) with calcineurin inhibitors FK506 and L-685,818 against Cryptococcus neoformans. Antimicrob. Agents Chemother. **44:**739–746.
141. **de Micheli, M., J. Bille, C. Schueller, and D. Sanglard.** 2002. A common drug-responsive element mediates the upregulation of the Candida albicans ABC transporters CDR1 and CDR2, two genes involved in antifungal drug resistance. Mol. Microbiol. **43:**1197–1214.
142. **d'Enfert, C.** 2006. Biofilms and their role in the resistance of pathogenic Candida to antifungal agents. Curr. Drug Targets **7:**465–470.
143. **Denning, D. W.** 2003. Echinocandin antifungal drugs. Lancet **362:**1142–1151.
144. **Denning, D. W.** 1998. Invasive aspergillosis. Clin. Infect. Dis. **26:**781–803.
145. **Denning, D. W., et al.** 2006. Micafungin (FK463), alone or in combination with other systemic antifungal agents, for the treatment of acute invasive aspergillosis. J. Infect. **53:**337–349.
146. **Denning, D. W., R. M. Tucker, L. H. Hanson, and D. A. Stevens.** 1989. Treatment of invasive aspergillosis with itraconazole. Am. J. Med. **86:**791–800.
147. **Denning, D. W., et al.** 1997. Itraconazole resistance in *Aspergillus fumigatus*. Antimicrob. Agents Chemother. **41:**1364–1368.
148. **Dermoumi, H.** 1994. In vitro susceptibility of fungal isolates of clinically important specimens to itraconazole, fluconazole and amphotericin B. Chemotherapy **40:**92–98.
149. **Desnos-Ollivier, M., et al.** 2008. Mutations in the fks1 gene in Candida albicans, C. tropicalis, and C. krusei correlate with elevated caspofungin MICs uncovered in AM3 medium using the method of the European Committee on Antibiotic Susceptibility Testing. Antimicrob. Agents Chemother. **52:**3092–3098.
150. **De Virgilio, C., and R. Loewith.** 2006. Cell growth control: little eukaryotes make big contributions. Oncogene **25:**6392–6415.
151. **Diamond, R. D., and R. A. Clark.** 1982. Damage to Aspergillus fumigatus and Rhizopus oryzae hyphae by oxidative and nonoxidative microbicidal products of human neutrophils in vitro. Infect. Immun. **38:**487–495.
152. **Diaz-Guerra, T. M., E. Mellado, M. Cuenca-Estrella, and J. L. Rodriguez-Tudela.** 2003. A point mutation in the 14alpha-sterol demethylase gene cyp51A contributes to itraconazole resistance in *Aspergillus fumigatus*. Antimicrob. Agents Chemother. **47:**1120–1124.
153. **Dirr, F., et al.** 2010. AfMkk2 is required for cell wall integrity signaling, adhesion, and full virulence of the human pathogen Aspergillus fumigatus. Int. J. Med. Microbiol. **300:**496–502.
154. **Do, J. H., R. Yamaguchi, and S. Miyano.** 2009. Exploring temporal transcription regulation structure of Aspergillus fumigatus in heat shock by state space model. BMC Genomics **10:**306.
155. **Doedt, T., et al.** 2004. APSES proteins regulate morphogenesis and metabolism in Candida albicans. Mol. Biol. Cell **15:**3167–3180.
156. **d'Ostiani, C. F., et al.** 2000. Dendritic cells discriminate between yeasts and hyphae of the fungus Candida albicans. Implications for initiation of T helper cell immunity in vitro and in vivo. J. Exp. Med. **191:**1661–1674.
157. **Douglas, C. M., et al.** 1997. Identification of the FKS1 gene of Candida albicans as the essential target of 1,3-beta-D-glucan synthase inhibitors. Antimicrob. Agents Chemother. **41:**2471–2479.
158. **Douglas, L. J.** 2003. Candida biofilms and their role in infection. Trends Microbiol. **11:**30–36.
159. **Douglas, L. J.** 2002. Medical importance of biofilms in Candida infections. Rev. Iberoam. Micol. **19:**139–143.
160. **D'Souza, C. A., et al.** 2001. Cyclic AMP-dependent protein kinase controls virulence of the fungal pathogen Cryptococcus neoformans. Mol. Cell. Biol. **21:**3179–3191.
161. **Du, W., M. Coaker, J. D. Sobel, and R. A. Akins.** 2004. Shuttle vectors for Candida albicans: control of plasmid copy number and elevated expression of cloned genes. Curr. Genet. **45:**390–398.
162. **Dumitru, R., et al.** 2007. In vivo and in vitro anaerobic mating in Candida albicans. Eukaryot. Cell **6:**465–472.
163. **Dunkel, N., J. Blass, P. D. Rogers, and J. Morschhauser.** 2008. Mutations in the multi-drug resistance regulator MRR1, followed by loss of heterozygosity, are the main cause of MDR1 overexpression in fluconazole-resistant Candida albicans strains. Mol. Microbiol. **69:**827–840.
164. **Dunkel, N., et al.** 2008. A gain-of-function mutation in the transcription factor Upc2p causes upregulation of ergosterol biosynthesis genes and increased fluconazole resistance in a clinical Candida albicans isolate. Eukaryot. Cell **7:**1180–1190.
165. **Eisman, B., et al.** 2006. The Cek1 and Hog1 mitogen-activated protein kinases play complementary roles in cell wall biogenesis and chlamydospore formation in the fungal pathogen Candida albicans. Eukaryot. Cell **5:**347–358.
166. **Ejzykowicz, D. E., et al.** 2009. The Aspergillus fumigatus transcription factor Ace2 governs pigment production, conidiation and virulence. Mol. Microbiol. **72:**155–169.
167. **El Barkani, A., et al.** 2000. Dominant active alleles of RIM101 (PRR2) bypass the pH restriction on filamentation of Candida albicans. Mol. Cell. Biol. **20:**4635–4647.
168. **Enjalbert, B., and M. Whiteway.** 2005. Release from quorum-sensing molecules triggers hyphal formation during Candida albicans resumption of growth. Eukaryot. Cell **4:**1203–1210.
169. **Enloe, B., A. Diamond, and A. P. Mitchell.** 2000. A single-transformation gene function test in diploid Candida albicans. J. Bacteriol. **182:**5730–5736.
170. **Epp, E., et al.** 2010. Reverse genetics in Candida albicans predicts ARF cycling is essential for drug resistance and virulence. PLoS Pathog. **6:**e1000753.
171. **Eschertzhuber, S., C. Velik-Salchner, C. Hoermann, D. Hoefer, and C. Lass-Florl.** 2008. Caspofungin-resistant Aspergillus flavus after heart transplantation and mechanical circulatory support: a case report. Transpl. Infect. Dis. **10:**190–192.
172. **Fan, W., A. Idnurm, J. Breger, E. Mylonakis, and J. Heitman.** 2007. EcaI, a sarcoplasmic/endoplasmic reticulum Ca2+-ATPase, is involved in stress tolerance and virulence in Cryptococcus neoformans. Infect. Immun. **75:**3394–3405.
173. **Fang, H. M., and Y. Wang.** 2006. RA domain-mediated interaction of Cdc35 with Ras1 is essential for increasing cellular cAMP level for Candida albicans hyphal development. Mol. Microbiol. **61:**484–496.
174. **Fang, W., et al.** 2010. Reduced expression of the O-mannosyltransferase 2 (AfPmt2) leads to deficient cell wall and abnormal polarity in Aspergillus fumigatus. Glycobiology **20:**542–552.
175. **Fang, W., et al.** 2009. Characterization of the Aspergillus fumigatus phosphomannose isomerase Pmi1 and its impact on cell wall synthesis and morphogenesis. Microbiology **155:**3281–3293.
176. **Fanos, V., and L. Cataldi.** 2000. Amphotericin B-induced nephrotoxicity: a review. J. Chemother. **12:**463–470.
177. **Feldmesser, M., Y. Kress, A. Mednick, and A. Casadevall.** 2000. The effect of the echinocandin analogue caspofungin on cell wall glucan synthesis by Cryptococcus neoformans. J. Infect. Dis. **182:**1791–1795.
178. **Felk, A., et al.** 2002. Candida albicans hyphal formation and the expression of the Efg1-regulated proteinases Sap4 to Sap6 are required for the invasion of parenchymal organs. Infect. Immun. **70:**3689–3700.
179. **Feng, Q., E. Summers, B. Guo, and G. Fink.** 1999. Ras signaling is required for serum-induced hyphal differentiation in Candida albicans. J. Bacteriol. **181:**6339–6346.
180. **Finley, K. R., and J. Berman.** 2005. Microtubules in Candida albicans hyphae drive nuclear dynamics and connect cell cycle progression to morphogenesis. Eukaryot. Cell **4:**1697–1711.
181. **Fontaine, T., et al.** 2000. Molecular organization of the alkali-insoluble fraction of Aspergillus fumigatus cell wall. J. Biol. Chem. **275:**27594–27607.
182. **Fonzi, W. A.** 1999. PHR1 and PHR2 of Candida albicans encode putative glycosidases required for proper cross-linking of beta-1,3- and beta-1,6-glucans. J. Bacteriol. **181:**7070–7079.
183. **Forche, A., et al.** 2008. The parasexual cycle in Candida albicans provides an alternative pathway to meiosis for the formation of recombinant strains. PLoS Biol. **6:**e110.
184. **Fortwendel, J. R., et al.** 2008. Aspergillus fumigatus RasA regulates asexual development and cell wall integrity. Eukaryot. Cell **7:**1530–1539.
185. **Fortwendel, J. R., et al.** 2010. Transcriptional regulation of chitin synthases by calcineurin controls paradoxical growth of Aspergillus fumigatus in response to caspofungin. Antimicrob. Agents Chemother. **54:**1555–1563.
186. **Fortwendel, J. R., et al.** 2009. Differential effects of inhibiting chitin and 1,3-beta-D-glucan synthesis in ras and calcineurin mutants of Aspergillus fumigatus. Antimicrob. Agents Chemother. **53:**476–482.
187. **Fortwendel, J. R., J. C. Panepinto, A. E. Seitz, D. S. Askew, and J. C. Rhodes.** 2004. Aspergillus fumigatus rasA and rasB regulate the timing and morphology of asexual development. Fungal Genet. Biol. **41:**129–139.
188. **Fortwendel, J. R., et al.** 2005. A fungus-specific ras homolog contributes to the hyphal growth and virulence of Aspergillus fumigatus. Eukaryot. Cell **4:**1982–1989.
189. **Fox, D. S., and J. Heitman.** 2002. Good fungi gone bad: the corruption of calcineurin. Bioessays **24:**894–903.
190. **Franzot, S. P., and A. Casadevall.** 1997. Pneumocandin L-743,872 enhances the activities of amphotericin B and fluconazole against Cryptococcus neoformans in vitro. Antimicrob. Agents Chemother. **41:**331–336.
191. **Fraser, J. A., et al.** 2005. Same-sex mating and the origin of the Vancouver Island Cryptococcus gattii outbreak. Nature **437:**1360–1364.
192. **Fries, B. C., C. P. Taborda, E. Serfass, and A. Casadevall.** 2001. Phenotypic switching of Cryptococcus neoformans occurs in vivo and influences the outcome of infection. J. Clin. Invest. **108:**1639–1648.
193. **Fu, Y., et al.** 2002. Candida albicans Als1p: an adhesin that is a downstream effector of the EFG1 filamentation pathway. Mol. Microbiol. **44:**61–72.
194. **Fu, Y., et al.** 1998. Expression of the Candida albicans gene ALS1 in Saccharomyces cerevisiae induces adherence to endothelial and epithelial cells. Infect. Immun. **66:**1783–1786.
195. **Fuchs, B. B., and E. Mylonakis.** 2009. Our paths might cross: the role of the fungal cell wall integrity pathway in stress response and cross talk with other stress response pathways. Eukaryot. Cell **8:**1616–1625.

196. **Fuller, K. K., et al.** 2011. Divergent protein kinase A isoforms co-ordinately regulate conidial germination, carbohydrate metabolism and virulence in Aspergillus fumigatus. Mol. Microbiol. **79:**1045–1062.
197. **Fuller, K. K., W. Zhao, D. S. Askew, and J. C. Rhodes.** 2009. Deletion of the protein kinase A regulatory subunit leads to deregulation of mitochondrial activation and nuclear duplication in Aspergillus fumigatus. Eukaryot. Cell **8:**271–277.
198. **Gantner, B. N., R. M. Simmons, and D. M. Underhill.** 2005. Dectin-1 mediates macrophage recognition of Candida albicans yeast but not filaments. EMBO J. **24:**1277–1286.
199. **Garcia-Sanchez, S., et al.** 2005. Global roles of Ssn6 in Tup1- and Nrg1-dependent gene regulation in the fungal pathogen, Candida albicans. Mol. Biol. Cell **16:**2913–2925.
200. **Gardiner, R. E., P. Souteropoulos, S. Park, and D. S. Perlin.** 2005. Characterization of Aspergillus fumigatus mutants with reduced susceptibility to caspofungin. Med. Mycol. **43**(Suppl. 1)**:**299–305.
201. **Gehrke, A., T. Heinekamp, I. D. Jacobsen, and A. A. Brakhage.** 2010. Heptahelical receptors GprC and GprD of Aspergillus fumigatus are essential regulators of colony growth, hyphal morphogenesis, and virulence. Appl. Environ. Microbiol. **76:**3989–3998.
202. **Gerik, K. J., S. R. Bhimireddy, J. S. Ryerse, C. A. Specht, and J. K. Lodge.** 2008. PKC1 is essential for protection against both oxidative and nitrosative stresses, cell integrity, and normal manifestation of virulence factors in the pathogenic fungus Cryptococcus neoformans. Eukaryot. Cell **7:**1685–1698.
203. **Ghannoum, M. A., B. Spellberg, S. M. Saporito-Irwin, and W. A. Fonzi.** 1995. Reduced virulence of Candida albicans PHR1 mutants. Infect. Immun. **63:**4528–4530.
204. **Giacometti, R., F. Kronberg, R. M. Biondi, and S. Passeron.** 2009. Catalytic isoforms Tpk1 and Tpk2 of Candida albicans PKA have non-redundant roles in stress response and glycogen storage. Yeast **26:**273–285.
205. **Giusani, A. D., M. Vinces, and C. A. Kumamoto.** 2002. Invasive filamentous growth of Candida albicans is promoted by Czf1p-dependent relief of Efg1p-mediated repression. Genetics **160:**1749–1753.
206. **Goldberg, D., I. Marbach, E. Gross, A. Levitzki, and G. Simchen.** 1993. A Candida albicans homolog of CDC25 is functional in Saccharomyces cerevisiae. Eur. J. Biochem. **213:**195–204.
207. **Gonzalez-Novo, A., et al.** 2008. Sep7 is essential to modify septin ring dynamics and inhibit cell separation during Candida albicans hyphal growth. Mol. Biol. Cell **19:**1509–1518.
208. **Gorlach, J., et al.** 2000. Identification and characterization of a highly conserved calcineurin binding protein, CBP1/calcipressin, in Cryptococcus neoformans. EMBO J. **19:**3618–3629.
209. **Gravelat, F. N., et al.** 2010. Aspergillus fumigatus MedA governs adherence, host cell interactions and virulence. Cell. Microbiol. **12:**473–488.
210. **Graybill, J. R., S. Hernandez, R. Bocanegra, and L. K. Najvar.** 2004. Antifungal therapy of murine *Aspergillus terreus* infection. Antimicrob. Agents Chemother. **48:**3715–3719.
211. **Gruszecki, W. I., M. Gagos, M. Herec, and P. Kernen.** 2003. Organization of antibiotic amphotericin B in model lipid membranes. A mini review. Cell. Mol. Biol. Lett. **8:**161–170.
212. **Guerrero, A., N. Jain, X. Wang, and B. C. Fries.** Cryptococcus neoformans variants generated by phenotypic switching differ in virulence through effects on macrophage activation. Infect. Immun. **78:**1049–1057.
213. **Ha, K. C., and T. C. White.** 1999. Effects of azole antifungal drugs on the transition from yeast cells to hyphae in susceptible and resistant isolates of the pathogenic yeast Candida albicans. Antimicrob. Agents Chemother. **43:**763–768.
214. **Harcus, D., A. Nantel, A. Marcil, T. Rigby, and M. Whiteway.** 2004. Transcription profiling of cyclic AMP signaling in Candida albicans. Mol. Biol. Cell **15:**4490–4499.
215. **Hawksworth, D. L.** 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation Mycol. Res. **95:**641–655.
216. **Hawksworth, D. L.** 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. **105:**1422–1432.
217. **Hawser, S., and K. Islam.** 1999. Comparisons of the effects of fungicidal and fungistatic antifungal agents on the morphogenetic transformation of Candida albicans. J. Antimicrob. Chemother. **43:**411–413.
218. **Hawser, S. P., and L. J. Douglas.** 1995. Resistance of Candida albicans biofilms to antifungal agents in vitro. Antimicrob. Agents Chemother. **39:** 2128–2131.
219. **Hazan, I., and H. Liu.** 2002. Hyphal tip-associated localization of Cdc42 is F-actin dependent in Candida albicans. Eukaryot. Cell **1:**856–864.
220. **Hazan, I., M. Sepulveda-Becerra, and H. Liu.** 2002. Hyphal elongation is regulated independently of cell cycle in Candida albicans. Mol. Biol. Cell **13:**134–145.
221. **Heath, V. L., S. L. Shaw, S. Roy, and M. S. Cyert.** 2004. Hph1p and Hph2p, novel components of calcineurin-mediated stress responses in Saccharomyces cerevisiae. Eukaryot. Cell **3:**695–704.
222. **Heckman, D. S., et al.** 2001. Molecular evidence for the early colonization of land by fungi and plants. Science **293:**1129–1133.
223. **Heilmann, C. J., S. Schneider, K. S. Barker, P. D. Rogers, and J. Morschhauser.** 2010. An A643T mutation in the transcription factor Upc2p causes constitutive ERG11 upregulation and increased fluconazole resistance in Candida albicans. Antimicrob. Agents Chemother. **54:**353–359.
224. **Hernandez, S., et al.** 2004. Caspofungin resistance in Candida albicans: correlating clinical outcome with laboratory susceptibility testing of three isogenic isolates serially obtained from a patient with progressive Candida esophagitis. Antimicrob. Agents Chemother. **48:**1382–1383.
225. **Hicks, J. K., C. A. D'Souza, G. M. Cox, and J. Heitman.** 2004. Cyclic AMP-dependent protein kinase catalytic subunits have divergent roles in virulence factor production in two varieties of the fungal pathogen Cryptococcus neoformans. Eukaryot. Cell **3:**14–26.
226. **Hnisz, D., O. Majer, I. E. Frohner, V. Komnenovic, and K. Kuchler.** 2010. The Set3/Hos2 histone deacetylase complex attenuates cAMP/PKA signaling to regulate morphogenesis and virulence of Candida albicans. PLoS Pathog. **6:**e1000889.
227. **Hogan, D. A., A. Vik, and R. Kolter.** 2004. A Pseudomonas aeruginosa quorum-sensing molecule influences Candida albicans morphology. Mol. Microbiol. **54:**1212–1223.
228. **Homann, O. R., J. Dea, S. M. Noble, and A. D. Johnson.** 2009. A phenotypic profile of the Candida albicans regulatory network. PLoS Genet. **5:**e1000783.
229. **Hoot, S. J., R. P. Brown, B. G. Oliver, and T. C. White.** 2010. The UPC2 promoter in Candida albicans contains two cis-acting elements that bind directly to Upc2p, resulting in transcriptional autoregulation. Eukaryot. Cell **9:**1354–1362.
230. **Hope, H., S. Bogliolo, R. A. Arkowitz, and M. Bassilana.** 2008. Activation of Rac1 by the guanine nucleotide exchange factor Dck1 is required for invasive filamentous growth in the pathogen Candida albicans. Mol. Biol. Cell **19:**3638–3651.
231. **Hope, H., C. Schmauch, R. A. Arkowitz, and M. Bassilana.** 2010. The Candida albicans ELMO homologue functions together with Rac1 and Dck1, upstream of the MAP kinase Cek1, in invasive filamentous growth. Mol. Microbiol. **76:**1572–1590.
232. **Hornby, J. M., et al.** 2001. Quorum sensing in the dimorphic fungus Candida albicans is mediated by farnesol. Appl. Environ. Microbiol. **67:**2982–2992.
233. **Hornby, J. M., and K. W. Nickerson.** 2004. Enhanced production of farnesol by Candida albicans treated with four azoles. Antimicrob. Agents Chemother. **48:**2305–2307.
234. **Howard, S. J., et al.** 2006. Multi-azole resistance in *Aspergillus fumigatus*. Int. J. Antimicrob. Agents **28:**450–453.
235. **Hoyer, L. L., et al.** 1994. A Candida albicans cyclic nucleotide phosphodiesterase: cloning and expression in Saccharomyces cerevisiae and biochemical characterization of the recombinant enzyme. Microbiology **140**(Pt. 7)**:**1533–1542.
236. **Hsueh, Y. P., C. Xue, and J. Heitman.** 2009. A constitutively active GPCR governs morphogenic transitions in Cryptococcus neoformans. EMBO J. **28:**1220–1233.
237. **Hsueh, Y. P., C. Xue, and J. Heitman.** 2007. G protein signaling governing cell fate decisions involves opposing Galpha subunits in Cryptococcus neoformans. Mol. Biol. Cell **18:**3237–3249.
238. **Hu, G., and J. W. Kronstad.** A putative P-type ATPase, Apt1, is involved in stress tolerance and virulence in Cryptococcus neoformans. Eukaryot. Cell **9:**74–83.
239. **Hu, G., et al.** 2007. Transcriptional regulation by protein kinase A in Cryptococcus neoformans. PLoS Pathog. **3:**e42.
240. **Huang, G., et al.** 2006. Bistable expression of WOR1, a master regulator of white-opaque switching in Candida albicans. Proc. Natl. Acad. Sci. U. S. A. **103:**12813–12818.
241. **Huang, H., D. Harcus, and M. Whiteway.** 2008. Transcript profiling of a MAP kinase pathway in C. albicans. Microbiol. Res. **163:**380–393.
242. **Hube, B.** 2006. Infection-associated genes of Candida albicans. Future Microbiol. **1:**209–218.
243. **Hudson, D. A., et al.** 2004. Identification of the dialysable serum inducer of germ-tube formation in Candida albicans. Microbiology **150:**3041–3049.
244. **Hull, C. M., and A. D. Johnson.** 1999. Identification of a mating type-like locus in the asexual pathogenic yeast Candida albicans. Science **285:**1271–1275.
245. **Hull, C. M., R. M. Raisner, and A. D. Johnson.** 2000. Evidence for mating of the "asexual" yeast Candida albicans in a mammalian host. Science **289:**307–310.
246. **Idnurm, A., et al.** 2005. Deciphering the model pathogenic fungus Cryptococcus neoformans. Nat. Rev. Microbiol. **3:**753–764.
247. **Imai, J., and I. Yahara.** 2000. Role of HSP90 in salt stress tolerance via stabilization and regulation of calcineurin. Mol. Cell. Biol. **20:**9262–9270.
248. **Jain, P., I. Akula, and T. Edlind.** 2003. Cyclic AMP signaling pathway modulates susceptibility of Candida species and Saccharomyces cerevisiae to antifungal azoles and other sterol biosynthesis inhibitors. Antimicrob. Agents Chemother. **47:**3195–3201.
249. **James, T. Y., et al.** 2006. Reconstructing the early evolution of fungi using a six-gene phylogeny. Nature **443:**818–822.
250. **James, T. Y., et al.** 2009. Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. PLoS Pathog. **5:**e1000458.

251. **Jarosz, L. M., D. M. Deng, H. C. van der Mei, W. Crielaard, and B. P. Krom.** 2009. Streptococcus mutans competence-stimulating peptide inhibits Candida albicans hypha formation. Eukaryot. Cell **8:**1658–1664.
252. **Ji, H., et al.** 2000. A three-dimensional model of lanosterol 14alpha-demethylase of Candida albicans and its interaction with azole antifungals. J. Med. Chem. **43:**2493–2505.
253. **Jiang, H., H. Ouyang, H. Zhou, and C. Jin.** 2008. GDP-mannose pyrophosphorylase is essential for cell wall integrity, morphogenesis and viability of Aspergillus fumigatus. Microbiology **154:**2730–2739.
254. **Johnson, E. M., et al.** 2000. Lack of correlation of *in vitro* amphotericin B susceptibility testing with outcome in a murine model of *Aspergillus* infection. J. Antimicrob. Chemother. **45:**85–93.
255. **Johnston, S. A., and R. C. May.** 2010. The human fungal pathogen Cryptococcus neoformans escapes macrophages by a phagosome emptying mechanism that is inhibited by Arp2/3 complex-mediated actin polymerisation. PLoS Pathog. **6:**e1001041.
256. **Jones, D. L., et al.** 2003. Transcriptome profiling of a Saccharomyces cerevisiae mutant with a constitutively activated Ras/cAMP pathway. Physiol. Genomics **16:**107–118.
257. **Jones, L. A., and P. E. Sudbery.** 2010. Spitzenkorper, exocyst, and polarisome components in Candida albicans hyphae show different patterns of localization and have distinct dynamic properties. Eukaryot. Cell **9:**1455–1465.
258. **Joseph-Horne, T., D. Hollomon, R. S. Loeffler, and S. L. Kelly.** 1995. Cross-resistance to polyene and azole drugs in Cryptococcus neoformans. Antimicrob. Agents Chemother. **39:**1526–1529.
259. **Jung, K. W., S. Y. Kim, L. H. Okagaki, K. Nielsen, and Y. S. Bahn.** 2011. Ste50 adaptor protein governs sexual differentiation of Cryptococcus neoformans via the pheromone-response MAPK signaling pathway. Fungal Genet. Biol. **48:**154–165.
260. **Jung, W. H., and L. I. Stateva.** 2003. The cAMP phosphodiesterase encoded by CaPDE2 is required for hyphal development in Candida albicans. Microbiology **149:**2961–2976.
261. **Juvvadi, P. R., et al.** 2008. Calcineurin localizes to the hyphal septum in Aspergillus fumigatus: implications for septum formation and conidiophore development. Eukaryot. Cell **7:**1606–1610.
262. **Kadosh, D., and A. D. Johnson.** 2005. Induction of the Candida albicans filamentous growth program by relief of transcriptional repression: a genome-wide analysis. Mol. Biol. Cell **16:**2903–2912.
263. **Kadosh, D., and A. D. Johnson.** 2001. Rfg1, a protein related to the Saccharomyces cerevisiae hypoxic regulator Rox1, controls filamentous growth and virulence in Candida albicans. Mol. Cell. Biol. **21:**2496–2505.
264. **Kafadar, K. A., and M. S. Cyert.** 2004. Integration of stress responses: modulation of calcineurin signaling in Saccharomyces cerevisiae by protein kinase A. Eukaryot. Cell **3:**1147–1153.
265. **Kamran, M., et al.** 2004. Inactivation of transcription factor gene ACE2 in the fungal pathogen Candida glabrata results in hypervirulence. Eukaryot. Cell **3:**546–552.
266. **Kan, V. L., and J. E. Bennett.** 1991. Beta 1,4-oligoglucosides inhibit the binding of Aspergillus fumigatus conidia to human monocytes. J. Infect. Dis. **163:**1154–1156.
267. **Kan, V. L., and J. E. Bennett.** 1988. Lectin-like attachment sites on murine pulmonary alveolar macrophages bind Aspergillus fumigatus conidia. J. Infect. Dis. **158:**407–414.
268. **Karos, M., et al.** 2000. Mapping of the Cryptococcus neoformans MATalpha locus: presence of mating type-specific mitogen-activated protein kinase cascade homologs. J. Bacteriol. **182:**6222–6227.
269. **Kebaara, B. W., et al.** 2008. Candida albicans Tup1 is involved in farnesol-mediated inhibition of filamentous-growth induction. Eukaryot. Cell **7:**980–987.
270. **Keller, N. P., G. Turner, and J. W. Bennett.** 2005. Fungal secondary metabolism—from biochemistry to genomics. Nat. Rev. Microbiol. **3:**937–947.
271. **Kelly, M. T., et al.** 2004. The Candida albicans CaACE2 gene affects morphogenesis, adherence and virulence. Mol. Microbiol. **53:**969–983.
272. **Kelly, S. L., D. C. Lamb, D. E. Kelly, J. Loeffler, and H. Einsele.** 1996. Resistance to fluconazole and amphotericin in Candida albicans from AIDS patients. Lancet **348:**1523–1524.
273. **Kelly, S. L., et al.** 1997. Resistance to fluconazole and cross-resistance to amphotericin B in Candida albicans from AIDS patients caused by defective sterol delta5,6-desaturation. FEBS Lett. **400:**80–82.
274. **Kelly, S. L., et al.** 1994. Resistance to amphotericin B associated with defective sterol delta 8→7 isomerase in a Cryptococcus neoformans strain from an AIDS patient. FEMS Microbiol. Lett. **122:**39–42.
275. **Khalaf, R. A., and R. S. Zitomer.** 2001. The DNA binding protein Rfg1 is a repressor of filamentation in Candida albicans. Genetics **157:**1503–1512.
276. **Klengel, T., et al.** 2005. Fungal adenylyl cyclase integrates CO2 sensing with cAMP signaling and virulence. Curr. Biol. **15:**2021–2026.
277. **Ko, Y. J., et al.** 2009. Remodeling of global transcription patterns of Cryptococcus neoformans genes mediated by the stress-activated HOG signaling pathways. Eukaryot. Cell **8:**1197–1217.
278. **Kohler, J. R.** 2003. Mos10 (Vps60) is required for normal filament maturation in Saccharomyces cerevisiae. Mol. Microbiol. **49:**1267–1285.
279. **Kohler, J. R., and G. R. Fink.** 1996. Candida albicans strains heterozygous and homozygous for mutations in mitogen-activated protein kinase signaling components have defects in hyphal development. Proc. Natl. Acad. Sci. U. S. A. **93:**13223–13228.
280. **Kojima, K., Y. S. Bahn, and J. Heitman.** 2006. Calcineurin, Mpk1 and Hog1 MAPK pathways independently control fludioxonil antifungal sensitivity in Cryptococcus neoformans. Microbiology **152:**591–604.
281. **Kontoyiannis, D. P., R. E. Lewis, G. S. May, N. Osherov, and M. G. Rinaldi.** 2002. Aspergillus nidulans is frequently resistant to amphotericin B. Mycoses **45:**406–407.
282. **Kontoyiannis, D. P., R. E. Lewis, N. Osherov, N. D. Albert, and G. S. May.** 2003. Combination of caspofungin with inhibitors of the calcineurin pathway attenuates growth in vitro in Aspergillus species. J. Antimicrob. Chemother. **51:**313–316.
283. **Kontoyiannis, D. P., et al.** 2009. Micafungin alone or in combination with other systemic antifungal therapies in hematopoietic stem cell transplant recipients with invasive aspergillosis. Transpl. Infect. Dis. **11:**89–93.
284. **Korting, H. C., et al.** 2003. Reduced expression of the hyphal-independent Candida albicans proteinase genes SAP1 and SAP3 in the efg1 mutant is associated with attenuated virulence during infection of oral epithelium. J. Med. Microbiol. **52:**623–632.
285. **Kraus, P. R., D. S. Fox, G. M. Cox, and J. Heitman.** 2003. The Cryptococcus neoformans MAP kinase Mpk1 regulates cell integrity in response to antifungal drugs and loss of calcineurin function. Mol. Microbiol. **48:**1377–1387.
286. **Kremer, S. B., and D. S. Gross.** 2009. SAGA and Rpd3 chromatin modification complexes dynamically regulate heat shock gene structure and expression. J. Biol. Chem. **284:**32914–32931.
287. **Krishnarao, T. V., and J. N. Galgiani.** 1997. Comparison of the in vitro activities of the echinocandin LY303366, the pneumocandin MK-0991, and fluconazole against Candida species and Cryptococcus neoformans. Antimicrob. Agents Chemother. **41:**1957–1960.
288. **Krueger, K. E., A. K. Ghosh, B. P. Krom, and R. L. Cihlar.** 2004. Deletion of the NOT4 gene impairs hyphal development and pathogenicity in Candida albicans. Microbiology **150:**229–240.
289. **Kubler, E., H. U. Mosch, S. Rupp, and M. P. Lisanti.** 1997. Gpa2p, a G-protein alpha-subunit, regulates growth and pseudohyphal development in Saccharomyces cerevisiae via a cAMP-dependent mechanism. J. Biol. Chem. **272:**20321–20323.
290. **Kuhn, D. M., T. George, J. Chandra, P. K. Mukherjee, and M. A. Ghannoum.** 2002. Antifungal susceptibility of Candida biofilms: unique efficacy of amphotericin B lipid formulations and echinocandins. Antimicrob. Agents Chemother. **46:**1773–1780.
291. **Kullas, A. L., M. Li, and D. A. Davis.** 2004. Snf7p, a component of the ESCRT-III protein complex, is an upstream member of the RIM101 pathway in Candida albicans. Eukaryot. Cell **3:**1609–1618.
292. **Kumamoto, C. A.** 2005. A contact-activated kinase signals Candida albicans invasive growth and biofilm development. Proc. Natl. Acad. Sci. U. S. A. **102:**5576–5581.
293. **Kumamoto, C. A., and M. D. Vinces.** 2005. Contributions of hyphae and hypha-co-regulated genes to Candida albicans virulence. Cell. Microbiol. **7:**1546–1554.
294. **Kurtz, M. B., et al.** 1996. Characterization of echinocandin-resistant mutants of Candida albicans: genetic, biochemical, and virulence studies. Infect. Immun. **64:**3244–3251.
295. **Kwon-Chung, K. J., and J. E. Bennett.** 1978. Distribution of alpha and alpha mating types of Cryptococcus neoformans among natural and clinical isolates. Am. J. Epidemiol. **108:**337–340.
296. **Kwon-Chung, K. J., and J. E. Bennett.** 1984. Epidemiologic differences between the two varieties of Cryptococcus neoformans. Am. J. Epidemiol. **120:**123–130.
297. **Kwon-Chung, K. J., and J. E. Bennett.** 1984. High prevalence of Cryptococcus neoformans var. gattii in tropical and subtropical regions. Zentralbl. Bakteriol. Mikrobiol. Hyg. A **257:**213–218.
298. **Kwon-Chung, K. J., J. C. Edman, and B. L. Wickes.** 1992. Genetic association of mating types and virulence in Cryptococcus neoformans. Infect. Immun. **60:**602–605.
299. **Kwon-Chung, K. J., T. C. Sorrell, F. Dromer, E. Fung, and S. M. Levitz.** 2000. Cryptococcosis: clinical and biological aspects. Med. Mycol. **38**(Suppl. 1)**:**205–213.
300. **Kwon-Chung, K. J., and J. E. Bennett.** 1992. Medical Mycology. Lea & Febiger, Philadelphia, PA.
301. **LaFayette, S. L., et al.** 2010. PKC signaling regulates drug resistance of the fungal pathogen Candida albicans via circuitry comprised of Mkc1, calcineurin, and Hsp90. PLoS Pathog. **6:**e1001069.
302. **LaFleur, M. D., C. A. Kumamoto, and K. Lewis.** 2006. Candida albicans biofilms produce antifungal-tolerant persister cells. Antimicrob. Agents Chemother. **50:**3839–3846.
303. **Lamb, D. C., D. E. Kelly, T. C. White, and S. L. Kelly.** 2000. The R467K amino acid substitution in Candida albicans sterol 14alpha-demethylase causes drug resistance through reduced affinity. Antimicrob. Agents Chemother. **44:**63–67.

304. **Lane, S., C. Birse, S. Zhou, R. Matson, and H. Liu.** 2001. DNA array studies demonstrate convergent regulation of virulence factors by Cph1, Cph2, and Efg1 in Candida albicans. J. Biol. Chem. **276:**48988–48996.
305. **Laprade, L., V. L. Boyartchuk, W. F. Dietrich, and F. Winston.** 2002. Spt3 plays opposite roles in filamentous growth in Saccharomyces cerevisiae and Candida albicans and is required for C. albicans virulence. Genetics **161:** 509–519.
306. **Lass-Flörl, C., et al.** 1998. In-vitro testing of susceptibility to amphotericin B is a reliable predictor of clinical outcome in invasive aspergillosis. J. Antimicrob. Chemother. **42:**497–502.
307. **Latgé, J. P.** 1999. Aspergillus fumigatus and aspergillosis. Clin. Microbiol. Rev. **12:**310–350.
308. **Latgé, J. P., et al.** 2005. Specific molecular features in the organization and biosynthesis of the cell wall of Aspergillus fumigatus. Med. Mycol. **43**(Suppl. 1)**:**S15–S22.
309. **Laverdiere, M., et al.** 2006. Progressive loss of echinocandin activity following prolonged use for treatment of Candida albicans oesophagitis. J. Antimicrob. Chemother. **57:**705–708.
310. **Leal, S. M., et al.** 2010. Distinct roles for dectin-1 and TLR4 in the pathogenesis of Aspergillus fumigatus keratitis. PLoS Pathog. **6:**e1000976.
311. **Leberer, E., et al.** 1996. Signal transduction through homologs of the Ste20p and Ste7p protein kinases can trigger hyphal formation in the pathogenic fungus Candida albicans. Proc. Natl. Acad. Sci. U. S. A. **93:**13217–13222.
312. **Leberer, E., et al.** 2001. Ras links cellular morphogenesis to virulence by regulation of the MAP kinase and cAMP signalling pathways in the pathogenic fungus Candida albicans. Mol. Microbiol. **42:**673–687.
313. **Leberer, E., et al.** 1997. Functional characterization of the Cdc42p binding domain of yeast Ste20p protein kinase. EMBO J. **16:**83–97.
314. **Leberer, E., et al.** 1997. Virulence and hyphal formation of Candida albicans require the Ste20p-like protein kinase CaCla4p. Curr. Biol. **7:**539–546.
315. **Lee, I., et al.** 2009. HdaA, a class 2 histone deacetylase of Aspergillus fumigatus, affects germination and secondary metabolite production. Fungal Genet. Biol. **46:**782–790.
316. **Lee, K. L., H. R. Buckley, and C. C. Campbell.** 1975. An amino acid liquid synthetic medium for the development of mycelial and yeast forms of Candida albicans. Sabouraudia **13:**148–153.
317. **Lenardon, M. D., I. Lesiak, C. A. Munro, and N. A. Gow.** 2009. Dissection of the Candida albicans class I chitin synthase promoters. Mol. Genet. Genomics **281:**459–471.
318. **Leng, P., P. R. Lee, H. Wu, and A. J. Brown.** 2001. Efg1, a morphogenetic regulator in Candida albicans, is a sequence-specific DNA binding protein. J. Bacteriol. **183:**4090–4093.
319. **Lengeler, K. B., et al.** 2002. Mating-type locus of Cryptococcus neoformans: a step in the evolution of sex chromosomes. Eukaryot. Cell **1:**704–718.
320. **Lessing, F., et al.** 2007. The Aspergillus fumigatus transcriptional regulator AfYap1 represents the major regulator for defense against reactive oxygen intermediates but is dispensable for pathogenicity in an intranasal mouse infection model. Eukaryot. Cell **6:**2290–2302.
321. **Lettner, T., et al.** 2010. Candida albicans AGE3, the ortholog of the S. cerevisiae ARF-GAP-encoding gene GCS1, is required for hyphal growth and drug resistance. PLoS One **5:**e11993.
322. **Levin, D. E.** 2005. Cell wall integrity signaling in Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. **69:**262–291.
323. **Levitz, S. M., and T. P. Farrell.** 1990. Human neutrophil degranulation stimulated by Aspergillus fumigatus. J. Leukoc. Biol. **47:**170–175.
324. **Li, H., et al.** 2007. Glycosylphosphatidylinositol (GPI) anchor is required in Aspergillus fumigatus for morphogenesis and virulence. Mol. Microbiol. **64:**1014–1027.
325. **Li, L., et al.** 2007. Canonical heterotrimeric G proteins regulating mating and virulence of Cryptococcus neoformans. Mol. Biol. Cell **18:**4201–4209.
326. **Li, M., S. J. Martin, V. M. Bruno, A. P. Mitchell, and D. A. Davis.** 2004. Candida albicans Rim13p, a protease required for Rim101p processing at acidic and alkaline pHs. Eukaryot. Cell **3:**741–751.
327. **Li, Y., C. Su, X. Mao, F. Cao, and J. Chen.** 2007. Roles of Candida albicans Sfl1 in hyphal development. Eukaryot. Cell **6:**2112–2121.
328. **Li, Y., et al.** 2008. Deletion of the msdS/AfmsdC gene induces abnormal polarity and septation in Aspergillus fumigatus. Microbiology **154:**1960–1972.
329. **Liebmann, B., S. Gattung, B. Jahn, and A. A. Brakhage.** 2003. cAMP signaling in Aspergillus fumigatus is involved in the regulation of the virulence gene pksP and in defense against killing by macrophages. Mol. Genet. Genomics **269:**420–435.
330. **Liebmann, B., M. Müller, A. Braun, and A. A. Brakhage.** 2004. The cyclic AMP-dependent protein kinase A network regulates development and virulence in *Aspergillus fumigatus*. Infect. Immun. **72:**5193–5203.
331. **Lin, S. J., J. Schranz, and S. M. Teutsch.** 2001. Aspergillosis case-fatality rate: systematic review of the literature. Clin. Infect. Dis. **32:**358–366.
332. **Lin, X., and J. Heitman.** 2005. Chlamydospore formation during hyphal growth in Cryptococcus neoformans. Eukaryot. Cell **4:**1746–1754.
333. **Lin, X., and J. Heitman.** 2006. The biology of the Cryptococcus neoformans species complex. Annu. Rev. Microbiol. **60:**69–105.
334. **Lin, X., C. M. Hull, and J. Heitman.** 2005. Sexual reproduction between partners of the same mating type in Cryptococcus neoformans. Nature **434:**1017–1021.
335. **Lin, X., J. C. Jackson, M. Feretzaki, C. Xue, and J. Heitman.** 2010. Transcription factors Mat2 and Znf2 operate cellular circuits orchestrating opposite- and same-sex mating in Cryptococcus neoformans. PLoS Genet. **6:**e1000953.
336. **Lionakis, M. S., R. E. Lewis, G. Chamilos, and D. P. Kontoyiannis.** 2005. *Aspergillus* susceptibility testing in patients with cancer and invasive aspergillosis: difficulties in establishing correlation between *in vitro* susceptibility data and the outcome of initial amphotericin B therapy. Pharmacotherapy **25:**1174–1180.
337. **Lips, K. R., et al.** 2006. Emerging infectious disease and the loss of biodiversity in a neotropical amphibian community. Proc. Natl. Acad. Sci. U. S. A. **103:**3165–3170.
338. **Liu, H., J. Kohler, and G. R. Fink.** 1994. Suppression of hyphal formation in Candida albicans by mutation of a STE12 homolog. Science **266:**1723–1726.
339. **Liu, O. W., et al.** 2008. Systematic genetic analysis of virulence in the human fungal pathogen Cryptococcus neoformans. Cell **135:**174–188.
340. **Liu, T. T., et al.** 2005. Genome-wide expression profiling of the response to azole, polyene, echinocandin, and pyrimidine antifungal agents in Candida albicans. Antimicrob. Agents Chemother. **49:**2226–2236.
341. **Liu, T. T., et al.** 2007. Genome-wide expression and location analyses of the Candida albicans Tac1p regulon. Eukaryot. Cell **6:**2122–2138.
342. **Lo, H. J., et al.** 1997. Nonfilamentous C. albicans mutants are avirulent. Cell **90:**939–949.
343. **Lohse, M. B., and A. D. Johnson.** 2009. White-opaque switching in Candida albicans. Curr. Opin. Microbiol. **12:**650–654.
344. **Lopes da Rosa, J., V. L. Boyartchuk, L. J. Zhu, and P. D. Kaufman.** 2010. Histone acetyltransferase Rtt109 is required for Candida albicans pathogenesis. Proc. Natl. Acad. Sci. U. S. A. **107:**1594–1599.
345. **Loussert, C., et al.** 2010. In vivo biofilm composition of Aspergillus fumigatus. Cell. Microbiol. **12:**405–410.
346. **Love, G. L., G. D. Boyd, and D. L. Greer.** 1985. Large Cryptococcus neoformans isolated from brain abscess. J. Clin. Microbiol. **22:**1068–1070.
347. **Lupetti, A., R. Danesi, M. Campa, M. Del Tacca, and S. Kelly.** 2002. Molecular basis of resistance to azole antifungals. Trends Mol. Med. **8:**76–81.
348. **Ma, H., J. E. Croudace, D. A. Lammas, and R. C. May.** 2006. Expulsion of live pathogenic yeast by macrophages. Curr. Biol. **16:**2156–2160.
349. **Ma, Y., et al.** 2008. The sho1 sensor regulates growth, morphology, and oxidant adaptation in Aspergillus fumigatus but is not essential for development of invasive pulmonary aspergillosis. Infect. Immun. **76:**1695–1701.
350. **MacCallum, D. M., et al.** 2010. Genetic dissection of azole resistance mechanisms in Candida albicans and their validation in a mouse model of disseminated infection. Antimicrob. Agents Chemother. **54:**1476–1483.
351. **MacCallum, D. M., et al.** 2006. Different consequences of ACE2 and SWI5 gene disruptions for virulence of pathogenic and nonpathogenic yeasts. Infect. Immun. **74:**5244–5248.
352. **MacDougall, L., et al.** 2007. Spread of Cryptococcus gattii in British Columbia, Canada, and detection in the Pacific Northwest, USA. Emerg. Infect. Dis. **13:**42–50.
353. **Maeng, S., et al.** 2010. Comparative transcriptome analysis reveals novel roles of the Ras and cyclic AMP signaling pathways in environmental stress response and antifungal drug sensitivity in Cryptococcus neoformans. Eukaryot. Cell **9:**360–378.
354. **Magee, B. B., and P. T. Magee.** 2000. Induction of mating in Candida albicans by construction of MTLa and MTLalpha strains. Science **289:**310–313.
355. **Mai, A., et al.** 2007. Discovery of uracil-based histone deacetylase inhibitors able to reduce acquired antifungal resistance and trailing growth in Candida albicans. Bioorg. Med. Chem. Lett. **17:**1221–1225.
356. **Maidan, M. M., et al.** 2008. Combined inactivation of the Candida albicans GPR1 and TPS2 genes results in avirulence in a mouse model for systemic infection. Infect. Immun. **76:**1686–1694.
357. **Maidan, M. M., et al.** 2005. The G protein-coupled receptor Gpr1 and the Galpha protein Gpa2 act through the cAMP-protein kinase A pathway to induce morphogenesis in Candida albicans. Mol. Biol. Cell **16:**1971–1986.
358. **Maidan, M. M., J. M. Thevelein, and P. Van Dijck.** 2005. Carbon source induced yeast-to-hypha transition in Candida albicans is dependent on the presence of amino acids and on the G-protein-coupled receptor Gpr1. Biochem. Soc. Trans. **33:**291–293.
359. **Maligie, M. A., and C. P. Selitrennikoff.** 2005. Cryptococcus neoformans resistance to echinocandins: (1,3)beta-glucan synthase activity is sensitive to echinocandins. Antimicrob. Agents Chemother. **49:**2851–2856.
360. **Manavathu, E. K., J. Cutright, and P. H. Chandrasekar.** 1999. Comparative study of susceptibilities of germinated and ungerminated conidia of Aspergillus fumigatus to various antifungal agents. J. Clin. Microbiol. **37:**858–861.
361. **Manavathu, E. K., J. A. Vazquez, and P. H. Chandrasekar.** 1999. Reduced susceptibility in laboratory-selected mutants of *Aspergillus fumigatus* to itraconazole due to decreased intracellular accumulation of the antifungal agent. Int. J. Antimicrob. Agents **12:**213–219.

362. **Mann, P. A., et al.** 2003. Mutations in *Aspergillus fumigatus* resulting in reduced susceptibility to posaconazole appear to be restricted to a single amino acid in the cytochrome P450 14alpha-demethylase. Antimicrob. Agents Chemother. **47:**577–581.
363. **Mansfield, B. E., et al.** 2010. Azole drugs are imported by facilitated diffusion in Candida albicans and other pathogenic fungi. PLoS Pathog. **6:**e1001126.
364. **Marchetti, O., et al.** 2003. Fungicidal synergism of fluconazole and cyclosporine in Candida albicans is not dependent on multidrug efflux transporters encoded by the CDR1, CDR2, CaMDR1, and FLU1 genes. Antimicrob. Agents Chemother. **47:**1565–1570.
365. **Marichal, P., et al.** 1999. Contribution of mutations in the cytochrome P450 14alpha-demethylase (Erg11p, Cyp51p) to azole resistance in Candida albicans. Microbiology **145**(Pt. 10)**:**2701–2713.
366. **Markovich, S., A. Yekutiel, I. Shalit, Y. Shadkchan, and N. Osherov.** 2004. Genomic approach to identification of mutations affecting caspofungin susceptibility in Saccharomyces cerevisiae. Antimicrob. Agents Chemother. **48:**3871–3876.
367. **Marr, K. A., R. A. Carter, F. Crippa, A. Wald, and L. Corey.** 2002. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin. Infect. Dis. **34:**909–917.
368. **Martel, C. M., et al.** 2010. Identification and characterization of four azole-resistant erg3 mutants of Candida albicans. Antimicrob. Agents Chemother. **54:**4527–4533.
369. **Martin, S. W., and J. B. Konopka.** 2004. Lipid raft polarization contributes to hyphal growth in Candida albicans. Eukaryot. Cell **3:**675–684.
370. **Martinez, L. R., and A. Casadevall.** 2006. Susceptibility of Cryptococcus neoformans biofilms to antifungal agents in vitro. Antimicrob. Agents Chemother. **50:**1021–1033.
371. **Martinez-Lopez, R., L. Monteoliva, R. Diez-Orejas, C. Nombela, and C. Gil.** 2004. The GPI-anchored protein CaEcm33p is required for cell wall integrity, morphogenesis and virulence in *Candida albicans*. Microbiology **150:**3341–3354.
372. **Mateus, C., S. A. Crow, Jr., and D. G. Ahearn.** 2004. Adherence of Candida albicans to silicone induces immediate enhanced tolerance to fluconazole. Antimicrob. Agents Chemother. **48:**3358–3366.
373. **Matthews, R. C., et al.** 2003. Preclinical assessment of the efficacy of Mycograb, a human recombinant antibody against fungal HSP90. Antimicrob. Agents Chemother. **47:**2208–2216.
374. **Mattia, E., G. Carruba, L. Angiolella, and A. Cassone.** 1982. Induction of germ tube formation by N-acetyl-D-glucosamine in Candida albicans: uptake of inducer and germinative response. J. Bacteriol. **152:**555–562.
375. **May, G. S., T. Xue, D. P. Kontoyiannis, and M. C. Gustin.** 2005. Mitogen activated protein kinases of Aspergillus fumigatus. Med. Mycol. **43:**83–86.
376. **McClellan, A. J., et al.** 2007. Diverse cellular functions of the Hsp90 molecular chaperone uncovered using systems approaches. Cell **131:**121–135.
377. **Mellado, E., G. Garcia-Effron, L. Alcazar-Fuoli, M. Cuenca-Estrella, and J. L. Rodriguez-Tudela.** 2004. Substitutions at methionine 220 in the 14alpha-sterol demethylase (Cyp51A) of *Aspergillus fumigatus* are responsible for resistance in vitro to azole antifungal drugs. Antimicrob. Agents Chemother. **48:**2747–2750.
378. **Mellado, E., et al.** 2007. A new *Aspergillus fumigatus* resistance mechanism conferring in vitro cross-resistance to azole antifungals involves a combination of cyp51A alterations. Antimicrob. Agents Chemother. **51:**1897–1904.
379. **Melo, A. S., A. L. Colombo, and B. A. Arthington-Skaggs.** 2007. Paradoxical growth effect of caspofungin observed on biofilms and planktonic cells of five different Candida species. Antimicrob. Agents Chemother. **51:**3081–3088.
380. **Menon, V., et al.** 2006. Functional studies of the Ssk1p response regulator protein of Candida albicans as determined by phenotypic analysis of receiver domain point mutants. Mol. Microbiol. **62:**997–1013.
381. **Merson-Davies, L. A., and F. C. Odds.** 1989. A morphology index for characterization of cell shape in Candida albicans. J. Gen. Microbiol. **135:** 3143–3152.
382. **Miller, L. G., R. A. Hajjeh, and J. E. Edwards, Jr.** 2001. Estimating the cost of nosocomial candidemia in the United States. Clin. Infect. Dis. **32:**1110.
383. **Miller, M. G., and A. D. Johnson.** 2002. White-opaque switching in Candida albicans is controlled by mating-type locus homeodomain proteins and allows efficient mating. Cell **110:**293–302.
384. **Mircescu, M. M., L. Lipuma, N. van Rooijen, E. G. Pamer, and T. M. Hohl.** 2009. Essential role for neutrophils but not alveolar macrophages at early time points following Aspergillus fumigatus infection. J. Infect. Dis. **200:** 647–656.
385. **Mitchell, B. M., T. G. Wu, B. E. Jackson, and K. R. Wilhelmus.** 2007. Candida albicans strain-dependent virulence and Rim13p-mediated filamentation in experimental keratomycosis. Invest. Ophthalmol. Vis. Sci. **48:**774–780.
386. **Miwa, T., et al.** 2004. Gpr1, a putative G-protein-coupled receptor, regulates morphogenesis and hypha formation in the pathogenic fungus Candida albicans. Eukaryot. Cell **3:**919–931.
387. **Mondon, P., et al.** 1999. Heteroresistance to fluconazole and voriconazole in Cryptococcus neoformans. Antimicrob. Agents Chemother. **43:**1856–1861.
388. **Monge, R. A., E. Roman, C. Nombela, and J. Pla.** 2006. The MAP kinase signal transduction network in Candida albicans. Microbiology **152:**905–912.
389. **Moosa, M. Y., G. J. Alangaden, E. Manavathu, and P. H. Chandrasekar.** 2002. Resistance to amphotericin B does not emerge during treatment for invasive aspergillosis. J. Antimicrob. Chemother. **49:**209–213.
390. **Morschhauser, J.** 2010. Regulation of multidrug resistance in pathogenic fungi. Fungal Genet. Biol. **47:**94–106.
391. **Morschhauser, J., et al.** 2007. The transcription factor Mrr1p controls expression of the MDR1 efflux pump and mediates multidrug resistance in Candida albicans. PLoS Pathog. **3:**e164.
392. **Mortensen, K. L., et al.** 2010. Environmental study of azole-resistant Aspergillus fumigatus and other aspergilli in Austria, Denmark, and Spain. Antimicrob. Agents Chemother. **54:**4545–4549.
393. **Mouyna, I., et al.** 2010. Members of protein O-mannosyltransferase family in Aspergillus fumigatus differentially affect growth, morphogenesis and viability. Mol. Microbiol. **76:**1205–1221.
394. **Mouyna, I., et al.** 2005. Deletion of GEL2 encoding for a beta(1-3)glucanosyltransferase affects morphogenesis and virulence in Aspergillus fumigatus. Mol. Microbiol. **56:**1675–1688.
395. **Mowat, E., J. Butcher, S. Lang, C. Williams, and G. Ramage.** 2007. Development of a simple model for studying the effects of antifungal agents on multicellular communities of Aspergillus fumigatus. J. Med. Microbiol. **56:**1205–1212.
396. **Mowat, E., C. Williams, B. Jones, S. McChlery, and G. Ramage.** 2009. The characteristics of Aspergillus fumigatus mycetoma development: is this a biofilm? Med. Mycol. **47**(Suppl. 1)**:**S120–S126.
397. **Moyes, D. L., et al.** 2010. A biphasic innate immune MAPK response discriminates between the yeast and hyphal forms of Candida albicans in epithelial cells. Cell Host Microbe **8:**225–235.
398. **Muhlschlegel, F. A., and W. A. Fonzi.** 1997. PHR2 of Candida albicans encodes a functional homolog of the pH-regulated gene PHR1 with an inverted pattern of pH-dependent expression. Mol. Cell. Biol. **17:**5960–5967.
399. **Mukherjee, P. K., J. Chandra, D. M. Kuhn, and M. A. Ghannoum.** 2003. Mechanism of fluconazole resistance in Candida albicans biofilms: phase-specific role of efflux pumps and membrane sterols. Infect. Immun. **71:** 4333–4340.
400. **Mukherjee, P. K., L. Long, H. G. Kim, and M. A. Ghannoum.** 2009. Amphotericin B lipid complex is efficacious in the treatment of Candida albicans biofilms using a model of catheter-associated Candida biofilms. Int. J. Antimicrob. Agents **33:**149–153.
401. **Mulhern, S. M., M. E. Logue, and G. Butler.** 2006. Candida albicans transcription factor Ace2 regulates metabolism and is required for filamentation in hypoxic conditions. Eukaryot. Cell **5:**2001–2013.
402. **Mullbacher, A., and R. D. Eichner.** 1984. Immunosuppression in vitro by a metabolite of a human pathogenic fungus. Proc. Natl. Acad. Sci. U. S. A. **81:**3835–3837.
403. **Mullbacher, A., P. Waring, and R. D. Eichner.** 1985. Identification of an agent in cultures of Aspergillus fumigatus displaying anti-phagocytic and immunomodulating activity in vitro. J. Gen. Microbiol. **131:**1251–1258.
404. **Mullins, J., P. S. Hutcheson, and R. G. Slavin.** 1984. Aspergillus fumigatus spore concentration in outside air: Cardiff and St. Louis compared. Clin. Allergy **14:**351–354.
405. **Munro, C. A., et al.** 2007. The PKC, HOG and Ca2+ signalling pathways co-ordinately regulate chitin synthesis in Candida albicans. Mol. Microbiol. **63:**1399–1413.
406. **Murad, A. M., et al.** 2001. Transcript profiling in Candida albicans reveals new cellular functions for the transcriptional repressors CaTup1, CaMig1 and CaNrg1. Mol. Microbiol. **42:**981–993.
407. **Murad, A. M., et al.** 2001. NRG1 represses yeast-hypha morphogenesis and hypha-specific gene expression in Candida albicans. EMBO J. **20:**4742–4752.
408. **Naglik, J., A. Albrecht, O. Bader, and B. Hube.** 2004. Candida albicans proteinases and host/pathogen interactions. Cell. Microbiol. **6:**915–926.
409. **Naglik, J. R., S. J. Challacombe, and B. Hube.** 2003. Candida albicans secreted aspartyl proteinases in virulence and pathogenesis. Microbiol. Mol. Biol. Rev. **67:**400–428.
410. **Nantel, A., et al.** 2002. Transcription profiling of Candida albicans cells undergoing the yeast-to-hyphal transition. Mol. Biol. Cell **13:**3452–3465.
411. **Nascimento, A. M., et al.** 2003. Multiple resistance mechanisms among Aspergillus fumigatus mutants with high-level resistance to itraconazole. Antimicrob. Agents Chemother. **47:**1719–1726.
412. **Neill, J. M., I. Abrahams, and C. E. Kapros.** 1950. A comparison of the immunogenicity of weakly encapsulated and of strongly encapsulated strains of Cryptococcus neoformans (Torula histolytica). J. Bacteriol. **59:** 263–275.
413. **Nett, J., and D. Andes.** 2006. Candida albicans biofilm development, modeling a host-pathogen interaction. Curr. Opin. Microbiol. **9:**340–345.

414. **Nett, J., et al.** 2007. Putative role of beta-1,3 glucans in Candida albicans biofilm resistance. Antimicrob. Agents Chemother. **51:**510–520.
415. **Nichols, C. B., J. Ferreyra, E. R. Ballou, and J. A. Alspaugh.** 2009. Subcellular localization directs signaling specificity of the Cryptococcus neoformans Ras1 protein. Eukaryot. Cell **8:**181–189.
416. **Nichols, C. B., Z. H. Perfect, and J. A. Alspaugh.** 2007. A Ras1-Cdc24 signal transduction pathway mediates thermotolerance in the fungal pathogen Cryptococcus neoformans. Mol. Microbiol. **63:**1118–1130.
417. **Nielsen, K., et al.** 2005. Cryptococcus neoformans alpha strains preferentially disseminate to the central nervous system during coinfection. Infect. Immun. **73:**4922–4933.
418. **Nierman, W. C., et al.** 2005. Genomic sequence of the pathogenic and allergenic filamentous fungus Aspergillus fumigatus. Nature **438:**1151–1156.
419. **Niimi, K., et al.** 2006. Overexpression of Candida albicans CDR1, CDR2, or MDR1 does not produce significant changes in echinocandin susceptibility. Antimicrob. Agents Chemother. **50:**1148–1155.
420. **Nobbs, A. H., M. M. Vickerman, and H. F. Jenkinson.** 2010. Heterologous expression of Candida albicans cell wall-associated adhesins in Saccharomyces cerevisiae reveals differential specificities in adherence and biofilm formation and in binding oral Streptococcus gordonii. Eukaryot. Cell **9:**1622–1634.
421. **Nobile, C. J., et al.** 2006. Critical role of Bcr1-dependent adhesins in C. albicans biofilm formation in vitro and in vivo. PLoS Pathog. **2:**e63.
422. **Nobile, C. J., and A. P. Mitchell.** 2006. Genetics and genomics of Candida albicans biofilm formation. Cell. Microbiol. **8:**1382–1391.
423. **Nobile, C. J., and A. P. Mitchell.** 2005. Regulation of cell-surface genes and biofilm formation by the C. albicans transcription factor Bcr1p. Curr. Biol. **15:**1150–1155.
424. **Nobile, C. J., et al.** 2008. Candida albicans transcription factor Rim101 mediates pathogenic interactions through cell wall functions. Cell. Microbiol. **10:**2180–2196.
425. **Noble, S. M., S. French, L. A. Kohn, V. Chen, and A. D. Johnson.** 2010. Systematic screens of a Candida albicans homozygous deletion library decouple morphogenetic switching and pathogenicity. Nat. Genet. **42:**590–598.
426. **Odds, F. C.** 1985. Morphogenesis in Candida albicans. Crit. Rev. Microbiol. **12:**45–93.
427. **Odds, F. C., A. Cockayne, J. Hayward, and A. B. Abbott.** 1985. Effects of imidazole- and triazole-derivative antifungal compounds on the growth and morphological development of Candida albicans hyphae. J. Gen. Microbiol. **131:**2581–2589.
428. **Odds, F. C., et al.** 1998. Evaluation of possible correlation between antifungal susceptibilities of filamentous fungi in vitro and antifungal treatment outcomes in animal infection models. Antimicrob. Agents Chemother. **42:**282–288.
429. **Odds, F. C., N. A. Gow, and A. J. Brown.** 2001. Fungal virulence studies come of age. Genome Biol. **2:**REVIEWS1009. http://genomebiology.com/2001/2/3/reviews/1009.
430. **Odom, A., et al.** 1997. Calcineurin is required for virulence of Cryptococcus neoformans. EMBO J. **16:**2576–2589.
431. **Okagaki, L. H., et al.** 2010. Cryptococcal cell morphology affects host cell interactions and pathogenicity. PLoS Pathog. **6:**e1000953.
432. **Oliver, B. G., et al.** 2002. Cloning and expression of pkaC and pkaR, the genes encoding the cAMP-dependent protein kinase of *Aspergillus fumigatus*. Mycopathologica **154:**85–91.
433. **Oliver, B. G., J. L. Song, J. H. Choiniere, and T. C. White.** 2007. *cis*-Acting elements within the Candida albicans ERG11 promoter mediate the azole response through transcription factor Upc2p. Eukaryot. Cell **6:**2231–2239.
434. **Olson, G. M., D. S. Fox, P. Wang, J. A. Alspaugh, and K. L. Buchanan.** 2007. Role of protein O-mannosyltransferase Pmt4 in the morphogenesis and virulence of Cryptococcus neoformans. Eukaryot. Cell **6:**222–234.
435. **Olszewski, M. A., Y. Zhang, and G. B. Huffnagle.** 2010. Mechanisms of cryptococcal virulence and persistence. Future Microbiol. **5:**1269–1288.
436. **O'Meara, T. R., et al.** 2010. Interaction of Cryptococcus neoformans Rim101 and protein kinase A regulates capsule. PLoS Pathog. **6:**e1000776.
437. **Onyewu, C., J. R. Blankenship, M. Del Poeta, and J. Heitman.** 2003. Ergosterol biosynthesis inhibitors become fungicidal when combined with calcineurin inhibitors against Candida albicans, Candida glabrata, and Candida krusei. Antimicrob. Agents Chemother. **47:**956–964.
438. **Onyewu, C., F. L. Wormley, Jr., J. R. Perfect, and J. Heitman.** 2004. The calcineurin target, Crz1, functions in azole tolerance but is not required for virulence of Candida albicans. Infect. Immun. **72:**7330–7333.
439. **Orsi, C. F., et al.** 2009. The ABC transporter-encoding gene AFR1 affects the resistance of Cryptococcus neoformans to microglia-mediated antifungal activity by delaying phagosomal maturation. FEMS Yeast Res. **9:**301–310.
440. **Osherov, N., D. P. Kontoyiannis, A. Romans, and G. S. May.** 2001. Resistance to itraconazole in *Aspergillus nidulans* and *Aspergillus fumigatus* is conferred by extra copies of the *A. nidulans* P-450 14alpha-demethylase gene, *pdmA*. J. Antimicrob. Chemother. **48:**75–81.
441. **Ostrosky-Zeichner, L., A. Casadevall, J. N. Galgiani, F. C. Odds, and J. H. Rex.** 2010. An insight into the antifungal pipeline: selected new molecules and beyond. Nat. Rev. Drug Discov. **9:**719–727.
442. **Pachl, J., et al.** 2006. A randomized, blinded, multicenter trial of lipid-associated amphotericin B alone versus in combination with an antibody-based inhibitor of heat shock protein 90 in patients with invasive candidiasis. Clin. Infect. Dis. **42:**1404–1413.
443. **Pagano, L., et al.** 2001. Infections caused by filamentous fungi in patients with hematologic malignancies. A report of 391 cases by GIMEMA Infection Program. Haematologica **86:**862–870.
444. **Palmer, D. A., J. K. Thompson, L. Li, A. Prat, and P. Wang.** 2006. Gib2, a novel Gbeta-like/RACK1 homolog, functions as a Gbeta subunit in cAMP signaling and is essential in Cryptococcus neoformans. J. Biol. Chem. **281:**32596–32605.
445. **Paramonova, E., B. P. Krom, H. C. van der Mei, H. J. Busscher, and P. K. Sharma.** 2009. Hyphal content determines the compression strength of Candida albicans biofilms. Microbiology **155:**1997–2003.
446. **Park, B. J., et al.** 2009. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS **23:**525–530.
447. **Park, S., et al.** 2005. Specific substitutions in the echinocandin target Fks1p account for reduced susceptibility of rare laboratory and clinical Candida sp. isolates. Antimicrob. Agents Chemother. **49:**3264–3273.
448. **Park, Y. N., and J. Morschhauser.** 2005. Tetracycline-inducible gene expression and gene deletion in Candida albicans. Eukaryot. Cell **4:**1328–1342.
449. **Paugam, A., et al.** 1994. Increased fluconazole resistance of Cryptococcus neoformans isolated from a patient with AIDS and recurrent meningitis. Clin. Infect. Dis. **19:**975–976.
450. **Pearl, L. H., and C. Prodromou.** 2006. Structure and mechanism of the Hsp90 molecular chaperone machinery. Annu. Rev. Biochem. **75:**271–294.
451. **Perea, S., et al.** 2001. Prevalence of molecular mechanisms of resistance to azole antifungal agents in Candida albicans strains displaying high-level fluconazole resistance isolated from human immunodeficiency virus-infected patients. Antimicrob. Agents Chemother. **45:**2676–2684.
452. **Perfect, J. R., and A. Casadevall.** 2002. Cryptococcosis. Infect. Dis. Clin. North Am. **16:**837–874.
453. **Perfect, J. R., and A. Casadevall.** 2006. Fungal molecular pathogenesis: what can it do and why do we need it?, p. 3–11. *In* J. Heitman, S. G. Filler, J. E. Edwards, and A. P. Mitchell (ed.), Molecular principles of fungal pathogenesis. ASM Press, Washington, DC.
454. **Perfect, J. R., B. Wong, Y. C. Chang, K. J. Kwon-Chung, and P. R. Williamson.** 1998. Cryptococcus neoformans: virulence and host defences. Med. Mycol. **36**(Suppl. 1)**:**79–86.
455. **Perlin, D. S.** 2007. Resistance to echinocandin-class antifungal drugs. Drug Resist. Updat. **10:**121–130.
456. **Pfaller, M. A., and D. J. Diekema.** 2007. Epidemiology of invasive candidiasis: a persistent public health problem. Clin. Microbiol. Rev. **20:**133–163.
457. **Pfaller, M. A., and D. J. Diekema.** 2010. Epidemiology of invasive mycoses in North America. Crit. Rev. Microbiol. **36:**1–53.
458. **Pfaller, M. A., et al.** 2009. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: 10.5-year analysis of susceptibilities of noncandidal yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. J. Clin. Microbiol. **47:**117–123.
459. **Pfaller, M. A., et al.** 2003. Caspofungin activity against clinical isolates of fluconazole-resistant Candida. J. Clin. Microbiol. **41:**5729–5731.
460. **Pfaller, M. A., et al.** 2005. Global trends in the antifungal susceptibility of Cryptococcus neoformans (1990 to 2004). J. Clin. Microbiol. **43:**2163–2167.
461. **Pfaller, M. A., et al.** 2009. Activity of MGCD290, a Hos2 histone deacetylase inhibitor, in combination with azole antifungals against opportunistic fungal pathogens. J. Clin. Microbiol. **47:**3797–3804.
462. **Pinchai, N., et al.** 2009. Aspergillus fumigatus calcipressin CbpA is involved in hyphal growth and calcium homeostasis. Eukaryot. Cell **8:**511–519.
463. **Porta, A., A. M. Ramon, and W. A. Fonzi.** 1999. PRR1, a homolog of Aspergillus nidulans palF, controls pH-dependent gene expression and filamentation in Candida albicans. J. Bacteriol. **181:**7516–7523.
464. **Post, M. J., C. Lass-Floerl, G. Gastl, and D. Nachbaur.** 2007. Invasive fungal infections in allogeneic and autologous stem cell transplant recipients: a single-center study of 166 transplanted patients. Transpl. Infect. Dis. **9:**189–195.
465. **Posteraro, B., et al.** 2003. Identification and characterization of a Cryptococcus neoformans ATP binding cassette (ABC) transporter-encoding gene, CnAFR1, involved in the resistance to fluconazole. Mol. Microbiol. **47:**357–371.
466. **Prasad, R., P. De Wergifosse, A. Goffeau, and E. Balzi.** 1995. Molecular cloning and characterization of a novel gene of Candida albicans, CDR1, conferring multiple resistance to drugs and antifungals. Curr. Genet. **27:**320–329.
467. **Prasad, T., et al.** 2010. Morphogenic regulator EFG1 affects the drug susceptibilities of pathogenic Candida albicans. FEMS Yeast Res. **10:**587–596.
468. **Prasad, T., et al.** 2005. Functional analysis of CaIPT1, a sphingolipid bio-

synthetic gene involved in multidrug resistance and morphogenesis of Candida albicans. Antimicrob. Agents Chemother. **49:**3442–3452.
469. **Prill, S. K., et al.** 2005. PMT family of Candida albicans: five protein mannosyltransferase isoforms affect growth, morphogenesis and antifungal resistance. Mol. Microbiol. **55:**546–560.
470. **Pukkila-Worley, R., et al.** 2005. Transcriptional network of multiple capsule and melanin genes governed by the Cryptococcus neoformans cyclic AMP cascade. Eukaryot. Cell **4:**190–201.
471. **Qadota, H., et al.** 1996. Identification of yeast Rho1p GTPase as a regulatory subunit of 1,3-beta-glucan synthase. Science **272:**279–281.
472. **Qiao, J., W. Liu, and R. Li.** 2008. Antifungal resistance mechanisms of Aspergillus. Nippon Ishinkin Gakkai Zasshi **49:**157–163.
473. **Qiao, J., W. Liu, and R. Li.** 2010. Truncated Afyap1 attenuates antifungal susceptibility of Aspergillus fumigatus to voriconazole and confers adaptation of the fungus to oxidative stress. Mycopathologia **170:**155–160.
474. **Queitsch, C., T. A. Sangster, and S. Lindquist.** 2002. Hsp90 as a capacitor of phenotypic variation. Nature **417:**618–624.
475. **Ramage, G., S. Bachmann, T. F. Patterson, B. L. Wickes, and J. L. Lopez-Ribot.** 2002. Investigation of multidrug efflux pumps in relation to fluconazole resistance in Candida albicans biofilms. J. Antimicrob. Chemother. **49:**973–980.
476. **Ramage, G., E. Mowat, B. Jones, C. Williams, and J. Lopez-Ribot.** 2009. Our current understanding of fungal biofilms. Crit. Rev. Microbiol. **35:**340–355.
477. **Ramage, G., S. P. Saville, D. P. Thomas, and J. L. Lopez-Ribot.** 2005. Candida biofilms: an update. Eukaryot. Cell **4:**633–638.
478. **Ramage, G., S. P. Saville, B. L. Wickes, and J. L. Lopez-Ribot.** 2002. Inhibition of Candida albicans biofilm formation by farnesol, a quorum-sensing molecule. Appl. Environ. Microbiol. **68:**5459–5463.
479. **Ramage, G., K. Vande Walle, B. L. Wickes, and J. L. Lopez-Ribot.** 2001. Standardized method for in vitro antifungal susceptibility testing of Candida albicans biofilms. Antimicrob. Agents Chemother. **45:**2475–2479.
480. **Ramezani-Rad, M.** 2003. The role of adaptor protein Ste50-dependent regulation of the MAPKKK Ste11 in multiple signalling pathways of yeast. Curr. Genet. **43:**161–170.
481. **Ramon, A. M., and W. A. Fonzi.** 2003. Diverged binding specificity of Rim101p, the Candida albicans ortholog of PacC. Eukaryot. Cell **2:**718–728.
482. **Ramon, A. M., A. Porta, and W. A. Fonzi.** 1999. Effect of environmental pH on morphological development of Candida albicans is mediated via the PacC-related transcription factor encoded by PRR2. J. Bacteriol. **181:**7524–7530.
483. **Rao, U. S., and G. A. Scarborough.** 1994. Direct demonstration of high affinity interactions of immunosuppressant drugs with the drug binding site of the human P-glycoprotein. Mol. Pharmacol. **45:**773–776.
484. **Reinoso-Martin, C., C. Schuller, M. Schuetzer-Muehlbauer, and K. Kuchler.** 2003. The yeast protein kinase C cell integrity pathway mediates tolerance to the antifungal drug caspofungin through activation of Slt2p mitogen-activated protein kinase signaling. Eukaryot. Cell **2:**1200–1210.
485. **Renwick, J., P. Daly, E. P. Reeves, and K. Kavanagh.** 2006. Susceptibility of larvae of Galleria mellonella to infection by Aspergillus fumigatus is dependent upon stage of conidial germination. Mycopathologia **161:**377–384.
486. **Richard, M. L., C. J. Nobile, V. M. Bruno, and A. P. Mitchell.** 2005. Candida albicans biofilm-defective mutants. Eukaryot. Cell **4:**1493–1502.
487. **Richie, D. L., et al.** 2009. A role for the unfolded protein response (UPR) in virulence and antifungal susceptibility in Aspergillus fumigatus. PLoS Pathog. **5:**e1000258.
488. **Riggle, P. J., K. A. Andrutis, X. Chen, S. R. Tzipori, and C. A. Kumamoto.** 1999. Invasive lesions containing filamentous forms produced by a Candida albicans mutant that is defective in filamentous growth in culture. Infect. Immun. **67:**3649–3652.
489. **Robbins, N., C. Collins, J. Morhayim, and L. E. Cowen.** 2010. Metabolic control of antifungal drug resistance. Fungal Genet. Biol. **47:**81–93.
490. **Rocha, C. R., et al.** 2001. Signaling through adenylyl cyclase is essential for hyphal growth and virulence in the pathogenic fungus Candida albicans. Mol. Biol. Cell **12:**3631–3643.
491. **Rocha, E. M., G. Garcia-Effron, S. Park, and D. S. Perlin.** 2007. A Ser678Pro substitution in Fks1p confers resistance to echinocandin drugs in *Aspergillus fumigatus*. Antimicrob. Agents Chemother. **51:**4174–4176.
492. **Rodero, L., et al.** 2003. G484S amino acid substitution in lanosterol 14-alpha demethylase (ERG11) is related to fluconazole resistance in a recurrent Cryptococcus neoformans clinical isolate. Antimicrob. Agents Chemother. **47:**3653–3656.
493. **Rodrigues, A. G., R. Araujo, and C. Pina-Vaz.** 2005. Human albumin promotes germination, hyphal growth and antifungal resistance by Aspergillus fumigatus. Med. Mycol. **43:**711–717.
494. **Roman, E., et al.** 2009. The Cek1 MAPK is a short-lived protein regulated by quorum sensing in the fungal pathogen Candida albicans. FEMS Yeast Res. **9:**942–955.
495. **Roman, E., D. M. Arana, C. Nombela, R. Alonso-Monge, and J. Pla.** 2007. MAP kinase pathways as regulators of fungal virulence. Trends Microbiol. **15:**181–190.
496. **Roman, E., F. Cottier, J. F. Ernst, and J. Pla.** 2009. Msb2 signaling mucin controls activation of Cek1 mitogen-activated protein kinase in Candida albicans. Eukaryot. Cell **8:**1235–1249.
497. **Roman, E., C. Nombela, and J. Pla.** 2005. The Sho1 adaptor protein links oxidative stress to morphogenesis and cell wall biosynthesis in the fungal pathogen Candida albicans. Mol. Cell. Biol. **25:**10611–10627.
498. **Romano, J., et al.** 2006. Disruption of the *Aspergillus fumigatus ECM33* homologue results in rapid conidial germination, antifungal resistance and hypervirulence. Microbiology **152:**1919–1928.
499. **Russell, C. L., and A. J. Brown.** 2005. Expression of one-hybrid fusions with Staphylococcus aureus lexA in Candida albicans confirms that Nrg1 is a transcriptional repressor and that Gcn4 is a transcriptional activator. Fungal Genet. Biol. **42:**676–683.
500. **Russell, N. J., D. Kerridge, and E. F. Gale.** 1975. Polyene sensitivity during germination of conidia of Aspergillus fumigatus. J. Gen. Microbiol. **87:**351–358.
501. **Rutherford, S. L., and S. Lindquist.** 1998. Hsp90 as a capacitor for morphological evolution. Nature **396:**336–342.
502. **Sanchez-Martinez, C., and J. Perez-Martin.** 2002. Gpa2, a G-protein alpha subunit required for hyphal development in Candida albicans. Eukaryot. Cell **1:**865–874.
503. **Sanglard, D., F. Ischer, L. Koymans, and J. Bille.** 1998. Amino acid substitutions in the cytochrome P-450 lanosterol 14alpha-demethylase (CYP51A1) from azole-resistant Candida albicans clinical isolates contribute to resistance to azole antifungal agents. Antimicrob. Agents Chemother. **42:**241–253.
504. **Sanglard, D., F. Ischer, O. Marchetti, J. Entenza, and J. Bille.** 2003. Calcineurin A of Candida albicans: involvement in antifungal tolerance, cell morphogenesis and virulence. Mol. Microbiol. **48:**959–976.
505. **Sanglard, D., F. Ischer, M. Monod, and J. Bille.** 1997. Cloning of Candida albicans genes conferring resistance to azole antifungal agents: characterization of CDR2, a new multidrug ABC transporter gene. Microbiology **143**(Pt. 2):405–416.
506. **Sanglard, D., F. Ischer, M. Monod, and J. Bille.** 1996. Susceptibilities of Candida albicans multidrug transporter mutants to various antifungal agents and other metabolic inhibitors. Antimicrob. Agents Chemother. **40:**2300–2305.
507. **Sanglard, D., et al.** 1995. Mechanisms of resistance to azole antifungal agents in Candida albicans isolates from AIDS patients involve specific multidrug transporters. Antimicrob. Agents Chemother. **39:**2378–2386.
508. **Sanguinetti, M., et al.** 2006. Role of AFR1, an ABC transporter-encoding gene, in the in vivo response to fluconazole and virulence of Cryptococcus neoformans. Infect. Immun. **74:**1352–1359.
509. **Santos, M., and I. F. de Larrinoa.** 2005. Functional characterization of the Candida albicans CRZ1 gene encoding a calcineurin-regulated transcription factor. Curr. Genet. **48:**88–100.
510. **Saporito-Irwin, S. M., C. E. Birse, P. S. Sypherd, and W. A. Fonzi.** 1995. PHR1, a pH-regulated gene of Candida albicans, is required for morphogenesis. Mol. Cell. Biol. **15:**601–613.
511. **Saville, S. P., A. L. Lazzell, C. Monteagudo, and J. L. Lopez-Ribot.** 2003. Engineered control of cell morphology in vivo reveals distinct roles for yeast and filamentous forms of Candida albicans during infection. Eukaryot. Cell **2:**1053–1060.
512. **Schmelzle, T., and M. N. Hall.** 2000. TOR, a central controller of cell growth. Cell **103:**253–262.
513. **Schuetzer-Muehlbauer, M., B. Willinger, R. Egner, G. Ecker, and K. Kuchler.** 2003. Reversal of antifungal resistance mediated by ABC efflux pumps from Candida albicans functionally expressed in yeast. Int. J. Antimicrob. Agents **22:**291–300.
514. **Schuetzer-Muehlbauer, M., et al.** 2003. The Candida albicans Cdr2p ATP-binding cassette (ABC) transporter confers resistance to caspofungin. Mol. Microbiol. **48:**225–235.
515. **Schweizer, A., S. Rupp, B. N. Taylor, M. Rollinghoff, and K. Schroppel.** 2000. The TEA/ATTS transcription factor CaTec1p regulates hyphal development and virulence in Candida albicans. Mol. Microbiol. **38:**435–445.
516. **Segarra, I., D. A. Movshin, and L. Zarif.** 2002. Pharmacokinetics and tissue distribution after intravenous administration of a single dose of amphotericin B cochleates, a new lipid-based delivery system. J. Pharm. Sci. **91:**1827–1837.
517. **Seidler, M. J., S. Salvenmoser, and F.-M. C. Müller.** 2008. Aspergillus fumigatus forms biofilms with reduced antifungal drug susceptibility on bronchial epithelial cells. Antimicrob. Agents Chemother. **52:**4130–4136.
518. **Sellam, A., et al.** 2009. Genome-wide mapping of the coactivator Ada2p yields insight into the functional roles of SAGA/ADA complex in Candida albicans. Mol. Biol. Cell **20:**2389–2400.
519. **Sellam, A., et al.** 2010. Role of transcription factor CaNdt80p in cell separation, hyphal growth, and virulence in Candida albicans. Eukaryot. Cell **9:**634–644.
520. **Sellam, A., F. Tebbji, and A. Nantel.** 2009. Role of Ndt80p in sterol metabolism regulation and azole resistance in Candida albicans. Eukaryot. Cell **8:**1174–1183.
521. **Selmecki, A., A. Forche, and J. Berman.** 2006. Aneuploidy and isochromosome formation in drug-resistant Candida albicans. Science **313:**367–370.

522. **Selmecki, A., M. Gerami-Nejad, C. Paulson, A. Forche, and J. Berman.** 2008. An isochromosome confers drug resistance in vivo by amplification of two genes, ERG11 and TAC1. Mol. Microbiol. **68:**624–641.
523. **Selmecki, A. M., K. Dulmage, L. E. Cowen, J. B. Anderson, and J. Berman.** 2009. Acquisition of aneuploidy provides increased fitness during the evolution of antifungal drug resistance. PLoS Genet. **5:**e1000705.
524. **Setiadi, E. R., T. Doedt, F. Cottier, C. Noffz, and J. F. Ernst.** 2006. Transcriptional response of Candida albicans to hypoxia: linkage of oxygen sensing and Efg1p-regulatory networks. J. Mol. Biol. **361:**399–411.
525. **Sevilla, M. J., and F. C. Odds.** 1986. Development of Candida albicans hyphae in different growth media—variations in growth rates, cell dimensions and timing of morphogenetic events. J. Gen. Microbiol. **132:**3083–3088.
526. **Shahbazian, M. D., and M. Grunstein.** 2007. Functions of site-specific histone acetylation and deacetylation. Annu. Rev. Biochem. **76:**75–100.
527. **Shapiro, R. S., et al.** 2009. Hsp90 orchestrates temperature-dependent Candida albicans morphogenesis via Ras1-PKA signaling. Curr. Biol. **19:** 621–629.
528. **Sharkey, L. L., M. D. McNemar, S. M. Saporito-Irwin, P. S. Sypherd, and W. A. Fonzi.** 1999. HWP1 functions in the morphological development of Candida albicans downstream of EFG1, TUP1, and RBF1. J. Bacteriol. **181:**5273–5279.
529. **Shen, G., Y. L. Wang, A. Whittington, L. Li, and P. Wang.** 2008. The RGS protein Crg2 regulates pheromone and cyclic AMP signaling in Cryptococcus neoformans. Eukaryot. Cell **7:**1540–1548.
530. **Shen, J., L. E. Cowen, A. M. Griffin, L. Chan, and J. R. Kohler.** 2008. The Candida albicans pescadillo homolog is required for normal hypha-to-yeast morphogenesis and yeast proliferation. Proc. Natl. Acad. Sci. U. S. A. **105:**20918–20923.
531. **Sheng, C., et al.** 2009. Three-dimensional model of lanosterol 14 alpha-demethylase from Cryptococcus neoformans: active-site characterization and insights into azole binding. Antimicrob. Agents Chemother. **53:**3487–3495.
532. **Sheppard, D. C., et al.** 2005. The Aspergillus fumigatus StuA protein governs the up-regulation of a discrete transcriptional program during the acquisition of developmental competence. Mol. Biol. Cell **16:**5866–5879.
533. **Shi, Q. M., Y. M. Wang, X. D. Zheng, R. T. Lee, and Y. Wang.** 2007. Critical role of DNA checkpoints in mediating genotoxic-stress-induced filamentous growth in Candida albicans. Mol. Biol. Cell **18:**815–826.
534. **Silver, P. M., B. G. Oliver, and T. C. White.** 2004. Role of Candida albicans transcription factor Upc2p in drug resistance and sterol metabolism. Eukaryot. Cell **3:**1391–1397.
535. **Singh, N., et al.** 2007. Cryptococcus neoformans in organ transplant recipients: impact of calcineurin-inhibitor agents on mortality. J. Infect. Dis. **195:**756–764.
536. **Singh, S. D., et al.** 2009. Hsp90 governs echinocandin resistance in the pathogenic yeast Candida albicans via calcineurin. PLoS Pathog. **5:**e1000532.
537. **Sionov, E., Y. C. Chang, H. M. Garraffo, and K. J. Kwon-Chung.** 2009. Heteroresistance to fluconazole in Cryptococcus neoformans is intrinsic and associated with virulence. Antimicrob. Agents Chemother. **53:**2804–2815.
538. **Sionov, E., H. Lee, Y. C. Chang, and K. J. Kwon-Chung.** 2010. Cryptococcus neoformans overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes. PLoS Pathog. **6:**e1000848.
539. **Slaven, J. W., et al.** 2002. Increased expression of a novel *Aspergillus fumigatus* ABC transporter gene, *atrF*, in the presence of itraconazole in an itraconazole resistant clinical isolate. Fungal Genet. Biol. **36:**199–206.
540. **Smith, W. L., and T. D. Edlind.** 2002. Histone deacetylase inhibitors enhance Candida albicans sensitivity to azoles and related antifungals: correlation with reduction in CDR and ERG upregulation. Antimicrob. Agents Chemother. **46:**3532–3539.
541. **Snelders, E., et al.** 2009. Possible environmental origin of resistance of Aspergillus fumigatus to medical triazoles. Appl. Environ. Microbiol. **75:** 4053–4057.
542. Reference deleted.
543. **Snelders, E., A. Karawajczyk, G. Schaftenaar, P. E. Verweij, and W. J. Melchers.** 2010. Azole resistance profile of amino acid changes in Aspergillus fumigatus CYP51A based on protein homology modeling. Antimicrob. Agents Chemother. **54:**2425–2430.
544. **Soll, D. R.** 2004. Mating-type locus homozygosis, phenotypic switching and mating: a unique sequence of dependencies in Candida albicans. Bioessays **26:**10–20.
545. **Sonneborn, A., et al.** 2000. Protein kinase A encoded by TPK2 regulates dimorphism of Candida albicans. Mol. Microbiol. **35:**386–396.
546. **Soriani, F. M., et al.** 2008. Functional characterization of the Aspergillus fumigatus CRZ1 homologue, CrzA. Mol. Microbiol. **67:**1274–1291.
547. **Spiering, M. J., et al.** 2010. Comparative transcript profiling of Candida albicans and Candida dubliniensis identifies SFL2, a C. albicans gene required for virulence in a reconstituted epithelial infection model. Eukaryot. Cell **9:**251–265.
548. **Srivastava, P.** 2002. Roles of heat-shock proteins in innate and adaptive immunity. Nat. Rev. Immunol. **2:**185–194.
549. **Staab, J. F., Y. S. Bahn, and P. Sundstrom.** 2003. Integrative, multifunctional plasmids for hypha-specific or constitutive expression of green fluorescent protein in Candida albicans. Microbiology **149:**2977–2986.
550. **Staab, J. F., S. D. Bradway, P. L. Fidel, and P. Sundstrom.** 1999. Adhesive and mammalian transglutaminase substrate properties of Candida albicans Hwp1. Science **283:**1535–1538.
551. **Stathopoulos, A. M., and M. S. Cyert.** 1997. Calcineurin acts through the CRZ1/TCN1-encoded transcription factor to regulate gene expression in yeast. Genes Dev. **11:**3432–3444.
552. **Stathopoulos-Gerontides, A., J. J. Guo, and M. S. Cyert.** 1999. Yeast calcineurin regulates nuclear localization of the Crz1p transcription factor through dephosphorylation. Genes Dev. **13:**798–803.
553. **Steenbergen, J. N., and A. Casadevall.** 2003. The origin and maintenance of virulence for the human pathogenic fungus Cryptococcus neoformans. Microbes Infect. **5:**667–675.
554. **Steenbergen, J. N., J. D. Nosanchuk, S. D. Malliaris, and A. Casadevall.** 2003. Cryptococcus neoformans virulence is enhanced after growth in the genetically malleable host Dictyostelium discoideum. Infect. Immun. **71:** 4862–4872.
555. **Steenbergen, J. N., H. A. Shuman, and A. Casadevall.** 2001. Cryptococcus neoformans interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic strategy in macrophages. Proc. Natl. Acad. Sci. U. S. A. **98:**15245–15250.
556. **Steinbach, W. J., et al.** 2004. Infections due to *Aspergillus terreus*: a multicenter retrospective analysis of 83 cases. Clin. Infect. Dis. **39:**192–198.
557. **Steinbach, W. J., et al.** 2006. Calcineurin controls growth, morphology, and pathogenicity in Aspergillus fumigatus. Eukaryot. Cell **5:**1091–1103.
558. **Steinbach, W. J., et al.** 2007. Calcineurin inhibition or mutation enhances cell wall inhibitors against Aspergillus fumigatus. Antimicrob. Agents Chemother. **51:**2979–2981.
559. **Steinbach, W. J., J. L. Reedy, R. A. Cramer, Jr., J. R. Perfect, and J. Heitman.** 2007. Harnessing calcineurin as a novel anti-infective agent against invasive fungal infections. Nat. Rev. Microbiol. **5:**418–430.
560. **Steinbach, W. J., et al.** 2004. In vitro interactions between antifungals and immunosuppressants against Aspergillus fumigatus. Antimicrob. Agents Chemother. **48:**1664–1669.
561. **Sterling, T. R., and W. G. Merz.** 1998. Resistance to amphotericin B: emerging clinical and microbiological patterns. Drug Resist. Updat. **1:**161–165.
562. **Stevens, D. A., M. Ichinomiya, Y. Koshi, and H. Horiuchi.** 2006. Escape of Candida from caspofungin inhibition at concentrations above the MIC (paradoxical effect) accomplished by increased cell wall chitin; evidence for beta-1,6-glucan synthesis inhibition by caspofungin. Antimicrob. Agents Chemother. **50:**3160–3161.
563. **Stewart, E., N. A. Gow, and D. V. Bowen.** 1988. Cytoplasmic alkalinization during germ tube formation in Candida albicans. J. Gen. Microbiol. **134:** 1079–1087.
564. **Stewart, E., S. Hawser, and N. A. Gow.** 1989. Changes in internal and external pH accompanying growth of Candida albicans: studies of non-dimorphic variants. Arch. Microbiol. **151:**149–153.
565. **Stewart, G. R., and D. B. Young.** 2004. Heat-shock proteins and the host-pathogen interaction during bacterial infection. Curr. Opin. Immunol. **16:** 506–510.
566. **Stoldt, V. R., A. Sonneborn, C. E. Leuker, and J. F. Ernst.** 1997. Efg1p, an essential regulator of morphogenesis of the human pathogen Candida albicans, is a member of a conserved class of bHLH proteins regulating morphogenetic processes in fungi. EMBO J. **16:**1982–1991.
567. **Stuart, S. N., et al.** 2004. Status and trends of amphibian declines and extinctions worldwide. Science **306:**1783–1786.
568. **Su, C., Y. Li, Y. Lu, and J. Chen.** 2009. Mss11, a transcriptional activator, is required for hyphal development in Candida albicans. Eukaryot. Cell **8:**1780–1791.
569. **Su, Z., H. Li, Y. Li, and F. Ni.** 2007. Inhibition of the pathogenically related morphologic transition in Candida albicans by disrupting Cdc42 binding to its effectors. Chem. Biol. **14:**1273–1282.
570. **Sudbery, P., N. Gow, and J. Berman.** 2004. The distinct morphogenic states of Candida albicans. Trends Microbiol. **12:**317–324.
571. **Sudbery, P. E.** 2001. The germ tubes of Candida albicans hyphae and pseudohyphae show different patterns of septin ring localization. Mol. Microbiol. **41:**19–31.
572. **Sugui, J. A., et al.** 2008. Genes differentially expressed in conidia and hyphae of Aspergillus fumigatus upon exposure to human neutrophils. PLoS One **3:**e2655.
573. **Sundstrom, P., E. Balish, and C. M. Allen.** 2002. Essential role of the Candida albicans transglutaminase substrate, hyphal wall protein 1, in lethal oroesophageal candidiasis in immunodeficient mice. J. Infect. Dis. **185:**521–530.
574. **Sutton, D. A., S. E. Sanche, S. G. Revankar, A. W. Fothergill, and M. G. Rinaldi.** 1999. In vitro amphotericin B resistance in clinical isolates of

Aspergillus terreus, with a head-to-head comparison to voriconazole. J. Clin. Microbiol. **37:**2343–2345.

575. **Suwannapong, G., T. Yemor, C. Boonpakdee, and M. E. Benbow.** 2011. Nosema ceranae, a new parasite in Thai honeybees. J. Invertebr. Pathol. **106:**236–241.
576. **Taipale, M., D. F. Jarosz, and S. Lindquist.** 2010. HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nat. Rev. Mol. Cell Biol. **11:**515–528.
577. **Thewes, S., et al.** 2007. In vivo and ex vivo comparative transcriptional profiling of invasive and non-invasive Candida albicans isolates identifies genes associated with tissue invasion. Mol. Microbiol. **63:**1606–1628.
578. **Thompson, J. R., et al.** 1999. A glucan synthase FKS1 homolog in cryptococcus neoformans is single copy and encodes an essential function. J. Bacteriol. **181:**444–453.
579. **Thornewell, S. J., R. B. Peery, and P. L. Skatrud.** 1997. Cloning and characterization of CneMDR1: a Cryptococcus neoformans gene encoding a protein related to multidrug resistance proteins. Gene **201:**21–29.
580. **Timpel, C., S. Zink, S. Strahl-Bolsinger, K. Schroppel, and J. Ernst.** 2000. Morphogenesis, adhesive properties, and antifungal resistance depend on the Pmt6 protein mannosyltransferase in the fungal pathogen Candida albicans. J. Bacteriol. **182:**3063–3071.
581. **Tolkacheva, T., P. McNamara, E. Piekarz, and W. Courchesne.** 1994. Cloning of a Cryptococcus neoformans gene, GPA1, encoding a G-protein alpha-subunit homolog. Infect. Immun. **62:**2849–2856.
582. **Torosantucci, A., L. Angiolella, C. Filesi, and A. Cassone.** 1984. Protein synthesis and amino acid pool during yeast-mycelial transition induced by N-acetyl-D-glucosamine in Candida albicans. J. Gen. Microbiol. **130:**3285–3293.
583. **Tripathi, G., et al.** 2002. Gcn4 co-ordinates morphogenetic and metabolic responses to amino acid starvation in Candida albicans. EMBO J. **21:**5448–5456.
584. **Trunk, K., et al.** 2009. Depletion of the cullin Cdc53p induces morphogenetic changes in Candida albicans. Eukaryot. Cell **8:**756–767.
585. **Tsao, C. C., Y. T. Chen, and C. Y. Lan.** 2009. A small G protein Rhb1 and a GTPase-activating protein Tsc2 involved in nitrogen starvation-induced morphogenesis and cell wall integrity of Candida albicans. Fungal Genet. Biol. **46:**126–136.
586. **Tscharke, R. L., M. Lazera, Y. C. Chang, B. L. Wickes, and K. J. Kwon-Chung.** 2003. Haploid fruiting in Cryptococcus neoformans is not mating type alpha-specific. Fungal Genet. Biol. **39:**230–237.
587. **Tuch, B. B., et al.** 2010. The transcriptomes of two heritable cell types illuminate the circuit governing their differentiation. PLoS Genet. **6:**e1001070.
588. **Tucker, S. C., and A. Casadevall.** 2002. Replication of Cryptococcus neoformans in macrophages is accompanied by phagosomal permeabilization and accumulation of vesicles containing polysaccharide in the cytoplasm. Proc. Natl. Acad. Sci. U. S. A. **99:**3165–3170.
589. **Twumasi-Boateng, K., et al.** 2009. Transcriptional profiling identifies a role for BrlA in the response to nitrogen depletion and for StuA in the regulation of secondary metabolite clusters in Aspergillus fumigatus. Eukaryot. Cell **8:**104–115.
590. **Umeyama, T., A. Kaneko, M. Niimi, and Y. Uehara.** 2006. Repression of CDC28 reduces the expression of the morphology-related transcription factors, Efg1p, Nrg1p, Rbf1p, Rim101p, Fkh2p and Tec1p and induces cell elongation in Candida albicans. Yeast **23:**537–552.
591. **Uppuluri, P., et al.** 2010. Dispersion as an important step in the Candida albicans biofilm developmental cycle. PLoS Pathog. **6:**e1000828.
592. **Uppuluri, P., J. Nett, J. Heitman, and D. Andes.** 2008. Synergistic effect of calcineurin inhibitors and fluconazole against Candida albicans biofilms. Antimicrob. Agents Chemother. **52:**1127–1132.
593. **Uppuluri, P., et al.** 2010. The transcriptional regulator Nrg1p controls Candida albicans biofilm formation and dispersion. Eukaryot. Cell **9:**1531–1537.
594. **Upton, A., et al.** 2007. First contemporary case of human infection with Cryptococcus gattii in Puget Sound: evidence for spread of the Vancouver Island outbreak. J. Clin. Microbiol. **45:**3086–3088.
595. **Ushinsky, S. C., et al.** 2002. CDC42 is required for polarized growth in human pathogen Candida albicans. Eukaryot. Cell **1:**95–104.
596. **Valiante, V., T. Heinekamp, R. Jain, A. Härtl, and A. A. Brakhage.** 2008. The mitogen-activated protein kinase MpkA of Aspergillus fumigatus regulates cell wall signaling and oxidative stress response. Fungal Genet. Biol. **45:**618–627.
597. **Vallim, M. A., C. B. Nichols, L. Fernandes, K. L. Cramer, and J. A. Alspaugh.** 2005. A Rac homolog functions downstream of Ras1 to control hyphal differentiation and high-temperature growth in the pathogenic fungus Cryptococcus neoformans. Eukaryot. Cell **4:**1066–1078.
598. **Vandeputte, P., et al.** 2007. Reduced susceptibility to polyenes associated with a missense mutation in the ERG6 gene in a clinical isolate of Candida glabrata with pseudohyphal growth. Antimicrob. Agents Chemother. **51:**982–990.
599. **van de Sande, W. W., M. Tavakol, W. van Vianen, and I. A. Bakker-Woudenberg.** 2010. The effects of antifungal agents to conidial and hyphal forms of Aspergillus fumigatus. Med. Mycol. **48:**48–55.
600. **Varma, A., and K. J. Kwon-Chung.** 2010. Heteroresistance of Cryptococcus gattii to fluconazole. Antimicrob. Agents Chemother. **54:**2303–2311.
601. **Velagapudi, R., Y. P. Hsueh, S. Geunes-Boyer, J. R. Wright, and J. Heitman.** 2009. Spores as infectious propagules of Cryptococcus neoformans. Infect. Immun. **77:**4345–4355.
602. **Venkateswarlu, K., M. Taylor, N. J. Manning, M. G. Rinaldi, and S. L. Kelly.** 1997. Fluconazole tolerance in clinical isolates of Cryptococcus neoformans. Antimicrob. Agents Chemother. **41:**748–751.
603. **Verweij, P. E., E. Snelders, G. H. J. Kema, E. Mellado, and W. J. G. Melchers.** 2009. Azole resistance in Aspergillus fumigatus: a side-effect of environmental fungicide use? Lancet Infect. Dis. **9:**789–795.
604. **Vinces, M. D., C. Haas, and C. A. Kumamoto.** 2006. Expression of the Candida albicans morphogenesis regulator gene CZF1 and its regulation by Efg1p and Czf1p. Eukaryot. Cell **5:**825–835.
605. **Virag, A., and S. D. Harris.** 2006. The Spitzenkorper: a molecular perspective. Mycol. Res. **110:**4–13.
606. **Voelz, K., and R. C. May.** 2010. Cryptococcal interactions with the host immune system. Eukaryot. Cell **9:**835–846.
607. **Walker, L. A., et al.** 2008. Stimulation of chitin synthesis rescues Candida albicans from echinocandins. PLoS Pathog. **4:**e1000040.
608. **Walsh, T. J., et al.** 2003. Experimental pulmonary aspergillosis due to *Aspergillus terreus*: pathogenesis and treatment of an emerging fungal pathogen resistant to amphotericin B. J. Infect. Dis. **188:**305–319.
609. **Wang, A., P. P. Raniga, S. Lane, Y. Lu, and H. Liu.** 2009. Hyphal chain formation in Candida albicans: Cdc28-Hgc1 phosphorylation of Efg1 represses cell separation genes. Mol. Cell. Biol. **29:**4406–4416.
610. **Wang, P., M. E. Cardenas, G. M. Cox, J. R. Perfect, and J. Heitman.** 2001. Two cyclophilin A homologs with shared and distinct functions important for growth and virulence of Cryptococcus neoformans. EMBO Rep. **2:**511–518.
611. **Wang, P., J. Cutler, J. King, and D. Palmer.** 2004. Mutation of the regulator of G protein signaling Crg1 increases virulence in Cryptococcus neoformans. Eukaryot. Cell **3:**1028–1035.
612. **Wang, P., and J. Heitman.** 1999. Signal transduction cascades regulating mating, filamentation, and virulence in Cryptococcus neoformans. Curr. Opin. Microbiol. **2:**358–362.
613. **Wang, P., et al.** 2002. Mating-type-specific and nonspecific PAK kinases play shared and divergent roles in Cryptococcus neoformans. Eukaryot. Cell **1:**257–272.
614. **Wang, P., J. R. Perfect, and J. Heitman.** 2000. The G-protein beta subunit GPB1 is required for mating and haploid fruiting in Cryptococcus neoformans. Mol. Cell. Biol. **20:**352–362.
615. **Warenda, A. J., and J. B. Konopka.** 2002. Septin function in Candida albicans morphogenesis. Mol. Biol. Cell **13:**2732–2746.
616. **Warn, P. A., G. Morrissey, J. Morrissey, and D. W. Denning.** 2003. Activity of micafungin (FK463) against an itraconazole-resistant strain of *Aspergillus fumigatus* and a strain of *Aspergillus terreus* demonstrating *in vivo* resistance to amphotericin B. J. Antimicrob. Chemother. **51:**913–919.
617. **Warn, P. A., A. Sharp, G. Morrissey, and D. W. Denning.** 2010. Activity of aminocandin (IP960; HMR3270) compared with amphotericin B, itraconazole, caspofungin and micafungin in neutropenic murine models of disseminated infection caused by itraconazole-susceptible and -resistant strains of Aspergillus fumigatus. Int. J. Antimicrob. Agents **35:**146–151.
618. **Warrilow, A. G., et al.** 2010. Expression, purification, and characterization of Aspergillus fumigatus sterol 14-alpha demethylase (CYP51) isoenzymes A and B. Antimicrob. Agents Chemother. **54:**4225–4234.
619. **Waugh, M. S., et al.** 2002. Ras1 and Ras2 contribute shared and unique roles in physiology and virulence of Cryptococcus neoformans. Microbiology **148:**191–201.
620. **Wheeler, R. T., and G. R. Fink.** 2006. A drug-sensitive genetic network masks fungi from the immune system. PLoS Pathog. **2:**e35.
621. **Wheeler, R. T., D. Kombe, S. D. Agarwala, and G. R. Fink.** 2008. Dynamic, morphotype-specific Candida albicans beta-glucan exposure during infection and drug treatment. PLoS Pathog. **4:**e1000227.
622. **White, S. J., et al.** 2007. Self-regulation of Candida albicans population size during GI colonization. PLoS Pathog. **3:**e184.
623. **White, T. C., K. A. Marr, and R. A. Bowden.** 1998. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clin. Microbiol. Rev. **11:**382–402.
624. **White, T. C., M. A. Pfaller, M. G. Rinaldi, J. Smith, and S. W. Redding.** 1997. Stable azole drug resistance associated with a substrain of Candida albicans from an HIV-infected patient. Oral Dis. **3**(Suppl. 1):S102–S109.
625. **Whiteway, M., and U. Oberholzer.** 2004. Candida morphogenesis and host-pathogen interactions. Curr. Opin. Microbiol. **7:**350–357.
626. **Whittington, A., and P. Wang.** 2011. The RGS protein Crg2 is required for establishment and progression of murine pulmonary cryptococcosis. Med. Mycol. **49:**263–275.
627. **Wickes, B. L., U. Edman, and J. C. Edman.** 1997. The Cryptococcus neoformans STE12alpha gene: a putative Saccharomyces cerevisiae STE12 homologue that is mating type specific. Mol. Microbiol. **26:**951–960.

628. **Wiederhold, N. P., D. P. Kontoyiannis, R. A. Prince, and R. E. Lewis.** 2005. Attenuation of the activity of caspofungin at high concentrations against Candida albicans: possible role of cell wall integrity and calcineurin pathways. Antimicrob. Agents Chemother. **49:**5146–5148.
629. **Willger, S. D., et al.** 2008. A sterol-regulatory element binding protein is required for cell polarity, hypoxia adaptation, azole drug resistance, and virulence in Aspergillus fumigatus. PLoS Pathog. **4:**e1000200.
630. **Wilson, D., A. Fiori, K. D. Brucker, P. V. Dijck, and L. Stateva.** 2010. Candida albicans Pde1p and Gpa2p comprise a regulatory module mediating agonist-induced cAMP signalling and environmental adaptation. Fungal Genet. Biol. **47:**742–752.
631. **Wilson, D., et al.** 2007. Deletion of the high-affinity cAMP phosphodiesterase encoded by PDE2 affects stress responses and virulence in Candida albicans. Mol. Microbiol. **65:**841–856.
632. **Wilson, L. S., et al.** 2002. The direct cost and incidence of systemic fungal infections. Value Health **5:**26–34.
633. **Wullschleger, S., R. Loewith, and M. N. Hall.** 2006. TOR signaling in growth and metabolism. Cell **124:**471–484.
634. **Wurtele, H., et al.** 2010. Modulation of histone H3 lysine 56 acetylation as an antifungal therapeutic strategy. Nat. Med. **16:**774–780.
635. **Xu, X. L., et al.** 2008. Bacterial peptidoglycan triggers Candida albicans hyphal growth by directly activating the adenylyl cyclase Cyr1p. Cell Host Microbe **4:**28–39.
636. **Xu, Z., et al.** 2006. cDNA microarray analysis of differential gene expression and regulation in clinically drug-resistant isolates of Candida albicans from bone marrow transplanted patients. Int. J. Med. Microbiol. **296:**421–434.
637. **Xue, C., Y. S. Bahn, G. M. Cox, and J. Heitman.** 2006. G protein-coupled receptor Gpr4 senses amino acids and activates the cAMP-PKA pathway in Cryptococcus neoformans. Mol. Biol. Cell **17:**667–679.
638. **Xue, T., C. K. Nguyen, A. Romans, and G. S. May.** 2004. A mitogen-activated protein kinase that senses nitrogen regulates conidial germination and growth in Aspergillus fumigatus. Eukaryot. Cell **3:**557–560.
639. **Yamazumi, T., et al.** 2003. Characterization of heteroresistance to fluconazole among clinical isolates of Cryptococcus neoformans. J. Clin. Microbiol. **41:**267–272.
640. **Yang, X. J., and E. Seto.** 2008. The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. Nat. Rev. Mol. Cell Biol. **9:**206–218.
641. **Yoshimoto, H., et al.** 2002. Genome-wide analysis of gene expression regulated by the calcineurin/Crz1p signaling pathway in Saccharomyces cerevisiae. J. Biol. Chem. **277:**31079–31088.
642. **Yuan, X., B. M. Mitchell, X. Hua, D. A. Davis, and K. R. Wilhelmus.** 2010. The RIM101 signal transduction pathway regulates Candida albicans virulence during experimental keratomycosis. Invest. Ophthalmol. Vis. Sci. **51:**4668–4676.
643. **Yue, C., et al.** 1999. The STE12alpha homolog is required for haploid filamentation but largely dispensable for mating and virulence in Cryptococcus neoformans. Genetics **153:**1601–1615.
644. **Zaragoza, O., C. de Virgilio, J. Ponton, and C. Gancedo.** 2002. Disruption in Candida albicans of the TPS2 gene encoding trehalose-6-phosphate phosphatase affects cell integrity and decreases infectivity. Microbiology **148:**1281–1290.
645. **Zaragoza, O., et al.** 2010. Fungal cell gigantism during mammalian infection. PLoS Pathog. **6:**e1000945.
646. **Zhang, L., H. Zhou, H. Ouyang, Y. Li, and C. Jin.** 2008. Afcwh41 is required for cell wall synthesis, conidiation, and polarity in Aspergillus fumigatus. FEMS Microbiol. Lett. **289:**155–165.
647. **Zhang, Y. Q., et al.** 2010. Requirement for ergosterol in V-ATPase function underlies antifungal activity of azole drugs. PLoS Pathog. **6:**e1000939.
648. **Zhao, R., et al.** 2005. Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the Hsp90 chaperone. Cell **120:**715–727.
649. **Zhao, W., et al.** 2006. Deletion of the regulatory subunit of protein kinase A in *Aspergillus fumigatus* alters morphology, sensitivity to oxidative damage, and virulence. Infect. Immun. **74:**4865–4874.
650. **Zhao, X., R. Mehrabi, and J. R. Xu.** 2007. Mitogen-activated protein kinase pathways and fungal pathogenesis. Eukaryot. Cell **6:**1701–1714.
651. **Zhao, X., et al.** 2004. ALS3 and ALS8 represent a single locus that encodes a Candida albicans adhesin; functional comparisons between Als3p and Als1p. Microbiology **150:**2415–2428.
652. **Zheng, X., Y. Wang, and Y. Wang.** 2004. Hgc1, a novel hypha-specific G1 cyclin-related protein regulates Candida albicans hyphal morphogenesis. EMBO J. **23:**1845–1856.
653. **Zheng, X. D., R. T. Lee, Y. M. Wang, Q. S. Lin, and Y. Wang.** 2007. Phosphorylation of Rga2, a Cdc42 GAP, by CDK/Hgc1 is crucial for Candida albicans hyphal growth. EMBO J. **26:**3760–3769.
654. **Zhou, H., et al.** 2007. O-Mannosyltransferase 1 in Aspergillus fumigatus (AfPmt1p) is crucial for cell wall integrity and conidium morphology, especially at an elevated temperature. Eukaryot. Cell **6:**2260–2268.
655. **Zhu, W., and S. G. Filler.** 2010. Interactions of Candida albicans with epithelial cells. Cell. Microbiol. **12:**273–282.
656. **Zhu, Y., et al.** 2009. Ras1 and Ras2 play antagonistic roles in regulating cellular cAMP level, stationary-phase entry and stress response in Candida albicans. Mol. Microbiol. **74:**862–875.
657. **Zimbeck, A. J., et al.** 2010. FKS mutations and elevated echinocandin MIC values among Candida glabrata isolates from U.S. population-based surveillance. Antimicrob. Agents Chemother. **54:**5042–5047.
658. **Zordan, R. E., D. J. Galgoczy, and A. D. Johnson.** 2006. Epigenetic properties of white-opaque switching in Candida albicans are based on a self-sustaining transcriptional feedback loop. Proc. Natl. Acad. Sci. U. S. A. **103:**12807–12812.
659. **Zou, H., H. M. Fang, Y. Zhu, and Y. Wang.** 2010. Candida albicans Cyr1, Cap1 and G-actin form a sensor/effector apparatus for activating cAMP synthesis in hyphal growth. Mol. Microbiol. **75:**579–591.

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