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# Efflux-Mediated Antifungal Drug Resistance†

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## INTRODUCTION

The fungal kingdom comprises an estimated 1.5 million species, about 200 of which have been associated with humans (40). Some of these fungi coexist with humans as commensals without causing harm, and others are overt pathogens. Certain commensal fungi, such as *Candida* species, however, are also opportunistic pathogens and cause infections when their human hosts become immunocompromised (43). These infections can be superficial and affect the skin or mucous membranes or can be hematogenously disseminated with serious consequences. Paradoxically, medical advances over the last 30 years have led to a significant increase in the incidence of life-threatening invasive fungal infections (IFIs) (275), a result of factors such as the AIDS epidemic, the rise in the number of people receiving organ transplantations, and the burgeoning range of new treatment options for cancer patients (171, 321, 327).

Systemic fungal infections are often hard to diagnose, which contributes to their high attributable mortality. In addition, there are far fewer classes of antifungal agents (Table 1) than antibacterial drugs, limiting therapeutic options. The azole antifungals are commonly used to treat fungal infections, as they are conveniently administered and have few side effects (325). Fungal azole drug resistance, however, can be a problem in some patient groups (380). The major mechanism responsible for high-level azole resistance in clinical *Candida* isolates is overexpression of plasma membrane efflux pumps (135, 300, 307, 378). There are two main families of efflux proteins, the ATP-binding cassette (ABC) pumps and the major facilitator superfamily (MFS) transporters (4, 23, 74, 89, 212, 267, 299, 334). MFS pumps appear to have a limited range of substrates, whereas ABC transporters have, in general, broader specificity (Table 2) and are of greater clinical significance, and they will be the focus of this review. The heterologous expression of efflux pumps in model systems, such as *Saccharomyces cerevisiae*, has enabled the functional analysis of efflux pumps from a variety of fungi (87, 90, 91, 109, 159, 181, 190, 306, 318, 362).

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TABLE 1. Antifungal drugs, their targets and possible resistance mechanisms

| Antifungal class and members | Primary target (mode of action) | Resistance mechanisms |
|---|---|---|
| Fluorinated pyrimidine analogs<br>5-FC | RNA and DNA synthesis (misincorporation of 5-fluorouracil) | Mutation in Fur1p (uracil phosphoribosyl transferase) |
| Polyenes<br>Nystatin<br>AMB | Cell membrane ergosterol (increased permeability and oxidative damage) | Induction of low membrane ergosterol content detected in some fungi |
| Allylamines<br>Terbinafine<br>Naftifine | Ergosterol biosynthesis (inhibition of squalene epoxidase; accumulation of toxic sterol intermediates) | Mutations in Erg1p, efflux via ABC transporters, stress tolerance induction, induction of detoxification |
| Imidazoles<br>MCZ, etc.<br>Triazoles<br>FLC<br>ITC<br>VCZ<br>POS<br>Ravuconazole<br>Isavuconazole<br>Pramiconazole<br>Albaconazole | Ergosterol biosynthesis (inhibition of Erg11p, the rate-limiting step in ergosterol biosynthesis; conversion of Erg11p substrate into toxic methylated sterols) | Mutations in Erg11p, induced overexpression of Erg11p, efflux via ABC and MFS transporters, tolerance to methylated sterols via mutation in *ERG3*, LOH[a] for mutant *ERG11* and *TAC1*, aneuploidy (*C. albicans* chromosome 5), stress tolerance induction, import of host cholesterol (*C. glabrata*) |
| Echinocandins<br>Caspofungin<br>Micafungin<br>Anidulafungin | Cell wall biosynthesis [inhibition of β(1,3)-glucan synthase] | Mutation in β(1,3)-glucan synthase, LOH for β(1,3)-glucan synthase (*C. albicans*) |

[a] LOH, loss of heterozygosity.

This has indicated the range of substrates for individual pumps (74, 169, 334) and has begun to identify the amino acid residues involved in substrate and inhibitor recognition (87, 90, 91, 306, 318, 362). There are several ways in which the clinical significance of efflux-mediated antifungal drug resistance can be mitigated. Alternative antifungal drugs, such as the echinocandins, for which efflux-mediated resistance is not an issue, may be used, but not in all cases. Future development of new therapeutic approaches targeting modulation by the efflux pump transcriptional regulators or the fungal stress response pathways may prevent resistance from developing. It may also be possible to overcome azole resistance by inhibiting efflux pumps directly, a scenario that parallels efforts to develop inhibitors of human P-glycoprotein (ABCB1) (P-gp) (188).

## EPIDEMIOLOGY OF FUNGAL INFECTIONS

Fungi commonly cause superficial infections of the skin and mucous membranes. When they penetrate the tissues of an immunocompromised host, however, they can cause IFIs, which are associated with much greater morbidity and mortality. The fungal species most often associated with fatal IFIs belong to the genera *Candida*, *Aspergillus*, and *Cryptococcus*. *Candida* species are the fourth most common cause of nosocomial bloodstream infections (157) and the leading cause of IFIs. In the United States, *Candida albicans* causes most candidemias, followed by *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and, in fifth place, *Candida krusei* (275). The excess treatment cost attributable to candidemia in the United States is between $1 and $2 billion per year (281, 382). IFIs are also a leading cause of infection-related mortality among patients with cancer and prolonged neutropenia and among allogeneic hematopoietic stem cell transplant (HSCT) (bone marrow transplant) recipients with graft-versus-host disease (321). The occurrence of IFIs in such patients is increasing as a result of changes in clinical procedures, for example, multiple transplantation, treatment of patients in higher age groups, and chemotherapy that affects the integrity of the gastrointestinal tract. The widespread use of fluconazole (FLC) prophylaxis in HSCT patients means that invasive aspergillosis (IA) and other filamentous fungal infections, rather than infections by *Candida* species, cause the majority of deaths from IFI (215, 321). In this patient population, the risk for invasive *Aspergillus* infection is declining but would range from 10% to 20% if no prophylaxis was employed. Fungal infections are also a serious complication for burn patients, for whom *Aspergillus* and *Candida* species are the most common cause of infections, with an attributable mortality of 33% (236).

Geography affects the fungal species detected in the clinic. *Cryptococcus gattii*, for example, is found predominantly in tropical and subtropical regions (27). A notable exception was a recent cryptococcosis outbreak caused by *C. gattii* on Vancouver Island in the Canadian Pacific Northwest (165). *C. glabrata* has emerged as an important opportunistic pathogen in the United States, whereas other non-*albicans Candida* species, such as *C. parapsilosis* and *C. tropicalis*, commonly cause infections in other countries, notably *C. parapsilosis* in Latin America (275).

The patient population and the clinical setting also influence the types of fungi detected (275). For example, pediatric patients are most likely to be infected with *C. albicans* or *C. parapsilosis* (262) and patients with leukemia with *C. albicans* or *C. tropicalis*, while HSCT patients are likely to be infected

TABLE 2. Substrates and inhibitors of clinically relevant efflux pumps

| Fungal species and pumps | Representative drug-like substrates | Inhibitors |
|---|---|---|
| *C. albicans* | | |
| CaCdr1p (ABC) | Azoles, R6G, cycloheximide (181, 237); rhodamine 123 (181); cerulenin, trifluoperazine, nigericin, tamoxifine, verapamil (237) | Milbemycins[a] (135, 181); enniatin (135, 181); FK506 (181); FK520 (109); unnarmicins (346) |
| CaCdr2p (ABC) | Azoles, R6G (109, 181); cycloheximide, cerulenin, (181); diamide (109) | Milbemycins[a] (135, 181) |
| CaMdr1p (MFS) | Benomyl, methotrexate (99); FLC (133, 181, 232, 243), VCZ (243) but not ITC or MCZ (181); cycloheximide (181); cerulenin (133, 181); brefeldin A (133); 4-nitroquinoline 1-oxide (133); diamide (232) | |
| *C. glabrata* | | |
| CgCdr1p (ABC) | Azoles (24, 146, 181, 372); R6G (24, 146, 181, 372); cycloheximide, chloramphenicol, 5-FC; terbinafine, cerulenin, cycloheximide, rhodamine 123, staurosporin (372) | FK506 (181, 372); oligomycin, verapamil (372); unnarmicins (346) |
| CgPdh1p (ABC) | Azoles (24, 146, 181, 372); R6G (24, 146, 181, 372); cycloheximide (24, 372); chloramphenicol (24, 372); 5-FC, terbinafine, cerulenin, cycloheximide, rhodamine 123, adriamycin (372) | Milbemycins[a] (181); FK506 (181, 372); unnarmicins (346) |
| *C. krusei* | | |
| CkAbc1p (ABC) | Azoles (158, 181, 182); albendazole (158); cycloheximide (158, 182); cerulenin, rhodamine 123 (182) | Milbemycins[a]; enniatin (181, 182); FK506, oligomycin (181) |
| CkAbc2p (ABC) | FLC (158) | None reported |
| *A. fumigatus*[b] | | |
| AfuMdr3p (MFS) | ITC[c] (240) | None reported |
| AfuMdr4p (ABC) | ITC[c] (240) | None reported |
| AtrFp (ABC) | ITC[c] (335) | None reported |
| *C. neoformans* | | |
| CneMdr1p (ABC) | Azoles, R6G (181) | None reported |
| CneAfr1p (ABC) | Azoles, R6G (284) | None reported |

[a] There are two structural classes of milbemycins and multiple members of each class. Each pump was inhibited by a unique range of milbemycins (see references 181 and 135 for more details of milbemycin specificities).
[b] There is little evidence that AfuMdr1p or AfuMdr2p is involved in clinically relevant drug resistance.
[c] May induce expression of the pump.

with naturally azole-resistant species such as *C. krusei*, *C. glabrata*, or *Candida lusitaniae* (275) which occurs mainly in AIDS patients or otherwise severely immunocompromised individuals. Moreover, previous exposure to, or the prophylactic use of, fungistatic azoles such as the well-tolerated drugs FLC and voriconazole (VCZ) is associated with an increasing frequency of novel drug-resistant clinical isolates, including non-*albicans Candida* species, non-*fumigatus Aspergillus* species, zygomycetes, and hyaline molds (172, 275). Clinically significant azole resistance is mainly due either to acquired resistance in commensal opportunistic fungal pathogens or to the selection of strains of species that show innate resistance.

## Candidiasis

*C. albicans* can cause serious infections of the oral mucosa, as well as disseminated infection in debilitated patients. Severe oropharyngeal candidiasis (OPC) afflicts many AIDS patients (15) and is a significant infection in cancer patients being treated with chemotherapy and/or radiotherapy (66, 327). OPC is frequently the first clinical symptom recognized in human immunodeficiency virus-positive patients prior to the onset of overt AIDS (327). In cancer patients, the increased incidence of OPC results both from the debilitating effects of the cancer itself and from the immunosuppressive treatment for the cancer. Administration of broad-spectrum antibiotics for the management of bacterial infections in these patients may further predispose them to OPC (338). Radiotherapy for oral cancer results in permanent damage to the salivary glands and frequently to lifelong xerostomia (115), another predisposing factor for OPC.

In the United States, *C. glabrata* is the most prevalent yeast pathogen after *C. albicans*, and it causes both superficial (oral, esophageal, vaginal, or urinary) infections and IFIs in humans. Systemic *Candida* infections caused by non-*albicans Candida* species have increased in the past 2 decades; *C. glabrata* and *C. parapsilosis* currently rank as the second and third most frequently isolated species from reported cases of candidiasis (53, 96, 123, 274). Bloodstream infection caused by *C. glabrata* accounts for approximately 16% of *Candida* fungemia worldwide (277) and occurs predominantly in patients with solid tumors and lymphoma (371) or in HSCT recipients (383, 384). *C. krusei* is the fifth most common cause of candidiasis (278) and accounts for about 2% of all clinical *Candida* isolates. *C. krusei* infections are more prevalent in the elderly (276).

## Aspergillosis

*Aspergillus* species are widely distributed in the environment and are often found in association with rotting vegetation. They are also opportunistic pathogens of humans that can cause primary invasive lung infections and disseminate to other organs. Their spores can be present in high concentrations in

the atmosphere, and *Aspergillus* species grow rapidly at elevated temperatures (>40°C). These attributes, together with the weak defenses of the immunocompromised host, are considered the main reasons for their pathogenicity, rather than specific virulence traits (348). IA is the most common form of invasive mold infection, accounting for 60% to 80% of all reported cases (172). A systematic review of the literature has identified the most common underlying disease or predisposing factor among patients with IA as leukemia (43%), followed by HSCT (26%), lung disease (20%), organ transplantation (13%), and human immunodeficiency virus/AIDS (4%) (191). The leading cause of IA is *Aspergillus fumigatus* (85%), followed by *Aspergillus flavus* (5 to 10%) and *Aspergillus terreus* (2 to 10%) (47, 120, 170, 281, 332). *Aspergillus niger* (2 to 3%), *Aspergillus nidulans*, and *Aspergillus ustus* are only rarely isolated. IA affects a narrower range of patients than invasive candidiasis, and one of the major risk factors is severe neutropenia (281, 325). Although the crude mortality rates of both IA and invasive candidiasis remained constant over the period 1997 to 2003 (275), the rates are high, with IA mortality exceeding 50% in most reports, and there are higher values in HSCT patients than in solid-organ transplant recipients (170, 191, 325, 332). The efficacies of current antifungal therapies for IA have improved with the introduction of newer antifungals, such as VCZ (343), or combinations of antifungals (47, 234, 331) and immunomodulatory strategies (332, 343), but they are still suboptimal. VCZ is superior to amphotericin B (AMB) for the treatment of IA and has become the primary treatment choice (129).

### Cryptococcosis

Cryptococcosis is an IFI caused predominantly by *Cryptococcus neoformans* and *C. gattii*. Cryptococcal meningitis is the most common fungal disease of the central nervous system (80, 192, 285). Excellent reviews of cryptococcal biology, life cycle, and virulence attributes and the different manifestations of cryptococcosis are available elsewhere (145, 192). *C. gattii* is rarely isolated from immunocompromised hosts but instead causes most (about 80%) cases of cryptococcal infection in the general population in tropical and subtropical regions, such as Australia, Papua New Guinea, and parts of Africa (46).

*C. neoformans* can be classified into three serotypes based on capsular agglutination: A (*C. neoformans* var. *grubii*), D (*C. neoformans* var. *neoformans*), and AD hybrids (145, 192, 193). *C. neoformans* var. *grubii* (serotype A) is the most pathogenic form, causing the vast majority of crytpococcal infections worldwide (95%) (46). In Europe, about 5 to 30% of cryptococcal infections are caused by AD hybrids, and this is likely an underestimate due to limitations in the serotyping technique (54, 193). *C. neoformans* is a heterothallic yeast with two mating types, *MAT***a** and *MAT*α (178, 179, 192). While most (>95%) *C. neoformans* isolates of serotypes A and D are haploid and of the α mating type, some have been determined to be diploid (54, 193). In contrast, most serotype AD strains are diploid. Alpha mating-type isolates are also more virulent (178, 179). *C. gattii* (serotype B and C) strains from diverse sources are predominantly sterile in the laboratory, and no evidence of recombination between isolates has been observed (124). Notable exceptions are the *C. gattii* isolates from a recent Vancouver outbreak, which are clonal, mainly of the α mating type, and able to mate robustly (101, 102, 165).

Although the number of cryptococcal infections in AIDS patients has remained low or even decreased in most developed countries since the introduction of highly active antiretroviral therapy (HAART) in the mid 1990s (7, 36, 139, 208, 209, 222, 279), the disease remains responsible for up to 30% of the attributable mortality in AIDS patients in regions such as Southeast Asia, Africa, and Spain (28, 126, 138, 177, 271, 317). Cryptococcosis is also a significant opportunistic infection in solid-organ transplant recipients, with a prevalence rate ranging from 0.26% to 5% and overall mortality of 42% (330). In the most severe cases of cryptococcal infections of the central nervous system, combination therapy for 2 weeks with AMB and flucytosine (5-FC) is the gold standard, followed by treatment with FLC for a minimum of 10 weeks, which should be continued for life (305). Cryptococcal infections are considered incurable because the fungal cells can remain dormant for many years and relapse occurs when the host becomes immunocompromised (192).

## DIAGNOSIS OF FUNGAL INFECTIONS

Some of the main factors contributing to the high mortality rates associated with IFIs are problems with slow diagnosis and choosing the appropriate treatment. The early symptoms of IFI are nonspecific, and the most widely used detection methods make timely diagnosis difficult. In addition, species-specific variations in antifungal susceptibilities make selection of an effective therapy problematic. Conventional fungal detection methods include direct microscopy of clinical specimens and culture-based, and non-culture-based, techniques (297). Visual examination of fungi in tissue samples allows presumptive identification based on cellular morphology and staining properties. However such identification requires a skilled mycologist, and identification can often be equivocal. Culture-based techniques can employ chromogenic primary isolation media, such as Chromagar, for the presumptive identification of the most prominent pathogenic *Candida* species, including *C. albicans*, *Candida dubliniensis*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and, in some instances, *C. parapsilosis* (249, 344). Other culture-based growth, morphology, and biochemical tests are available in kit format for the identification of fungi isolated from clinical samples. These kits include API ID 32C, API 20C AUX, and RapID Yeast Plus (297). Although the kits are relatively easy to use, the results often show poor discrimination between possible species, and the process involves two culturing steps that can take 36 to 48 h. In addition, *Aspergillus* cannot be cultured from a significant proportion of sputum (66 to 92%) or bronchoalveolar lavage (38 to 55%) samples from patients with IA (265).

Non-culture-based identification techniques include immunological detection of antigens and molecular detection of DNA or RNA. The galactomannan from the cell wall of *Aspergillus* species can be detected with a double-sandwich enzyme-linked immunosorbent assay (332). In HSCT patients, this method has been shown to diagnose IA with a sensitivity of 67 to 100% and a specificity of 86 to 99% (332). The cryptococcal capsular polysaccharide can be detected in patients with cryptococcal meningitis by latex agglutination with a sensitivity

of 90% and a specificity of 95% (377). Immunological methods that detect *Candida* β(1,3)-glucan, mannan, or the Cand-Tec test antigen are of limited value because the levels of circulating antigens are low and the transient nature of the antigenemia requires sensitive assays and frequent sampling of at-risk patients (283).

The prospect of highly specific, highly sensitive, and rapid fungal detection and identification is offered by a range of molecular methods that are currently being tested in the laboratory. These methods include quantitative real-time PCR (166), fluorescence in situ hybridization (256; reviewed in reference 356), and multilocus sequence typing (MLST). Quantitative real-time PCR can be used to measure the amount of fungal DNA present, and the use of primers, or molecular beacons, specific for mutations that confer antifungal resistance can rapidly detect resistant fungal isolates (106). MLST, which uses PCR and DNA sequence analysis to detect nucleotide polymorphisms within several housekeeping genes, provides a rigorous molecular method for species and strain identification. The typing of strains can identify relationships between isolates that can help trace sources of infection and their transmission and microevolution and indicate if a drug-resistant infection is likely to occur (147, 252, 253). Thus, molecular detection methods, combined with short-term culture of clinical samples, has the potential not only to accurately and rapidly identify fungal pathogens, but also to indicate whether the pathogen is likely to respond to conventional antifungal treatment.

## ANTIFUNGAL AGENTS

In order to evaluate the impact of efflux-mediated antifungal drug resistance, it is necessary to be aware of the range of antifungal agents currently available and the mechanisms of antifungal drug resistance. These topics are reviewed elsewhere (4, 61, 122, 171, 196, 251, 307) and can be summarized as follows. There are five main antifungal drug classes (Table 1). The fluorinated pyrimidine analog 5-FC causes aberrant RNA synthesis and interferes with DNA replication (4, 307). The polyenes, such as nystatin and AMB, are heterocyclic amphipathic molecules that insert into lipid bilayers, bind to ergosterol, and aggregate in annuli to form pores. These pores disrupt plasma membrane integrity and permit the efflux of cations, such as $K^+$, which results in cell death. Polyenes are also thought to cause oxidative damage (171, 196, 307). The allylamines terbinafine and naftifine inhibit squalene epoxidase (encoded by *ERG1*), which catalyzes the first step in the biosynthesis of ergosterol from squalene (Table 1). Although they have the greatest potency against dermatophytes, they are fungistatic for the majority of *Candida* species (171, 251, 307). The azole antifungals, such as FLC, also interfere with sterol biosynthesis (Table 1). They inhibit the cytochrome P450 14α-lanosterol demethylase, encoded by the *ERG11* gene (also known as *CYP51*), which is the rate-limiting step of the ergosterol biosynthetic pathway. Inhibition of Erg11p depletes the membranes of ergosterol and results in the increase of toxic sterol pathway intermediates, which inhibit growth (4, 307). Azoles are thus usually fungistatic for *C. albicans*. The first azole drugs developed were the imidazoles such as miconazole (MCZ) and ketoconazole (KTC) (326). These drugs have problems with solubility. The triazole FLC has increased water solubility and improved pharmacokinetic properties but is ineffective against *A. fumigatus*. Itraconazole (ITC) and VCZ are more effective and also fungicidal against *Aspergillus*, and the newer triazoles, such as posaconazole (POS), ravuconazole, and albaconazole, appear to be effective against *Aspergillus* species, *Cryptococcus* species, and other fungi, such as *Malassezia* species (263, 325). The most recently developed class of antifungals is the cyclic lipopeptides, the echinocandins. They were originally obtained from soil fungi in the 1970s, and semisynthetic derivatives have been developed, such as caspofungin, micafungin, and anidulafungin. These drugs interfere with wall biosynthesis by inhibiting β(1,3)-glucan synthase (Table 1). The echinocandins are fungicidal for *C. albicans* but are not active against *Cryptococcus* species and are fungistatic for *Aspergillus* and other filamentous fungi (154, 251, 307). Despite weak fungistatic activity against *A. fumigatus*, caspofungin has been approved as salvage therapy for patients with IA (202).

## ANTIFUNGAL DRUG RESISTANCE

The extents of antifungal drug resistance vary for the different drug classes (Table 1). There is fairly limited resistance to the polyenes, allylamines, and echinocandins, whereas resistance to 5-FC, imidazoles, and triazoles is more common. The rare occurrence of resistance to polyenes can be caused by a reduction in the amount of plasma membrane ergosterol, to which polyenes bind. There is primary resistance in some isolates of *C. lusitaniae*, *Candida lipolytica*, and *Candida guilliermondii* (196). *A. terreus* and *A. flavus* are frequently associated with AMB resistance in both in vitro and in vivo studies (47, 118, 340, 341, 374). Although the molecular mechanisms are not well understood, it is clear that *A. terreus* has a much lower ergosterol content than most other fungal species (47, 374), and alterations in cell wall glucans have been shown to lead to AMB resistance in *A. flavus* (196). Mutations in *C. albicans ERG3*, which encodes a C-5 sterol desaturase, an enzyme in the ergosterol biosynthetic pathway, lower the concentration of ergosterol in the membrane and cause AMB resistance (161). These mutations also confer cross-resistance to azoles (161, 307). There is little evidence of fungal resistance to allylamines. This may reflect the fact that these drugs target an early step in ergosterol biosynthesis, precluding compensatory mutations elsewhere in the pathway conferring resistance. Echinocandin resistance also appears to be rare (154). This may be due to their limited use to date, because resistance events are inherently uncommon, or because mutations in multiple genes are required to obtain clinically significant resistance. Echinocandin-resistant *Candida* isolates have point mutations in the β(1,3)-glucan synthase subunit that is orthologous to *S. cerevisiae* Fks1p (17, 272).

There is significant intrinsic and acquired resistance of *Aspergillus* and *Candida* species to 5-FC, limiting its utility. Resistance of clinical *C. albicans* isolates to 5-FC most often correlates with mutations in the enzyme uracil phosphoribosyltransferase (Fur1p) that prevent the conversion of 5-fluorouracil to 5-fluorouridine monophosphate (4). *C. albicans* strains can be grouped into clades according to their genetic relatedness based on DNA fingerprinting and MLST analysis (250, 337). Clade I clinical isolates (equivalent to the general-purpose genotype described by Schmid et al. [319]) are the

most prevalent group of strains isolated from patients in all geographic regions studied (250, 288, 319). Interestingly, 5-FC resistance caused by mutations in *C. albicans* Fur1p (CaFur1p) are restricted to clade I isolates (81, 250, 288). Mutations in cytosine deaminase (CaFca1p) may also contribute to resistance (154). The incidence of 5-FC resistance in fungi has led to its use primarily in combination with other antifungals, such as AMB, which has become the gold standard for the treatment of cryptococcosis (305).

There are multiple mechanisms that can give rise to azole resistance in fungi (Table 1), and different combinations of these mechanisms operate in different fungi. The drug target, Erg11p, can be overexpressed or can develop point mutations that reduce FLC binding (4, 61, 307, 380). Common mutations in CaErg11p that confer moderate azole resistance are Y132H, S405F, G464S, and R467K (214, 269, 310). Azole-induced *C. albicans* growth inhibition is caused by reduction in the ergosterol content of membranes and also by the accumulation of toxic ergosterol precursors, such as 14α-methylergosta-8,24(28)-dien-3β,6α-diol. If Erg3p is inactivated by mutation, in the presence of FLC, these cells accumulate the nontoxic sterol 14α-methylfecosterol. High-level azole resistance correlates with overexpression in the plasma membrane of proteins that pump the drug out of the cell, thus reducing intracellular azole concentrations to levels at which Erg11p is not inhibited (269, 380). Transcriptional analysis has demonstrated that some azole-resistant *C. albicans* strains express multiple pumps in vitro (300, 378). These pumps may have an additive effect on drug efflux, but the level of pump expression in vivo and the levels of expression required to achieve clinically significant resistance have not been determined.

While it could be expected that the expression of efflux pumps might confer a fitness cost in terms of protein expression or energy utilization, there is some evidence that this may not be the case. *C. albicans* cells grown in the presence of FLC developed resistance by a number of mechanisms, including drug efflux, and any significant cost of resistance in terms of fitness was eliminated with further evolution in the presence of FLC (59). In *C. glabrata*, strains with hyperactive *C. glabrata PDR1* (Cg*PDR1*) alleles that upregulate Cg*CDR1*, Cg*PDH1*, and Cg*SNQ2* expression were more virulent in mice than strains with wild-type alleles and gained fitness in the animal model (93).

Some fungi form biofilms on host tissues or prostheses. *C. albicans*, for example, establishes biofilms on catheters and voice prostheses, and it is well known that such biofilms are resistant to azole antifungals (235, 293). Some studies have shown upregulation of *C. albicans* efflux pump genes and gene products during biofilm formation (216, 233), and others have not (107). In addition, *C. albicans* strains with the efflux pump genes *CDR1*, *CDR2*, and *MDR1* deleted still formed azole-resistant biofilms (273, 293). Therefore, the azole resistance of biofilms is complex and multifactorial and is unlikely to be solely dependent on efflux pump expression.

## CLASSES OF EFFLUX PUMPS

There are two main classes of efflux pumps, ABC proteins and MFS pumps. These membrane proteins actively translocate compounds across cell membranes using different energy sources. The ABC proteins are primary transporters that use the hydrolysis of ATP. The MFS pumps are secondary transporters that utilize the proton-motive force across the plasma membrane. Both types of transporter contain distinctive protein domains: nucleotide binding domains (NBDs) in ABC pumps and transmembrane domains (TMDs) in both ABC and MFS pumps that confer substrate specificity (Fig. 1).

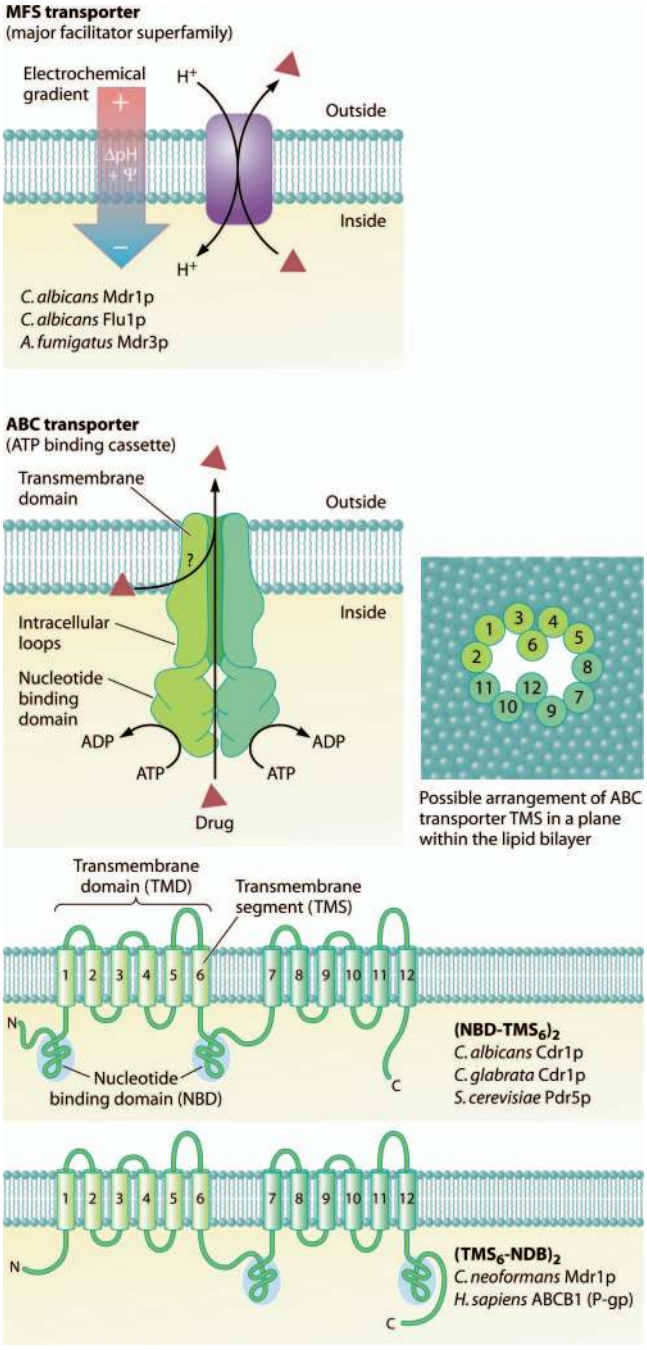


FIG. 1. Domain arrangements of ABC and MFS transporters. The schematic representation of the ABC TMS in the plane of the membrane is based on the crystal structure of Sav1866 (67). *H. sapiens*, *Homo sapiens*.

### MFS Transporters

MFS transporters, like ABC transporters, comprise large superfamilies of proteins with high sequence similarity found in plants, animals, bacteria, and fungi. There are two subfamilies of MFS transporters involved in drug efflux that are defined by the number of transmembrane spans (TMS) within the TMD: DHA1 (drug:$H^+$ antiporter 1; 12 TMS) and DHA2 (14 TMS) (110, 267). The first MFS transporter gene to be characterized from a pathogenic fungus was Ca*MDR1* (also named *BEN*$^r$). This gene was cloned by its ability to confer benomyl and methotrexate resistance on *S. cerevisiae* (99). Expression of Ca*MDR1* has been detected in both in vitro-derived FLC-resistant mutants (6) and azole-resistant clinical isolates (269, 378). CaMdr1p is a DHA1 MFS transporter, and heterologous overexpression in *S. cerevisiae* conferred resistance to FLC and KTC, but not to MCZ or ITC (181, 264). Experimental overexpression of CaMdr1p in *C. albicans* conferred resistance to cerulenin and brefeldin A, but high levels of expression were required to confer FLC resistance (133). Structural and functional analyses of CaMdr1p have indicated that amino acid residues located in TMS5 are critical for drug/$H^+$ transport (264). Another DHA1 MFS gene from *C. albicans* is *FLU1* (42). Disruption of *FLU1* in *C. albicans* had little effect on FLC susceptibility but made cells sensitive to mycophenolic acid, suggesting that it might be a pump substrate. There is no evidence of *FLU1* expression being associated with azole resistance in clinical isolates. Thus, despite the involvement of CaMdr1p in the azole resistance of certain clinical *C. albicans* isolates and a strong association between expression of the *C. dubliniensis* MFS transporter Mdr1p and FLC resistance (345), there is, in general, a much stronger association between azole resistance and the expression of ABC pumps (see below).

### ABC Transporters

ABC transporters are found in all cells of all organisms, often in the plasma membrane, but also in the membranes of organelles. Their function is to transport substances across the membrane. Some ABC proteins transport a specific ligand, while others, notably mammalian P-gp, which is responsible for the resistance of cancer cells to chemotherapeutic agents, have evolved broad specificity for hydrophobic compounds, including drugs, which is usually referred to as multidrug resistance (MDR) (132). The basic structure of ABC transporters consists of two cytoplasmic NBDs and two TMDs (108, 130, 260) (Fig. 1). The NBDs are involved in ATP binding and hydrolysis, and the TMDs span the membrane, usually six times, via putative α-helices. The arrangement of the NBDs and TMDs within the pump polypeptide varies according to the type of ABC protein (Fig. 1). ABC proteins in *S. cerevisiae* have been classified into three main subfamilies, the pleiotropic drug resistance (PDR), MDR, and multidrug resistance-associated protein (MRP) (cf. human CFTR) subfamilies (23, 71, 334). The domain arrangement in most MDR and MRP ABC proteins is, from the $NH_2$ terminus, $(TMD\text{-}NBD)_2$, and for most PDR pumps the arrangement is reversed, $(NBD\text{-}TMD)_2$ (71). An example of a PDR ABC protein from a pathogenic fungus is *C. albicans* Cdr1p $(NBD\text{-}TMS_6)_2$ (260), and *C. neoformans* Mdr1p $(TMS_6\text{-}NBD)_2$ is an MDR protein (Fig. 1). In these four-domain ABC transporters, there is often a high level of homology between the amino-terminal and carboxy-terminal halves of the protein, suggesting gene duplication and fusion. Indeed, in many organisms, there are "half-size" transporters consisting of one TMD and one NBD (260), although biochemical and crystallographic evidence indicates that they probably function as dimers (132). In several *S. cerevisiae* ABC transporters, for example, the MRP Ycf1p, there is an N-terminal extension containing an extra TMD that precedes the other four domains, giving the following arrangement: $TMS_5(TMS_6\text{-}NBD)_2$. In contrast to the significant differences in the primary sequences of TMDs, each NBD contains conserved amino acid sequences for ATP binding, such as the Walker A and Walker B motifs and the ABC signature sequence (287).

The subfamily of fungal ABC proteins most often associated with antifungal drug resistance is the PDR group of transporters, with the archetype being *S. cerevisiae* Pdr5p (20, 31, 134). These fungal PDR proteins appear to share common features on both sides of the two TMDs that separate the cytosolic from the extracytosolic space (Fig. 1). The cytosolic side consists of a large N-terminal domain including NBD1, followed by two small intracellular loops (IL-1 and IL-2), a second large domain including NBD2, and another two small loops (IL-3 and IL-4). On the extracytosolic side they all appear to have four small (EL1, EL2, EL4, and EL5) and two large (EL3, between TMS5/6, and EL6, between TMS11/12) extracellular loops (Fig. 1). While the amino acid sequences for most of the cytosolic portions appear to be highly conserved, the diversity of individual members of the PDR family resides mainly in the TMDs and the ELs. This probably reflects the fact that the cytosolic part is the motor that drives the transport of a variety of substrates across the lipid bilayer through the core of the protein (formed by the 12 TMS) into the extracytosolic space or the outer layer of the lipid bilayer. Sequence comparison of PDR proteins revealed another unique feature called the PDR-CDR signature motif. This motif spans the EL3, TMS6, and the cytosolic linker region preceding NBD2. So far, the biological significance of the motif has remained obscure, but its presence only in this subfamily suggests either a topological constraint or a role in drug efflux. Another unique feature of PDR transporters is that while most fungal, as well as human, MDR or MRP transporters contain two symmetrical NBDs (conserved Walker A, Walker B, and ABC signature motifs in both NBDs), most PDR ABC transporters display asymmetrical NBDs (20, 90, 109, 373). That is, their N-terminal NBD1 contains a highly conserved ABC signature motif flanked by degenerate Walker A and B motifs while their C-terminal NBD2 consists of two highly conserved Walker A and B motifs and a degenerate ABC signature motif (20, 90, 109). Many biochemical studies have investigated the contributions of the highly conserved NBDs, the ILs, and the TMDs to function, but little or no attention has been paid to the significance of the ELs. We have performed extensive molecular mapping of *S. cerevisiae* Pdr5p (ScPdr5p) and CaCdr1p and found that their ELs appear to be very important for the interaction of these efflux pumps with small-molecule inhibitors, such as D-peptide derivatives, FK506, and the milbemycins (M. Niimi, unpublished results). To map suppressor mutations that attenuated the inhibition of ScPdr5p and/or CaCdr1p, we employed our specially modified host *S. cerevisiae* ADΔ (181) (derived from

AD1-8 [see below]), which is exquisitely sensitive to a large array of xenobiotics. Overexpression of these efflux pumps in ADΔ led to highly FLC-resistant strains. We used these strains to identify broad-spectrum, as well as very specific, efflux pump inhibitors (181) (Table 2). Screening these strains for suppressor mutants that were no longer susceptible to the inhibitors allowed us to identify an array of point mutations in both ScPdr5p and CaCdr1p that were almost exclusively located in the TMDs and the ELs of these pumps (B. C. Monk, unpublished results).

There is much interest in how these proteins bind and transport substrates and in what their "normal" substrates are. Surprisingly, there is no evidence to indicate that any of the Pdrp proteins are essential. Even when as many as six *S. cerevisiae* PDR genes have been deleted, the cells grow normally, apart from a hypersensitivity to certain ions and xenobiotics, including the azole drugs (72). There are indications that particular ABC proteins, including CaCdr1p, CaCdr2p, and CaCdr3p (336), are involved in phospholipid transport across the lipid bilayer that may help maintain an asymmetry in the compositions of the two leaflets (131). Thus, the overexpression of these ABC proteins may indirectly cause antifungal resistance through the effects of membrane composition on membrane function and/or membrane protein activity.

An understanding of the role of fungal ABC transporters in drug resistance is hampered by the lack of high-resolution crystal structures for these proteins. Recently, however, a structure has been generated for the half-size ABC transporter Sav1866 of *Staphylococcus aureus* (67), which forms a homodimer in the membrane. It is thought that the two TMDs in the homodimer provide inward-facing sites that bind drugs from the lipid bilayer, or possibly the cytoplasm. The NBDs are then able to form interfacial contacts mediated by the binding of two ATP molecules to conserved features on both NBDs (including the Walker A and B motifs on one NBD and the signature motif on the other NBD). This induces the TMDs to undergo conformational change and results in a cavity that is open extracellularly and closed intracellularly. The bound drug is thus able to access the extracellular space and is effluxed from the cell. The structure of the transporter after the efflux of the drug will be similar to that observed when Sav1866 binds a nonhydrolyzable ATP analog. In the normal reaction cycle, hydrolysis of ATP then allows the transporter to reset to its drug-binding conformation (68). The schematic representation of the TMS in the ABC protein in Fig. 1 is based on the Sav1866 crystal structure but does not take into account the finding that isolated ScPdr5p may occur as dimers (95). Although biochemical analysis of the full-size ABC transporter P-gp suggests a slightly different arrangement of the TMS (198), the interactions between the two TMDs is also proposed to occur between TMS2 and TMS11 and between TMS5 and TMS8. Despite the advent of structural information on ABC transporters, much remains to be understood about individual pumps and the functions of their respective families. Many transporters extract their substrates from the inner leaflet of the bilayer (132), but it is not known whether this holds true for fungal pumps. One way to systematically study the structure and function of efflux pumps involved in drug resistance, using information that is already available, is to determine phylogenetic relationships among the PDR family of efflux pumps.

## Phylogeny of PDR Efflux Pumps

The best-studied families of fungal efflux pumps are those from *S. cerevisiae* (23, 71, 89), in part because it was the first eukaryote to have its genome sequenced (114). The group of *S. cerevisiae* ABC transporters most closely associated with drug resistance is the PDR subfamily. There are 28 ABC transporter genes in *S. cerevisiae*, and 9 of these encode PDR transporters. There are also large clusters of PDR genes in closely related fungal pathogens (Fig. 2). We have investigated the relationships between these PDR transporters using classical phylogenetic analysis involving data mining of sequences using the BLAST method with *S. cerevisiae* PDR genes as queries.

We identified a total of 123 PDR transporters in 14 fungal species for which nearly complete genome sequences were available (see Table S1 in the supplemental material). Nine of the representative species are prominent human pathogens (Table 3). The analyzed proteins are all full-size ABC transporters (1,241 to 1,564 amino acid residues) that are predicted to have the typical PDR topology: $(\text{NBD-TMD})_2$ (Fig. 1). The topology of the *A. terreus* transporter 063.1 is an exception because it has two additional TMDs (each containing six TMS) at its C terminus. All the Pdrp proteins analyzed (with the possible exception of *A. nidulans* 952.3, which requires further examination due to sequence uncertainties) (see Table S1 in the supplemental material) contain variants of the Walker A1 motif, LGXPG(S/A)G(C/K)STL (71). They are also predicted to include the two extended ECLs, ECL3 and ECL6, of at least 50 amino acid residues that connect TMS5/6 and TMS11/12, as previously detected in the founding member, ScPdr5p, which was identified simultaneously by several research groups, attesting to its broad substrate specificity (20, 31, 134, 173).

A phylogenetic tree was constructed from the 45 Pdr proteins identified in *S. cerevisiae* and five representative human pathogens: *C. albicans*, *C. lusitaniae*, *Coccidioides immitis*, *A. fumigatus*, and *C. neoformans* (Fig. 3). A total of eight Pdrp phylogenetic clusters, some of which had not been described before, were identified and named A to H. All 123 Pdrp proteins could be classified according to the clusters illustrated in Fig. 3. The number of proteins contributed to different clusters by each fungal species varied considerably, with clusters A, B, and H containing the largest numbers of members (Table 3). Cluster A contains the well-known *S. cerevisiae* members Pdr5p, Pdr10p, and Pdr15p and their closely related Saccharomycetes orthologs, including CaCdr1p and CaCdr2p. Cluster B shares a common ancestry with cluster A and contains only members from the Eurotiomycetes (e.g., *A. fumigatus* and *C. immitis*) and Basidiomycota (e.g., *C. neoformans*). They can therefore be considered to have emerged from "precursors" of the *Saccharomyces* and *Candida* Pdr5-like transporters. Cluster C contains only Eurotiomycetes members, none of which has been characterized. Cluster D includes the well-known *S. cerevisiae* member Snq2p, which shares many substrates and transcriptional regulators with ScPdr5p (168). The weak organic acid transporter ScPdr12p (282) shares an ancestor with ScSnq2p. Other Saccharomycetes orthologs belong to cluster D, but there are no Eurotiomycetes or Basidiomycota members of this cluster.

Cluster E members have some particular properties. The cluster includes the sterol importers ScAus1p and ScPdr11p

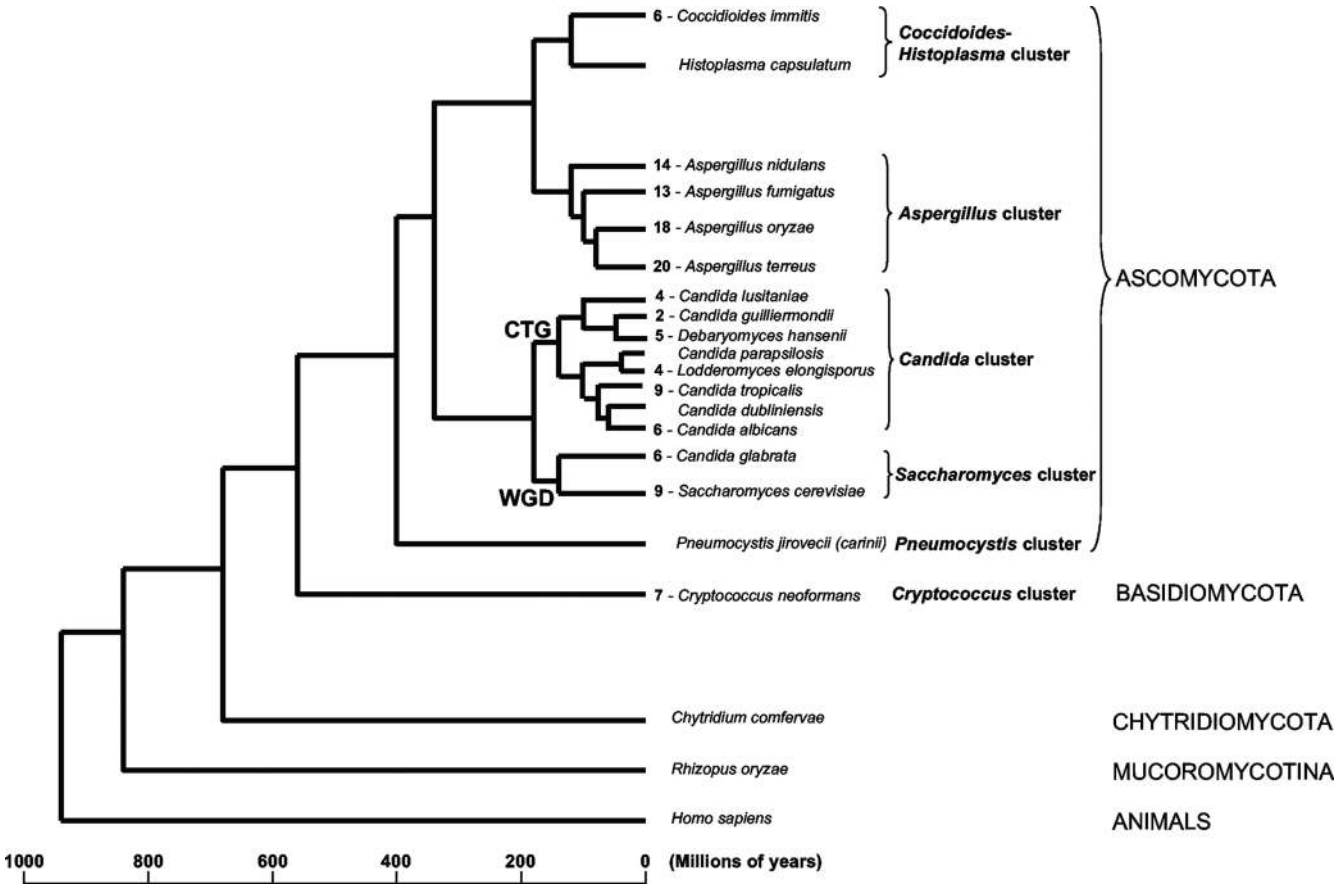


FIG. 2. Phylogeny of fungi. The numbers indicate the number of PDR ABC genes identified in each species. CTG indicates the reassignment of the CTG codon as serine in the majority of *Candida* spp. rather than encoding leucine as in other organisms. WGD indicates genomes that have undergone a whole-genome duplication (98). The tree is based on the work of Fitzpatrick et al. (98).

(190) and their ortholog CgAus1p in the closely related species *C. glabrata* (239). Both of these organisms are able to grow anaerobically when supplemented with unsaturated fatty acids and sterols. Interestingly, the GSGK/C residues of the Walker A1 motif are deleted in these three proteins. As the Walker A1 motif is involved in ATP binding, the deletion may have significant structural and mechanistic consequences. The Saccharomycetes are not represented in cluster H, which comprises members from Eurotiomycetes and Basidiomycota species. The large size of the cluster reflects the considerable expansion of Pdrp paralogs in the Eurotiomycetes. The basidiomycete *C. neoformans* contributes two Pdrp members that form cluster G, distant from the other Basidiomycota members of cluster H.

TABLE 3. Phylogenetic clusters of fungal Pdrps

| Fungal species[a] | Total no. of Pdrps | No. of Pdrps in phylogenetic cluster[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H |
| ***A. fumigatus*** | 13 | | 4 | 2 | | | 1 | | 6 |
| *A. nidulans* | 14 | | 5 | 2 | | | | | 7 |
| *A. oryzae* | 18 | | 4 | 3 | | | 1 | 1 | 9 |
| ***A. terreus*** | 20 | | 8 | 2 | | | 1 | | 9 |
| ***C. albicans*** | 6 | 4 | | | 1 | | 1 | | |
| ***C. glabrata*** | 6 | 2 | | | 2 | 1 | 1 | | |
| ***C. guilliermondii*** | 2 | 1 | | | | | 1 | | |
| ***C. lusitaniae*** | 4 | 3 | | | | | 1 | | |
| ***C. tropicalis*** | 9 | 6 | | | 2 | | 1 | | |
| ***C. immitis*** | 6 | | 2 | 1 | | | 1 | | 2 |
| ***C. neoformans*** | 7 | | 2 | | | | 1 | 2 | 2 |
| *Debaryomyces hansenii*; *Candida famata* | 5 | 4 | | | 1 | | | | |
| *Lodderomyces elongisporus* | 4 | 3 | | | 1 | | | | |
| *S. cerevisiae* | 9 | 3 | | | 3 | 2 | 1 | | |
| Total | 123 | 26 | 25 | 10 | 10 | 3 | 11 | 3 | 35 |

[a] Fungi pathogenic for humans are in boldface.
[b] See Fig. 3.

Of the eight discrete clusters identified by phylogenetic analysis of fungal pathogens, only cluster F contains representatives of all the Saccharomycetes, Eurotiomycetes, and Basidiomycota species tested. Although the function of its *S. cerevisiae* member Yol075p (SACE_L075C) (see Table S1 in the supplemental material) is unknown, all of its paralogs show the classical GSGK Walker A1 motif found in most ABC transporters instead of the K-to-C substitution (e.g., GSGC instead of GCGK) that characterizes the Pdrp members of the A, B, C, D, G, and H clusters (20, 71). We therefore consider cluster F to be the fungal Pdrp family ancestor. On the other hand, deletion of the GSGK core in Walker A1 and an established role in sterol import suggests that cluster E members are unlikely to be drug efflux pumps. Thus, only clusters A, B, C, D, G, and H can be considered in a strict sense Pdrp molecules.

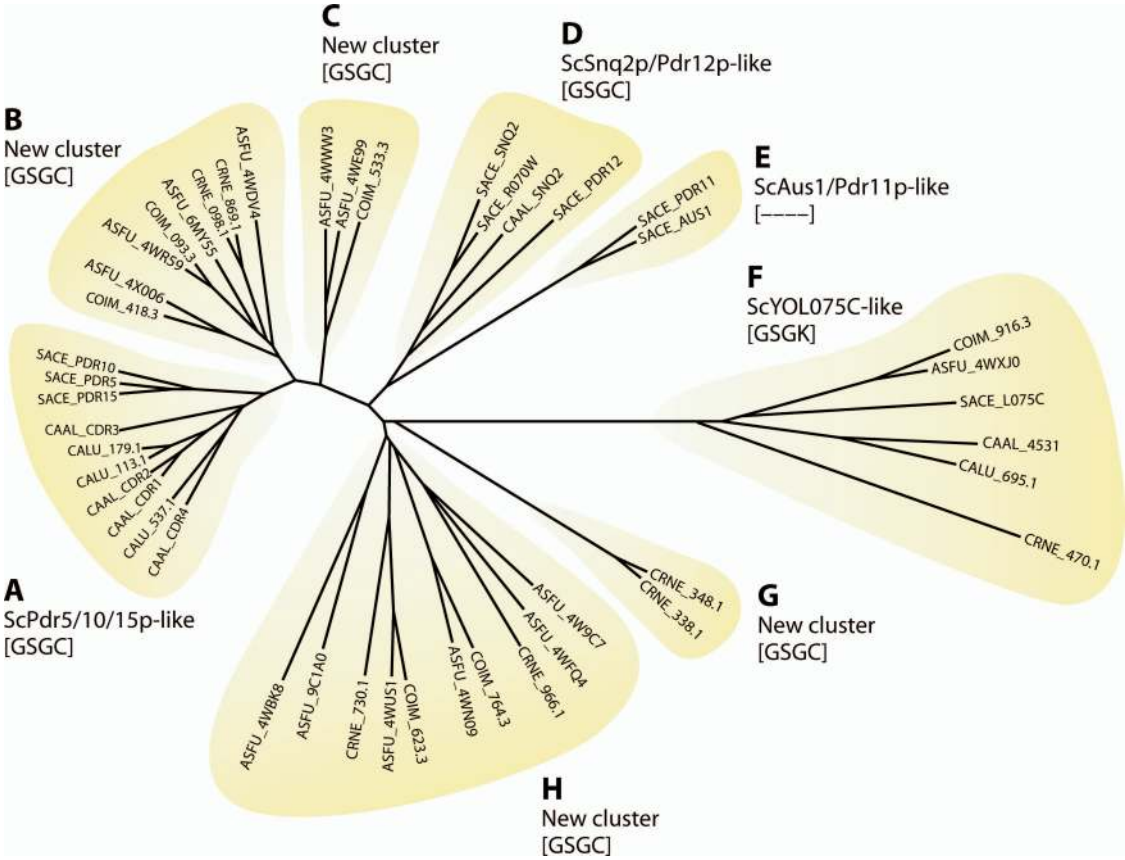


FIG. 3. Phylogenetic tree for 45 Pdr proteins from *S. cerevisiae* (SACE), *C. albicans* (CAAL), *C. lusitaniae* (CALU), *C. immitis* (COIM), *A. fumigatus* (ASFU), and *C. neoformans* (CRNE). The amino acid sequences of the Pdrps were aligned using MUSCLE (86). The Phylip suite of programs (92) was used to calculate distances between amino acid sequences (PROTDIST) and to draw trees by a neighbor-joining method (144). The proteins could be differentiated into eight clusters (A to H). The amino acids in the GSGK/C core of the Walker A1 motif, which are common to all members of each cluster except E, are shown in square brackets. New clusters are those in which there are no members from *Saccharomyces* or *Candida* species, and therefore, they were not identified until now. Details for individual Pdrps are given in Table S1 in the supplemental material.

The overall phylogenetic pattern is consistent with the occurrence of multiple independent expansions of PDR genes that have assisted the successful environmental adaptation of individual fungal pathogens. It remains now to assess systematically the responses of cluster families to xenobiotics, including antifungals, and to determine how individual clusters contribute to the transport of endogenous substrates.

## TRANSCRIPTIONAL CONTROL OF EFFLUX PUMPS

The ABC and MFS transporter families existed in ancestral fungal lineages that preceded a genome duplication event that occurred in the *S. cerevisiae* branch about 100 million years ago (Fig. 2) (110). In addition, elements determining transcriptional control of the PDR ABC subfamily found in *S. cerevisiae* appear to be conserved in several well-studied fungal pathogens.

### *S. cerevisiae*

PDR in *S. cerevisiae* is the best-understood fungal MDR mechanism. Many point mutations causing resistance to chemically diverse xenobiotics (including azoles) with differing targets have been mapped in isogenes encoding the Gal4-like zinc finger transcription factors ScPdr1p and ScPdr3p (45, 248). These gain-of-function mutations activate over 20 target genes, the major ones being drug efflux transporters of the ABC (Sc*PDR5*, Sc*SNQ2*, and Sc*YOR1*) or MFS (Sc*TPO1* and Sc*FLR1*) superfamilies (77, 299). Resistance to a wide range of drugs involves *trans*-activation of gene expression through the binding of the dimeric ScPdr1p and/or ScPdr3p transcription factor to promoters containing palindromic octanucleotide consensus binding sites (PDR elements [PDREs]) (160, 206). Mechanisms regulating the *trans*-activation include plasma membrane sphingolipid homeostasis, autoregulation of ScPdr3p and its specific activation on loss of mitochondrial respiration, chaperone-specific differential regulation of ScPdr1p and ScPdr3p (122), and ScPdr1p's ability to induce compensatory expression of efflux pumps (20, 387). Yeast cells incubated with antifungals or other xenobiotics transiently activate ScPdr1p/ScPdr3p (199), and this has been shown to be associated with drug efflux pump expression (349). Drugs like ITC and progesterone bind to a 250-amino-acid hydrophobic xenobiotic binding domain of ScPdr1p/ScPdr3p, enabling a specific association with the KIX domain (comprising three α helices) of the ScGal11p subunit of the mediator complex that recruits

RNA polymerase II for expression of the ScPdr1/ScPdr3p-controlled genes (113, 349). Another feature of ScPdr1p regulation of efflux pump expression is compensatory induction. If individual efflux pump genes, such as Sc*PDR5*, Sc*SNQ2*, or Sc*YOR1*, are deleted, there is a compensatory upregulation of the other drug efflux pumps (168). This induction requires ScPdr1p and is inhibited by ScPdr3p (168).

Other zinc finger transcription factors, such as ScYrr1p, ScStb5p, ScRdr1p, and ScYrm1p, also contribute, in a combinatorial fashion, to the expression of the various transporter genes (3, 186). Activation of a basic leucine zipper transcription factor, ScYap1p, controls a parallel MFS-mediated drug efflux pathway and protects yeast cells against oxidative damage (122).

### Pathogenic Fungi

In *C. albicans*, expression of the ABC transporters CaCdr1p and CaCdr2p is controlled by the ScPdr1/ScPdr3p-like zinc finger transcription factor CaTac1p (57). Expression is increased by gain-of-function mutations in CaTac1p, with high-level drug resistance occurring when this mutation is brought to homozygosity by loss of heterozygosity (55, 324). High doses of the female steroid hormone progesterone transiently upregulate, via steroid-specific PDREs, the same core of closely related ABC transporters induced by antifungal intervention or gain-of-function mutations in the transcription factors (21, 185). In some instances, FLC-resistant clinical isolates overexpress the MFS transporter CaMdr1p (232). Analogous to the resistance that requires homozygosity of a mutant CaTac1p, mutations in the zinc cluster transcription factor CaMrr1p, followed by loss of heterozygosity, cause Ca*MDR1*-mediated azole resistance (85). Ca*MRR1* deletion diminishes drug resistance more strongly than deletion of the efflux pump, indicating that additional protective cellular mechanisms are involved (122). There are differences between the PDR pathways of *C. albicans* and *S. cerevisiae.* CaTac1p and ScPdr1p/ScPdr3p show less than 20% sequence identity, they use significantly different PDRE motifs (57), and CaTac1p appears more focused in effect than ScPdr1p/ScPdr3p (21, 195).

*C. glabrata*, which is closely related to *S. cerevisiae*, uses only one ortholog of the ScPdr1p/Pdr3p transcription factor pairing to control expression of its major ABC pumps, CgCdr1p and CgPdh1p. As in *S. cerevisiae*, expression of these pumps is induced rapidly by treatment with diverse drugs, and they are highly expressed in mutants defective in respiratory function. Antifungal binding to the CgPdr1p xenobiotic binding domain induces PDR via a CgGal11p homolog in the *C. glabrata* transcription mediator complex (349). Mutants overexpressing CgPdr1p coordinately regulate 11 genes that are homologous to ScPdr1/ScPdr3p targets (368). However, the differential expression of other genes, which are functionally linked with transport, cell wall biosynthesis, lipid metabolism, subcellular trafficking, and cell stress, by CgPdr1p probably reflects the adaptation of the two species to different environmental niches.

Mechanistic data on azole and related PDR phenomena identified in *C. krusei* (182) and *C. neoformans* (E. Lamping, unpublished data) suggest that the various drug resistance mechanisms found in *C. albicans* may also operate in these pathogenic fungi. While there will be differences and commonalities in the mechanisms of resistance to azole antifungals among these less well-studied pathogens, Pdr1p/Pdr3p-like transcriptional control systems probably contribute to their PDR and are potential targets for overcoming efflux-mediated azole resistance.

## FUNGAL EFFLUX-MEDIATED DRUG RESISTANCE

### *C. albicans*

Analysis of the *C. albicans* genome has identified at least 27 ABC proteins classified into six distinct subfamilies (37, 108). Of these, there are seven open reading frames (*CDR1*, *CDR2*, *CDR3*, *CDR4*, *CDR11* [*CDR5*], *SNQ2*, and *YOR1*) that are annotated in the *Candida* genome database (CGD) (http://www.candidagenome.org/) (14) as having confirmed or inferred xenobiotic-transporting ATPase activity that could be associated with MDR. In transcriptional-array studies of *C. albicans* gene expression, several of these genes were shown to be upregulated in the presence of FLC, ITC, or fluphenazine (70, 155, 300, 390). Upregulation of Ca*CDR1* and Ca*CDR2* was shown for four FLC-resistant isolates, relative to their susceptible parental strains, in a study combining transcriptional-array analysis with a *TAC1* regulon location analysis (195). Indeed, only for Ca*CDR1* and Ca*CDR2* has functional transport of known antifungal drugs been demonstrated (37). Disruption of Ca*CDR1* makes *C. albicans* hypersusceptible to azoles (313), and controlled overexpression of CaCdr1p in a *C. albicans CDR1*-null mutant conferred resistance to FLC and other xenobiotics (245). When Ca*CDR2* was deleted in a *C. albicans* strain with Ca*CDR1* deleted, the resulting double *cdr1*Δ:*cdr2*Δ mutant was more susceptible to azoles than the single *cdr1*Δ mutant (312). Both Ca*CDR1* and Ca*CDR2*, when heterologously expressed in *S. cerevisiae*, confer resistance to azoles and other xenobiotics, including the fluorescent compound and PDR substrate rhodamine 6G (181), suggesting that they are ABC pump substrates (Fig. 4 and Table 2). Ca*CDR3* and Ca*CDR4* have been shown to encode phospholipid flippases. Despite a high degree of sequence conservation with CaCdr1p and CaCdr2p, CaCdr3p does not appear to be involved in resistance to antifungals, including FLC (19). Similarly, the results of gene disruption and cloning experiments showed that Ca*CDR4* was not involved in *C. albicans* FLC resistance (100). The annotation of putative xenobiotic-transporting activity for the other *C. albicans* ABC transporters is inferred by homology to transporter genes in other fungi; in *S. cerevisiae*, the orthologs of *SNQ2* and *YOR1* are involved in efflux-mediated resistance to 4-nitro-1-oxido-quinoline (63) and aureobasidin A, respectively (254). Ca*CDR11* is uncharacterized but was annotated in the CGD as an ABC transporter by sequence homology.

The other class of membrane transporters identified in *C. albicans* as putative drug efflux pumps is the MFS-type transporter proteins. Six genes are annotated as MFS-like in the CGD (*MDR1*, *FLU1*, *TPO3*, *orf19.2350*, *NAG3*, and *MDR97*), and of these, only CaMdr1p and CaFlu1p have substrates that are antifungals. Ca*FLU1* was identified by complementation of FLC hypersusceptibility in an *S. cerevisiae* strain lacking the ABC transporter gene *PDR5* but was shown not to be required

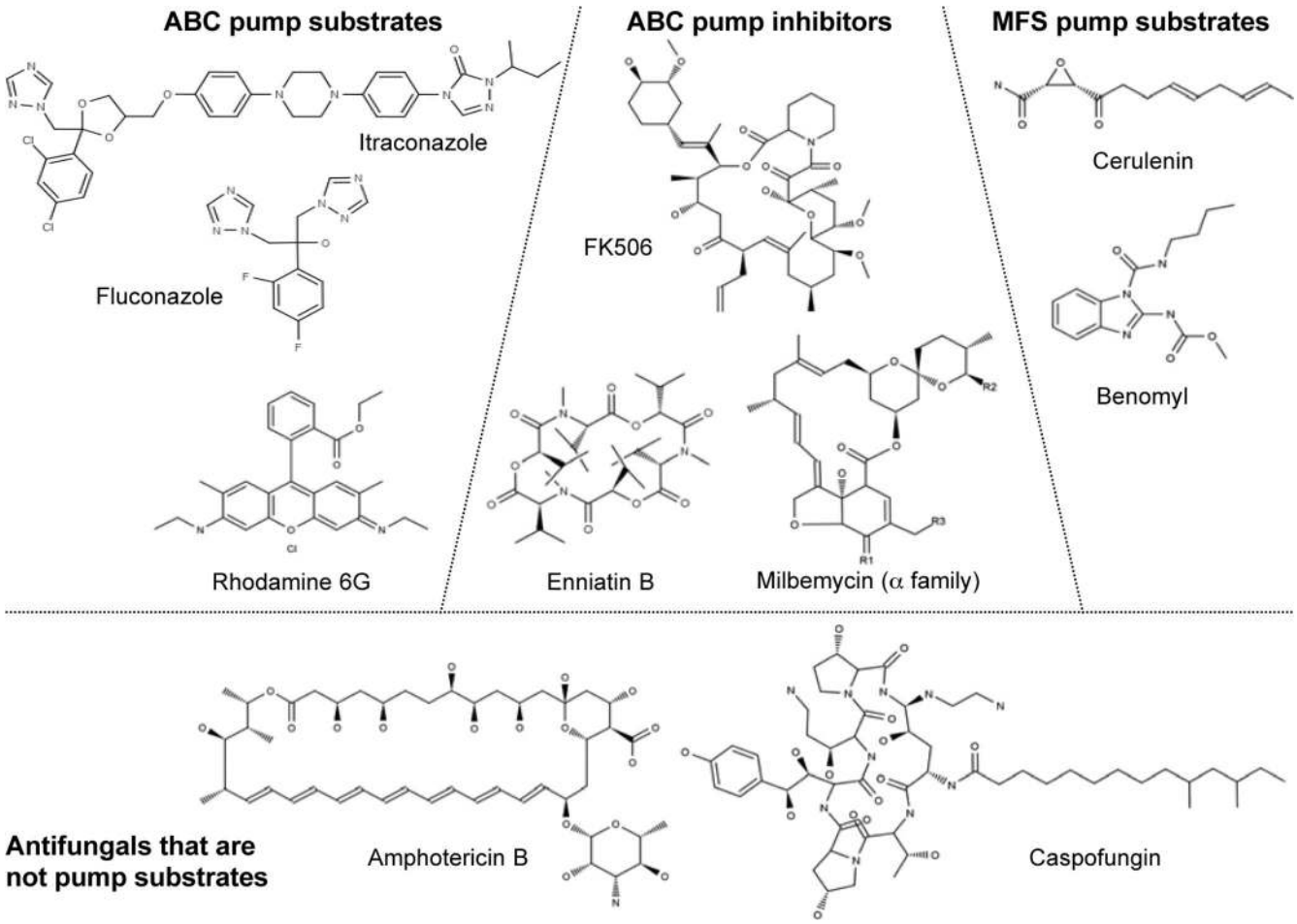


FIG. 4. Structures of representative ABC pump substrates, ABC pump inhibitors, MFS pump substrates, and antifungal drugs that are not pump substrates. All the structures except those of milbemycin and fluconazole were obtained from the ChemSpider database and were visualized using MarvinView. The milbemycin and fluconazole structures were built using the MarvinSketch v.5.1.3_2 editor (ChemAxon).

for the development of azole resistance in clinical isolates (42). Disruption of *MDR1* in *C. albicans* resulted in reduced FLC susceptibility, and Ca*MDR1* was shown to be upregulated in some *C. albicans* strains with reduced FLC susceptibility (386).

An important question related to strategies to overcome efflux-mediated antifungal resistance is the relative contribution of each efflux pump protein to clinically significant antifungal resistance in *C. albicans*. It is now clear that the transporters CaCdr1p, CaCdr2p, and CaMdr1p are the main efflux pumps mediating resistance of *C. albicans* to azole drugs. However, CaMdr1p is relatively specific for FLC (167, 307), whereas many azole drugs can act as substrates for CaCdr1p and CaCdr2p (237) (Table 2). Interestingly, a number of FLC-resistant isolates of *C. albicans* overexpress just Ca*CDR1* and Ca*CDR2*, but not Ca*MDR1*, whereas other strains overexpress only Ca*MDR1*, reflecting the existence of at least two different transcriptional pathways that are responsible for the upregulation of these genes in azole-resistant strains (395).

Although it is evident that multiple mechanisms contribute to clinical *C. albicans* FLC resistance (49, 269, 379), high-level resistance in clinical isolates most often correlates with overexpression of mRNA for Ca*CDR1* and Ca*CDR2*, rather than for Ca*MDR1* (49, 201, 269, 300, 314, 378), and evidence is accumulating that Ca*CDR1* expression may be more critical than Ca*CDR2* expression. Several studies (70, 155, 300, 390) have used whole-genome transcriptional-array analysis of expression, but they show only comparative, not absolute, levels of mRNAs. In a study using haploinsufficiency phenotype assays (389), FLC-induced haploinsufficiency was observed only for the Ca*CDR1* transporter, not for Ca*CDR2*. Ca*CDR1* was also the only transporter gene significantly upregulated in a study of *C. albicans* resistance development in FLC-treated, *C. albicans*-infected mice using genome-wide transcriptional analysis confirmed by Northern analysis (9, 10). Protein expression levels are more relevant to in vivo function than mRNA expression. A recent analysis of Cdr protein expression in a collection of *C. albicans* clinical isolates with reduced FLC susceptibilities demonstrated that CaCdr1p was expressed in greater amounts than CaCdr2p and that most FLC efflux function in these strains was mediated by CaCdr1p rather than CaCdr2p (135). An earlier study showing that the Ca*CDR2* gene possesses much higher heterozygosity than Ca*CDR1* (137) may reflect this differential function. It is possible that

the less conserved Ca*CDR2* gene may have a role in adaptation to varying environmental conditions in the human host.

Thus, there is strong evidence for the dominant role of CaCdr1p in clinically significant *C. albicans* FLC resistance. It therefore represents a good target for combination therapies to substantially reverse, or even prevent, FLC resistance.

### *C. glabrata*

A significant proportion (30%) of clinical *C. glabrata* isolates show moderate innate resistance to azole antifungals (384). The organism can also acquire increased azole resistance during the course of therapy, or prophylaxis, with azole drugs, usually with FLC (24, 364). The acquisition of azole resistance is rapid, and the resistance phenotype is stable after the removal of FLC (34). Increasing use of FLC for the treatment of *C. glabrata* infections apparently results in the selection of an azole-resistant population, and resistant clinical isolates possess cross-resistance to other azoles (ITC, KTC, or VCZ) (315). This makes it more difficult to treat patients with *C. glabrata* infections, and FLC prophylaxis for immunocompromised patients may be a risk factor for the development of *C. glabrata* infections (1).

As with *C. albicans*, azole-resistance in *C. glabrata* clinical isolates is associated with increased expression of PDR ABC drug efflux pumps, in this case, CgCdr1p and CgPdh1p (also called CgCdr2p) (24, 309). There is in vitro evidence that exposure of *C. glabrata* cells to FLC induces expression of the drug target, CgErg11p (34, 128, 301). Mass spectrometric fingerprint analysis confirmed that the induction of CgCdr1p and CgErg11p in laboratory strains occurred several hours after the cells were exposed to FLC (244). However, unlike *C. albicans*, there is little evidence for changes in Cg*ERG11* expression levels (369) or mutations in Cg*ERG11* in azole-resistant clinical isolates (315).

The *C. glabrata* genome was sequenced as part of the Genolevures program (84) (http//www.genolevures.org/). The genome of this haploid yeast comprises 13 chromosomes encoding a total of 5,283 genes and shows a high degree of synteny with the genome of the closely related yeast *S. cerevisiae* (Fig. 2). Comparison of the *C. glabrata* and *S. cerevisiae* genomes identified putative MDR transport proteins belonging to both the ABC and MFS families. There are predicted to be 18 ABC transporters (6 in the PDR subfamily) (Table 3), 6 half transporters and 12 full transporters, and 15 MFS transporters, 10 of subfamily DHA1 and 5 of subfamily DHA2, in *C. glabrata* (110). Of these transporter genes, only a few have been studied with regard to drug resistance or transport activities. The best-characterized transporter is Cg*CDR1* (309), an ortholog of Sc*PDR5.* Cg*CDR1* overexpression confers resistance to azole antifungals, and deletion of the gene renders cells susceptible to azoles. A wide range of structurally and functionally diverse compounds are substrates of this efflux pump (372) (Table 2). *C. glabrata* clinical isolates exhibiting azole resistance predominantly overexpress Cg*CDR1* (308) and Cg*PDH1* (146, 224). Both pumps require protein phosphorylation to pump xenobiotics out of the cells (372). The phosphorylation apparently affects drug efflux activity, and specifically CgCdr1p ATPase activity, as amino acid substitution in certain phosphorylation sites resulted in substantial reduction in the ATPase activity and cells became more susceptible to azoles (373). The ABC transporter Cg*SNQ2* is highly similar to Sc*SNQ2* and mediates resistance to azoles and an *SNQ2*-specific substrate, 4-nitroquinoline *N*-oxide (354).

Expression of these ABC transporters is controlled by the transcriptional regulator Cg*PDH1*, an ortholog of Sc*PDR1.* Azole-resistant clinical isolates of *C. glabrata* showed higher expression of Cg*CDR1* and Cg*PDR1* than the susceptible parent strains, indicating the importance of the MDR network to azole resistance in clinical isolates (93, 357, 368). The CgPdr1p from the resistant isolates also had amino acid substitutions that conferred upregulation of Cg*PDR1* and azole resistance (93, 357). Overexpression of the drug efflux transporters Cg*CDR1*, Cg*PDH1*, and Cg*SNQ2*, as well as the regulator Cg*PDR1*, was also demonstrated in a strain with deletion of the Cg*PGS1* gene, encoding phosphatidylglycerolphosphate synthase, an enzyme involved in the synthesis of phospholipids essential for functional mitochondria, possibly in response to an altered mitochondrial phospholipid composition (22). In *C. glabrata*, loss of mitochondrial function or respiratory deficiency (resulting in petite mutants) is also linked to the upregulation of Cg*CDR1* and Cg*PDH1* (38, 308).

Cg*AUS1*, an ortholog of the *S. cerevisiae* ABC transporters *AUS1* and *PDR11*, was recently described as a putative sterol importer that may help protect *C. glabrata* from azole toxicity (239). It has been proposed that cells use CgAus1p to incorporate exogenous sterol present in serum into cell membranes to compensate for the ergosterol depletion caused by azoles (238).

### *C. krusei*

While most *Candida* species, with the exception of *C. glabrata*, as discussed above, are susceptible to azole antifungals, *C. krusei* is generally considered innately resistant to FLC, with about 80% of strains being susceptible dose-dependent to ITC. *C. krusei* is, however, susceptible to the newer triazoles, such as VCZ and POS (278, 280). While the genomes of important fungal pathogens, such as *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. neoformans*, and *A. fumigatus*, have been sequenced and partially or fully annotated, the *C. krusei* genome remains largely undescribed. This is partly because of uncertainties surrounding the karyotypes of clinical isolates and because *C. krusei* is not amenable to genetic manipulation. Transformation protocols have yet to be reported, and no auxotrophic or dominant selection markers are available for the genetic modification of *C. krusei*. The innate azole resistance phenotype of *C. krusei* appears to be mainly due to the reduced susceptibility of the drug target Erg11p to azole antifungals (103, 258, 365). It has also been shown that efflux pumps can contribute to *C. krusei* FLC resistance (52, 158, 213, 296, 366). Using degenerate primers against a highly conserved region of NBDs, Katiyar and Edlind (158) isolated NBDs belonging to two ABC transporters, *C. krusei ABC1* (Ck*ABC1*) and Ck*ABC2* (158). These are the only *C. krusei* ABC transporters reported to date. While Ck*ABC2* was minimally expressed under all growth conditions tested, Ck*ABC1* was strongly induced with different azoles (158). We have isolated and characterized Ck*ABC1* in detail. Heterologous overexpression of CkAbc1p in the *S. cerevisiae* host AD$\Delta$ (181)

showed that Abc1p is indeed a multidrug efflux transporter able to transport a large array of xenobiotics (Table 2), including FLC, ITC, KTC, MCZ, and VCZ (182).

### *Aspergillus* Species

The genomes for *A. fumigatus* (241), the model filamentous fungus *A. nidulans*, and *Aspergillus oryzae* have recently been sequenced. The *A. fumigatus* genome is predicted to encode 49 ABC and 278 MFS transporters, more than four times the number of such transporters found in yeasts like *S. cerevisiae* or *Schizosaccharomyces pombe* (94). However, despite the large number of putative transporters, there is a dearth of evidence linking any particular ABC or MFS transporter with clinically relevant antifungal drug resistance. This is quite surprising and may indicate very limited specificity for all transporters (348) or simply that efflux is not necessary to confer antifungal resistance. However, the sequence data also revealed that all three species contain multiple copies of genes encoding several enzymes in the ergosterol biosynthesis pathway (94). It appears that many filamentous fungi possess two (218) or even three (e.g., *A. oryzae*) *CYP51* (equivalent to *ERG11*) genes belonging to two distinct *CYP51* gene clusters, the *CYP51A* or *CYP51B* cluster (94). They also possess two (*A. nidulans* and *A. oryzae*) or three (*A. fumigatus*) distinct *ERG3* genes belonging to three separate gene clusters (*ERG3A*, *ERG3B*, and *ERG3C*) (94). Much progress has been made in deciphering the azole resistance mechanisms of *A. fumigatus* (reviewed in detail in references 47, 61, 231, and 290). *A. fumigatus* is innately resistant to FLC and KTC (140, 221, 231). Gene knockout experiments have shown that Cyp51p is essential for *A. fumigatus* and that *A. fumigatus* Cyp51Ap (AfuCyp51Ap), not AfuCyp51Bp, is responsible for the innate resistance to FLC and KTC (140, 221). Although the prevalence of ITC- or VCZ-resistant clinical *A. fumigatus* isolates is low, several studies have demonstrated that resistance is usually due to point mutations in AfuCyp51Ap that lead to different patterns of azole resistance for long-side-chain-containing azoles, such as ITC and POS, as opposed to VCZ and FLC (50, 64, 210, 220, 240, 370). Often mutations in amino acids G54 (G54V, G54W, G54R, G54K, or G54E) or M220 (M220I, M220V, M220T, or M220K) are found in AfuCyp51Ap, both in clinical isolates that are resistant to ITC or POS (50, 79, 210, 219) and in *A. fumigatus* cells mutagenized in vitro (64, 210, 240). Mutation of CaErg11p A61, the equivalent of G54 in AfuCyp51Ap, and overexpression in *S. cerevisiae*, however, have no effect on azole susceptibility (388).

In contrast, there are few data associating ABC or MFS multidrug efflux pumps with antifungal drug resistance in *Aspergillus*. Most in vitro studies attempting to identify candidate efflux pumps that could contribute to azole resistance in *Aspergillus* have been performed in the model filamentous fungus *A. nidulans*. Candidate ABC transporters isolated and characterized thus far include *AtrA* (73) and *AtrB* (11, 73), *AtrC* (12), *AtrC2* (13), and *AtrD* (12). *AtrA* and *AtrB* are PDR ABC pumps; *AtrC*, *AtrC2*, and *AtrD* belong to the MDR class of transporters, sharing significant homology with human P-gp. *AtrB* (11, 73) and *AtrD* (12) are the pumps most likely to contribute to MDR in *A. nidulans*. *AtrB* (11) and *AtrD* (12) knockout strains were hypersusceptible to a number of fungicides and/or xenobiotics. In addition, overexpression of *AtrB* in either *S. cerevisiae* (73) or *A. nidulans* (11) caused increased resistance to a similar range of compounds. *AtrA*, *AtrC*, and/or *AtrC2* may be multidrug efflux pumps, but perhaps with narrower substrate specificity. They might also be less efficient transporters, so that any gene knockout is masked by *AtrB*, *AtrD*, or other endogenous efflux pumps.

Few azole-resistant *A. fumigatus* clinical isolates have been found to overexpress efflux pumps. *A. fumigatus* AF72 showed a reduced accumulation of ITC, possibly due to increased drug efflux (76). The PDR-type ABC transporter *atrF* (an ortholog of the *A. nidulans* transporters *AtrA* and *AtrB*) was cloned from this strain, and *atrF* mRNA levels were found to increase fivefold in AF72 cells in response to sub-MIC levels of ITC (335). Other studies have used in vitro evolution of ITC resistance by *A. fumigatus* to identify possible azole resistance mechanisms (64, 207, 240). In two cases, multiple resistance mechanisms were discovered involving drug target alterations, as well as other, uncharacterized mechanisms that possibly involved efflux pumps (64, 240). A third study concluded that reduced ITC uptake was responsible for the ITC-resistant phenotype of two variants (207).

Afu*MDR1* and Afu*MDR2* were isolated using ABC pump NBD-specific degenerate primers (352). Afu*MDR1* is an MDR-type transporter with a $(TMD\text{-}NBD)_2$ protein topology closely related to both *A. nidulans AtrD* (78% identical with conserved intron positions) and *A. flavus MDR1* (Afl*MDR1*) (352). Overexpression of AfuMdr1p in *S. cerevisiae* conferred increased resistance only to cilofungin, an echinocandin B analog (352). AfuMdr2p is a half-size transporter with a TMD-NBD topology orthologous to ScMdl1p and ScMdl2p (352). Neither transporter is associated with an antifungal resistance phenotype. A further transporter, *A. fumigatus abcA*, belongs to the PDR family of transporters and shows the highest homology to *ATR2* of *Mycosphaerella graminicola* (59% identity) and *ABC1* of *Pyricularia grisea* (54% identity) (184). However, deletion of *abcA* in *A. fumigatus* did not result in increased susceptibility to any of the antifungals tested (184).

In a study that generated 26 ITC-resistant *A. fumigatus* mutants by UV mutagenesis, 8 had point mutations in the previously described azole resistance-associated amino acid G54 of Afu*CYP51A* (240). About half of the mutants, however, appeared to be ITC resistant due to the overexpression of the efflux pumps Afu*MDR3*, an MFS-type transporter of the DHA2 family, and Afu*MDR*4, a typical MDR-type ABC transporter with the $(TMD\text{-}NBD)_2$ topology. However, there was no evidence linking either Afu*MDR3* or Afu*MDR4* directly with the ITC-resistant phenotype of any of the mutants analyzed. Although these in vitro studies can be used to infer a role for multidrug efflux in the development of *Aspergillus* azole resistance, all in vivo studies suggest that this resistance mechanism is unlikely to be of major clinical importance.

### *Cryptococcus* Species

*C. neoformans* and *C. gattii* are important human fungal pathogens. AMB-resistant clinical isolates (162, 367), as well as variants obtained in vitro (152), have been reported, but AMB-resistant cryptococcal infections are rare (7, 28, 36, 139, 271).

Analysis of a series of clinical isolates that showed cross-resistance between AMB and FLC (367) revealed that they all had defects in either *C. neoformans* Erg2p (CneErg2p) or CneErg3p and had reduced levels of ergosterol that could explain the AMB resistance. In another study, *C. neoformans* strains cross-resistant to AMB and FLC appeared to display a multidrug efflux pump-mediated phenotype (152). However, efflux pump-mediated FLC resistance has yet to be reported in clinical isolates. Cryptococci are considered resistant to echinocandins, such as caspofungin or micafungin. The mechanisms are not fully understood, given that the drug target Fks1p, (1-3)-β-D-glucan synthase, is both essential and sensitive to echinocandins in vitro (205). A possible role for the calcineurin stress response pathway mediating echinocandin resistance in *Cryptococcus* has been suggested (205).

FLC or ITC resistance can be acquired by *Cryptococcus* isolates during prolonged maintenance therapy (30, 65, 266, 268, 367). The mechanisms responsible for azole resistance are either mutations of the drug target, CneErg11p (180, 298, 367), or increased drug efflux (284, 316, 367). Although azole resistance development during maintenance therapy is rare in AIDS patients receiving HAART, these resistance mechanisms are particularly important for those patients with no access to HAART.

While clinical isolates with medium levels of FLC resistance mostly contain mutations in Cne*ERG11* (180, 298, 367), the highest levels of resistance are likely caused by increased drug efflux. The genomes of *C. neoformans* var. *grubii* (strain H99 *MAT*α) and var. *neoformans* (strains JEC21 *MAT*α, JEC20 *MAT***a**, and B3501 *MAT*α), as well as two *C. gattii* genomes (strains WM276 *MAT*α and R265 *MAT*α; serotype B) have recently been sequenced (for a review of the strains and links to websites, see reference 192). Analysis of the H99 genome sequence predicted 54 ABC transporters and 159 MFS transporters, suggesting enhanced transport capabilities of this environmental yeast (197). CneAfr1p (284, 316) and CneMdr1p (350) are the only two efflux pumps that have been linked to antifungal drug resistance in *C. neoformans*. CneAfr1p is a member of the PDR family of ABC transporters, with the highest homology to *A. nidulans* AtrBp, AfuAtrFp, ScSnq2p, and CgPdh1p with a $(NBD\text{-}TMD)_2$ protein topology (284). Overexpression of CneAfr1p leads to azole-resistant *C. neoformans* that cannot be treated with azole antifungals, as shown in mouse models that used inhalation or intravenous infection to produce systemic cryptococcosis (316). The overexpression of CneAfr1p led to increased virulence in the mouse models and significantly improved the survival of *C. neoformans* during in vitro macrophage infection (316). About 5% of clinical isolates (5 of 107 isolates tested) exhibited a so-called FLC heteroresistance phenotype (391). Heteroresistant *C. neoformans* isolates grow on solid media at four- to eightfold-higher FLC concentrations than their liquid MICs would suggest. Cne*AFR1* appears to be associated with this heteroresistant phenotype (E. Sionov, H. S. Lee, Y. C. Chang, J. E. Bennett, and J. Kwon-Chung, presented at the 7th International Conference on Cryptococcus and Cryptococcosis, Nagasaki, Japan, 2008). Heteroresistant strains are aneuploid (discussed further below) and have increased copy numbers of some chromosomes under selective pressure. Cne*AFR1* is located on one of these chromosomes, and deletion of Cne*AFR1* in a heteroresistant strain led to a reversion of the resistance phenotype (E. Sionov, H. S. Lee, Y. C. Chang, J. E. Bennett, and J. Kwon-Chung, presented at the 7th International Conference on Cryptococcus and Cryptococcosis, Nagasaki, Japan, 2008).

Another ABC transporter, CneMdr1p, thought to be associated with azole resistance, belongs to the MDR-type ABC transporter family with a $(TMD\text{-}NBD)_2$ protein topology (350). Cne*MDR1* shows high homology to Afl*MDR1*, Afu*MDR1*, and human ABCB1 and to a lesser extent to Sc*STE6* (350). However, Cne*MDR1* expression has yet to be linked with azole resistance in *C. neoformans* clinical isolates or in resistant mutants isolated in vitro.

Overexpression of both Cne*AFR1* and Cne*MDR1* homologs from *C. neoformans* strain CDC551 (serotype A) in *S. cerevisiae* ADΔ conferred MDR to a large array of xenobiotics, including all azole antifungals tested (181; E. Lamping, unpublished data) (Table 2). Despite differences in domain order placing these ABC transporters in different classes, their resistance profiles in *S. cerevisiae* were very similar.

Considering the mortality associated with cryptococcal meningitis and the incidence of azole-resistant breakthrough infections in AIDS patients without access to HAART, further investigation of the role of efflux-mediated azole resistance in *C. neoformans* is warranted.

## EFFLUX PUMP-MEDIATED DRUG RESISTANCE AS A STRESS RESPONSE

Fungi inhabit a range of environments, including various niches on humans. Their environment can change as the fungus colonizes and spreads within a host or due to medication given to the human host. These environmental changes cause physiological stress in fungal cells, and fungi have evolved responses to ameliorate the harmful effects of the stress (61, 176). While the response of microorganisms to stresses such as temperature, pH, and changes in osmolarity have been well studied, it is important to acknowledge that the administration of antifungal drugs also represents a stress to which fungi respond (44, 61). The nature of the fungal responses to antifungal drugs depends on the fungus, the dose, its duration, and the mechanism of drug action. For fungicidal drugs, such as echinocandins (for *Candida* species) and polyenes at concentrations significantly above the MIC, the responses fail to prevent cell death. With fungistatic drugs, such as the azoles, above the MIC, growth is inhibited but the cells are not killed, a phenomenon referred to in this context as drug tolerance (311).

Most of the established antifungal resistance mechanisms are due to genetic mutation, usually point mutations in drug targets or enzymes in metabolic pathways or in transcription factors, leading to gene overexpression. Such mutations are stable, take time to be acquired, and can be thought of as long-term stress responses. Likewise, azole resistance can be caused by genetic rearrangements (270) or aneuploidy (323) affecting the expression of drug targets, pumps, or transcription factors. However, antifungal drugs also stimulate classic, immediate stress responses. These can be thought of as reversible phenotypic responses that do not involve mutation or chromosomal rearrangement. The short-term phenotypic stress responses that lead to drug tolerance are important in

fungi because they may give cells time to develop long-term genetically stable resistance mechanisms that could also confer a fitness gain.

Antifungal drugs that target the wall or membrane sterol biosynthesis can induce osmotic or membrane stress. These stresses elicit responses through conserved signaling pathways, notably the mitogen-activated protein kinase (MAPK) signal transduction network (174, 227, 303) and the cyclic AMP-protein kinase A pathway (44, 61, 291). Membrane stress induced by azole exposure is transduced through the protein kinase C (Mkc1p) component of the MAPK pathway (J. Pla, personal communication). Oxidative stress in *C. albicans* induces responses, mainly via the transcription factor CaCap1p, which is also involved in MDR (5, 302). Activation of CaCap1p by a C-terminal truncation of the protein results in upregulation of the MFS transporter gene Ca*MDR1* (5). The cyclic AMP-protein kinase A signal transduction pathway is involved in the response of *C. albicans* to antifungal stress; adenylate cyclase (Ca*CDC35*) mutants no longer respond to azoles with upregulation of Ca*CDR1* (148).

The serine/threonine protein phosphatase calcineurin is highly conserved in eukaryotes and is activated in response to several stresses. It has important physiological roles in *C. albicans* and is essential for survival during membrane stress (62). *C. albicans* calcineurin is a heterodimer composed of a catalytic subunit A (encoded by Ca*CMP1* [also called Ca*CNA1*]) and a regulatory subunit B (encoded by Ca*CNB1*). The phosphatase activity of calcineurin is activated when it binds calmodulin in the presence of calcium ions, and it affects gene expression via transcriptional regulators, such as CaCrz1p. Calcineurin mediates tolerance for a variety of stresses, including salt and high pH, in addition to membrane stress (342). Calcineurin is essential for *C. albicans* survival in serum (16), and it is the calcium component of serum that is toxic to the yeast in the absence of functional calcineurin (32). Calcineurin also plays an important role in the tolerance of *C. albicans* for certain antifungal drugs, in particular, azoles. If *C. albicans* calcineurin activity is inhibited, the fungistatic azoles become fungicidal; tolerance is abolished. Thus, the immunosuppressants cyclosporine A (CsA) and tacrolimus (FK506) have been found to act synergistically with FLC (311). They bind with either cyclophilin A (Cyp1p) or Rbp1p, respectively, and are thought to inhibit calcineurin by binding at the subunit A and B interface (62, 311, 342). Synergism of FK506 with FLC was also seen with *C. tropicalis* and *C. parapsilosis*, but not with *S. cerevisiae* or *C. krusei* (62). Calcineurin is activated, following an increase in intracellular $Ca^{2+}$, by the binding of $Ca^{2+}$-calmodulin. As in *S. cerevisiae*, one of the major substrates of *C. albicans* calcineurin is the transcription factor Crz1p (156). Disruption of Ca*CRZ1*, however, did not completely remove azole tolerance, suggesting that the tolerance is mediated by other substrates of calcineurin (156, 257). Protein kinase CaCka2p is involved in FLC susceptibility, possibly by inhibiting Ca*CDR1* and Ca*CDR2* expression (39). In a Ca*CKA2* knockout strain, Ca*CDR1*, Ca*CDR2*, and Ca*RTA3* were overexpressed. Although calcineurin contributes to Cka2p-mediated FLC sensitivity, CaCrz1p is not the CaCka2p substrate. Thus, the precise mechanism of calcineurin-dependent azole tolerance remains to be discovered. Calcineurin responds to changes in calcium ion concentration, and it is possible that exposure to azoles causes an intracellular calcium spike that induces the calcineurin tolerance pathway (311). The calcineurin pathway is also involved in the growth and pathogenicity of *A. fumigatus* and *C. neoformans*, and it has been proposed as a novel target for antifungal agents (342).

Heat shock protein 90 (Hsp90) is a molecular chaperone that stabilizes a number of cellular proteins, many of which are involved in signaling pathways, including calcineurin (61). In vitro studies have demonstrated a role for Hsp90 in promoting the rapid acquisition of FLC resistance by *S. cerevisiae* and *C. albicans* (58, 60). Hsp90, together with calcineurin, could be targets for abolishing tolerance and thus preventing the development of azole resistance.

## ANEUPLOIDY AND EFFLUX-MEDIATED RESISTANCE

Population genetic analyses of *C. albicans*, *C. neoformans*, and *A. fumigatus* revealed that each fungus is mostly clonal with limited evidence of sexual recombination (192, 261, 351). Although these fungi contain the genetic machinery necessary for sexual reproduction (104, 192, 246), sexual recombination has been observed only in the laboratory (203, 255) or between modified strains in animal infection models (143). Thus, outside the laboratory, the fungi may rarely undertake sexual reproduction and will have to rely on other genetic mechanisms to adapt to changes in their environment. Whole-genome sequencing and physical mapping, chromosomal-genome hybridization, and haplotype analysis have shown that genetic recombination, chromosome deletions, and translocations, as well as the gain or loss of chromosomes, occur frequently in fungi. This can result in aneuploidy—an abnormal number of chromosomes—which can have profound effects on phenotypes through gene dosage or by uncovering recessive mutations. Widespread aneuploidy in *S. cerevisiae* has been revealed by using microarray profiling, which showed spurious gene expression for knockout strains (142). In this study, it was shown that 8% of 300 tested gene knockout strains contained chromosomal aneuploidies.

The genome of *C. albicans* is very "plastic," and it has been found that specific, nonrandom chromosomal rearrangements occur during selective pressure, such as exposure to antimicrobials or growth on different carbon sources (e.g., L-sorbose) or even the genetic manipulation of cells (transformation and selection on 5-fluoroorotic acid medium) (2, 149, 187, 270, 304, 322). Aneuploidy is also common in *C. albicans* clinical isolates, and this can result in azole resistance due to overexpression of Erg11p and the ABC pumps Cdr1p and Cdr2p. It has been shown that 36% of 42 FLC-resistant *C. albicans* isolates possessed an increased copy number of chromosome 5 (either a trisomy or a segmental aneuploidy caused by the formation of an isochromosome containing two left arms of chromosome 5 separated by a centromere) (323). Further studies revealed that the left arm of chromosome 5 contained both the azole drug target Ca*ERG11* and the transcription factor Ca*TAC1*, which regulates Ca*CDR1* and Ca*CDR2* expression. All strains contained two or more copies of the CaTac1p gain-of-function mutation, mentioned above, that led to constitutively high levels of CaCdr1p and CaCdr2p expression. This, together with the increase in the Ca*ERG11* copy number, was responsible for the FLC resistance phenotype observed in these clinical isolates (56, 324). We have recently reported aneuploidy in some

*C. krusei* isolates that possess three Ck*ERG11*-containing chromosomes, but in this case, it was not sufficient to increase FLC resistance significantly (182).

Most *C. neoformans* clinical isolates are haploid serotype A strains, but it has been shown that some strains can become aneuploid, leading to a transient heteroresistant phenotype (226, 391). These strains possess an extra copy of chromosome A, and their increased resistance may be associated with the increased copy number of the multidrug transporter Cne*AFR1*. Disruption of Cne*AFR1* removed the heteroresistant phenotype (E. Sionov, H. S. Lee, Y. C. Chang, J. E. Bennett, and J. Kwon-Chung, presented at the 7th International Conference on Cryptococcus and Cryptococcosis, Nagasaki, Japan, 2008).

Although aneuploidy can result in increased antifungal resistance, it usually confers a fitness cost that is more severe in haploid organisms. For example, haploid *S. cerevisiae* cells containing an extra copy of any of its chromosomes demonstrate a typical stress response that leads to reduced growth yields (355). Therefore, resistant aneuploid isolates are unstable and tend to lose these unfavorable chromosomal rearrangements in the absence of selective pressure. Indeed, the segmental aneuploidy responsible for the Tac1p-mediated FLC resistance of *C. albicans* is reversible. When FLC treatment ceases, isolates lose isochromosome 5 and become FLC sensitive (324). Chromosomal rearrangements are more prevalent in *C. albicans* than in *S. cerevisiae*, possibly because the fitness cost of aneuploidy in diploid organisms is significantly less than the cost to a haploid species and possibly because sexual recombination is not efficient in *C. albicans*. Therefore, aneuploidy, leading to overexpression of efflux pumps, may be a significant cause of drug resistance in diploid pathogenic fungi.

## EXPERIMENTAL ANALYSIS OF EFFLUX PUMPS

As efflux pumps play important roles in the biology of many eukaryotic organisms, not least the drug resistance of fungi, much effort has been expended in the study of pump structure and function. Although fungi have obtained multiple genes encoding efflux pumps during evolution, in common with most membrane proteins, the individual efflux pumps are normally expressed at low levels. In order to get sufficient transporter protein to study, they are often heterologously expressed in other organisms or in cell-free systems. The heterologous expression systems include *Escherichia coli*, insect cell lines, mammalian cell lines, and yeasts, such as *Pichia pastoris* or *S. cerevisiae*.

Significant differences in the synthesis of polypeptides in prokaryotes and eukaryotes place limitations on the use of *E. coli* for the expression of eukaryotic transporters. There has been some success in the expression of P-gp peptide motifs as antigens for antibody production (347), and the His-tagged NBD (NBD1) of murine P-gp has also been expressed and purified from *E. coli* (69). Although the expressed NBD was able to bind nucleotides, the authors reported low solubility and molecule half-life problems. Even when the full-length P-gp was expressed in *E. coli* deficient in OmpT protease, the problem of low yield and low activity (possibly due to the absence of glycosylation) persisted (26). Insect cell lines, such as *Spodoptera frugiperda* (18, 111) and *Trichoplusia ni* (cabbage looper moth) (163, 292), have also been used to express human P-gp with a higher yield of ~6 mg of purified protein from $4 \times 10^9$ baculovirus-infected insect cells.

Among cell-free expression technologies, the wheat germ system appears to be most suitable for eukaryotic proteins. This method, however, requires special techniques (such as separate mRNA synthesis), and despite substantial recent procedure improvements made by Promega (394), there have been few applications to membrane protein expression (247).

The yeast *P. pastoris* is considered by many a good choice for eukaryotic-protein expression and is widely used to produce secreted proteins (112, 200). It has advantages for fungal-protein expression due to its close relationship to the donor organisms and a similar membrane composition. It can use methanol as a sole carbon and energy source and grows under controlled fermentation conditions to very high cell densities of ~300 g (wet weight) of cells per liter of culture (51). The methylotropic pathway controlled by the *AOX1* (alcohol oxidase) gene is highly induced by methanol (358), and the *AOX1* promoter is often used to control the expression of heterologous genes (200). Disadvantages of using *AOX1* induction include difficulties in monitoring methanol concentrations during induction and the need to switch between carbon sources at a precise growth stage. Cloning genes of interest in *P. pastoris* can also be problematic, with individual clones showing different levels of heterologous gene expression. The *P. pastoris* expression system has been widely used for producing microbial (8, 393) as well as animal, including human, transporters (41, 51, 82, 121, 189, 211). Although many of these proteins show appropriate activity and kinetic characteristics, there has been little success in obtaining the levels of purity and homogeneity (monodispersity) required for structural analysis by X-ray crystallography. This may be due to the fact that the *Pichia* cells are harvested in the late exponential (or stationary) phase, which increases the diversity of posttranslational modifications.

There are numerous advantages that make *S. cerevisiae* (baker's yeast) an attractive host for the expression of eukaryotic membrane proteins (29). Like *P. pastoris*, it is nonpathogenic and easy and inexpensive to culture. *S. cerevisiae* is able to grow at a range of temperatures, at various pHs, and under hypoxic (anaerobic) conditions. Baker's yeast can often be used both for the production of recombinant proteins and for in vivo assays and screening, as the expression of the heterologous transporter can complement host mutations and often gives a measurable phenotype. The *S. cerevisiae* genome is comprehensively annotated, and microarray analysis has provided extensive information on the expression of many of its ~6,000 genes. Haploid and diploid forms of many well-characterized strains are available, and the ease of mating and sporulation facilitates a variety of classic genetic approaches that can be used to confirm the predictions and veracity of molecular biological experiments. In addition to a panel of haploid mutants in which each nonessential gene has been knocked out (385), there is a panel of haploid strains in which the activities of many essential genes can be titrated using the Tet promoter (141, 225), and there is a panel of heterozygous diploid mutants in which individual essential or nonessential genes have been deleted (http://www.openbiosystems.com/GeneExpression/Yeast). Such mutants enable insightful study of phenotypes and the detection of gene and chemogenomic

interactions. A number of other useful mutations/modifications are available in *S. cerevisiae*. They include the secretion/transport mutation *sec6-4*, which leads to the accumulation of post-Golgi network secretory vesicles (183, 375) or mutations that ensure protein quality based on the unfolded-protein response pathway (119). Strong evidence of the utility of *S. cerevisiae* as a host for heterologous membrane protein expression was obtained with the expression of functional rabbit $Ca2^{+}$ ATPase SERCA1a (150). The incorporation of a C-terminal biotinylation domain allowed the purification and crystallization of the correctly folded enzyme (150).

### Use of *S. cerevisiae* To Study ABC Transporter Function and To Screen for Pump Inhibitors

Several researchers have studied ABC pumps heterologously expressed in *S. cerevisiae* (73, 175, 286, 294, 309, 312, 314, 352, 353). The analysis of any heterologously expressed protein can be complicated by the presence of endogenous host proteins with similar activities. The study of ABC protein function in *S. cerevisiae* is therefore made more difficult by the presence of numerous ABC genes (71), as described above. In 1998, Decottignies and coworkers developed an *S. cerevisiae* mutant (AD12345678 [AD1-8]) in which seven ABC pump genes, predominantly of the PDR family, were deleted in order to reduce background drug transport activity due to endogenous transporters (72). In addition, the strain has *PDR3* deleted and has a gain-of-function mutation in *PDR1*. The *pdr1-3* mutation in AD1-8, together with the disruption of *pdr3*, leads to the constitutive hyperexpression of *PDR5* and the coordinated overexpression of other members of the PDR gene network that facilitate the biosynthesis and trafficking of membrane proteins (45, 77). This strain has been developed into an expression host in which heterologous genes are cloned downstream of the Sc*PDR5* promoter and then integrated into the *S. cerevisiae* genome at the *PDR5* locus from a transformation cassette by homologous recombination (181, 237). When the *C. albicans* ABC transporter CaCdr1p was expressed in this system, it contributed approximately 30% of the plasma membrane protein. The CaCdr1p was functional and conferred resistance to azoles and other xenobiotics on AD1-8 (181). The *S. cerevisiae* expression system has been used to study the functions of several fungal ABC proteins, including CaCdr1p, CaCdr2p, CgCdr1p, CgPdh1p, CkAbc1p, and CneMdr1p, as well as the *C. albicans* MFS pump Mdr1p (181, 264, 329). As the genetic distance from *S. cerevisiae* increased, the level of heterologous pump expression, in general, decreased (181). This drawback could possibly be addressed through codon optimization of heterologous genes or by altering the membrane composition of the host yeast.

Heterologous expression can be used to examine the effects of point mutations in specific domains of the ABC transporters on pump function. Site-directed mutation has shown that F774 in TMS6 of CaCdr1p affects trafficking and localization (329), and a T1351F mutation in CaCdr1p affects substrate specificity (328). Other amino acid residues important for transporter function have been identified by cloning CaCdr1p alleles mutated by low-fidelity amplification of the CaCdr1p open reading frame (136). Two serine residues involved in phosphorylation of CgCdr1p and CgPdh1p were identified by heterologous expression in *S. cerevisiae* (372, 373), and recently, we have confirmed that at least one homologous phosphorylation site (S312) is important for the efflux function of CaCdr1p (A. R. Holmes, unpublished data). Alanine scanning mutagenesis of Ca*CDR1* and expression in *S. cerevisiae* have revealed the importance of TMS11 for pump function (306). Heterologous expression in *S. cerevisiae* was also used to map naturally occurring single nucleotide polymorphisms and to analyze allelic variation in CaCdr1p and CaCdr2p (125, 136). CaCdr1p sequences were shown to be highly conserved, but an intra-allelic differential function was demonstrated for CaCdr2p, resulting from two adjacent nonsynonymous single nucleotide polymorphisms in TMS12, present in 81% of 61 clinical isolates (135).

As well as allowing a functional analysis of heterologous efflux pumps, *S. cerevisiae* can provide a robust in vivo screening platform. The expression of particular efflux pumps in ADΔ can confer antifungal drug resistance. If these pumps are inhibited, the cells can be chemosensitized to the antifungal. Thus, an efflux pump inhibitor will not inhibit the normal growth of cells expressing the efflux pump but will sensitize the cells to sub-MIC levels of their antifungal substrates. Screening for chemosensitization in agar diffusion assays or liquid growth assays has been used to identify pump inhibitors, as discussed further below. The functional overexpression of heterologous transporter molecules in a background depleted of endogenous transporters can also provide the high signal-to-noise ratios required for in vitro ATPase assays and the measurement of energy-dependent drug efflux in whole cells. These secondary screens provide experimental confirmation of ABC transporter inhibition and increase the robustness of the drug discovery process (181).

## OVERCOMING EFFLUX-MEDIATED ANTIFUNGAL DRUG RESISTANCE

An ideal antifungal agent, in addition to meeting pharmacological requirements, would not be susceptible to the development of resistance due to efflux mechanisms (48). There are four principal approaches to negating the impact of efflux, all of which depend on maintaining a high concentration of the antifungal agent at its site of action (Fig. 5). The simplest would be to use antifungals that are not substrates of efflux pumps; current examples are the polyenes and the echinocandins (Fig. 5, image 1). Another approach would be to protect the efficacy of antifungals that are subject to efflux by developing treatments that prevent efflux (Fig. 5, image 2a and b). A third approach would be to deplete cells of the energy required for drug efflux by inhibiting the plasma membrane $H^{+}$ ATPase (Fig. 5, image 3). Alternatively, it might be possible to design drugs with an enhanced rate of uptake and thus shift the balance between uptake and efflux so that a high intracellular concentration of the drug is maintained despite any upregulation of efflux (Fig. 5, image 4).

The polyene and echinocandin antifungals in clinical use are probably not substrates of fungal efflux pumps at therapeutic concentrations. There is one report that the echinocandin caspofungin is pumped by the CaCdr2p ABC transporter (320), although the efflux activity detected was very weak, was subject to highly specific conditions, and does not appear to be an important feature in clinical isolates (243). As the polyenes

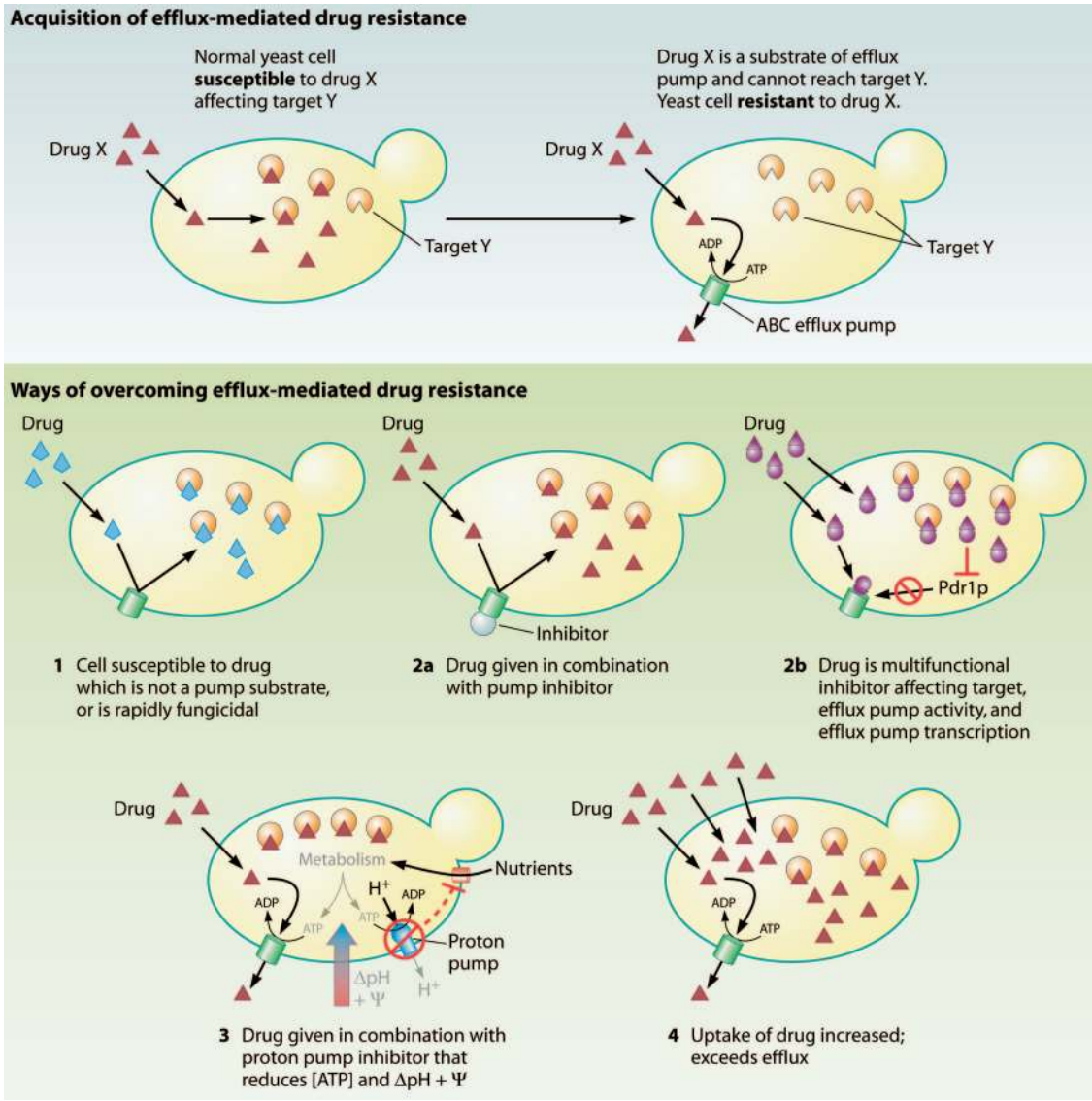


FIG. 5. Possible ways of overcoming efflux-mediated fungal drug resistance.

span the lipid bilayer, they may not be able to enter the drug-binding pocket of the transporter. Significant hydrophobicity is currently considered a prerequisite for substrate–multidrug-transporter interaction (204), and size is also reported to be a factor determining whether a hydrophobic compound can act as a substrate of at least one fungal ABC transporter, ScPdr5p (117). Echinocandins are large lipopeptide molecules with a molecular weight of about 1,200, and although they have a lipid side chain (Fig. 4) that presumably intercalates with the phospholipid bilayer of the cell membrane (75), they may not be sufficiently hydrophobic or the right size to interact with the efflux pump. Thus, new drug classes designed by taking into account such size and hydrophobicity constraints could avoid being ABC transporter substrates (Fig. 5, image 1). However, because efflux pumps have evolved large and flexible drug-binding sites to protect cells against a wide variety of toxic compounds (132), such rational drug design will be challenging.

Another approach has been to develop pump inhibitors that chemosensitize cells to existing effective drugs. A major impetus for this work has come from cancer research, because the human drug efflux pumps, such as P-gp, are important mediators of resistance to many anticancer drugs. Since the discovery of P-gp in 1976 (153), a wide range of compounds that are pump pseudosubstrates or that can modify pump activity by other mechanisms have been identified. The P-gp-inhibitory compounds identified include natural and synthetic polymers (88, 376, 392); modulators of H bond acceptor capacity (97); a globotriaosylceramide analog (78); P-gp substrates, such as FK506 (359); calcium channel modulators, such as verapamil (361); calmodulin inhibitors (105); and quinine analogs (360). Clinical trials of promising synthetic P-gp inhibitors, such as Tarquidar, have been undertaken but have been discontinued because of side effects that may be related to the protective function of P-gp in sites such as the blood-brain barrier (289). Strategies for the development of P-gp inhibitors have been

comprehensively reviewed by McDevitt and Callaghan (217) and include pharmacophore profiling, combinatorial chemistry, and structure-based design, in combination with high-throughput screening of lead compounds.

Such strategies can also be applied to the development of fungal ABC transporter inhibitors (Fig. 5, image 2). These inhibitors could affect the pump directly (Fig. 5, image 2a), either by binding as a pseudosubstrate (Pdr5p is thought to have at least three substrate-binding sites [116]), competitively or noncompetitively, and blocking access to the binding site, or by locking the molecule in a conformation that prevents the transport reaction cycle. A possible confounding factor for this approach is the observation that if one *S. cerevisiae* PDR transporter is inactivated, there is compensatory upregulation of other PDR pumps (168). In an alternative strategy, inhibitors could be designed that act indirectly on efflux, de-energizing the transporter by lowering the cytoplasmic ATP concentration (for ABC pumps) or depleting the electrochemical potential of the plasma membrane (for MFS transporters) (Fig. 5, image 3). The plasma membrane $H^+$ ATPase Pma1p is the primary proton pump in fungi. It maintains intracellular pH and generates the electrochemical gradient of the plasma membrane that is required for drug transport by MFS pumps. Pma1p drives a range of secondary transporters, such as those involved in ionic balance and nutrient uptake required for ATP synthesis, so it indirectly affects drug efflux by ABC transporters (Fig. 5, image 3). This approach has been validated: it has been demonstrated that inhibitors of *C. albicans* Pma1p also inhibit azole resistance at concentrations below the MIC (230). However, lowering the membrane potential could also result in decreased uptake of the antifungal drug if active diffusion/transport is involved.

The heterologous expression of fungal efflux pumps in *S. cerevisiae* has been used to screen for compounds that inhibit the transporters and thus chemosensitize the host yeast cells to pump substrates. The application of this screen to panels of xenobiotics (181), combinatorial peptide libraries (242), and libraries of natural products (346) has identified tacrolimus (FK506), milbemycins, enniatins (181), beauvericin (M. Niimi, unpublished), unnarmicins (346), and some octapeptides (242) as specific ABC pump inhibitors (Table 3 and Fig. 4). Ideally, these compounds will inhibit a range of clinically important efflux pumps, but not human orthologs. While some of the identified pump inhibitors may not be suitable for clinical application, they may be useful in pump protein crystallization and structural resolution. As the structures of fungal transporters become available, this will enable in silico modeling (25, 295) and structure-directed development of more effective pump inhibitors. It should be noted, however, that despite a great deal of promising investigation of pump inhibitors as chemosensitizers of tumor cells and bacteria, no successful products have entered clinical use.

Another way of inhibiting the activity of ABC transporter-mediated efflux, instead of directly targeting the ABC transporter protein, would be to modulate the transcription of the genes encoding ABC transporters (113, 349). In *S. cerevisiae* and *C. glabrata*, xenobiotic substrates (such as the antifungal azoles) of ABC transporters have recently been shown to directly bind to the Pdr1p family of transcription regulator proteins via a nuclear-receptor-like pathway, in a mechanism similar to the regulation of human MDR by the PXR (pregnane X receptor) nuclear receptor (349). The authors suggest that small-molecule antagonists could be developed that bind Pdr1p and its orthologs and thus prevent activation of the efflux pump genes (Fig. 5, image 2b). Alternatively, transcription of ABC transporter genes could be targeted by the use of RNA silencing (RNA interference [RNAi]). Human MDR genes have been successfully inhibited experimentally using RNAi (339). Limitations to anti-human MDR therapy with small RNAs include the longevity of small interfering RNA expression, the stability of the targeted message, and the efficient delivery of the RNAi. Delivery of RNAi as an adjunct to antifungal therapy with pump-susceptible drugs would have the same limitations. An additional problem is that although RNAi can be used in some fungi, such as *A. fumigatus* and *C. neoformans* (127, 194), it is ineffective in *C. albicans*. Target specificity might also be an issue. An advantage of directly inhibiting the pump protein is that inhibitors can be designed to act at the fungal cell surface and therefore are less likely to be subject to the development of efflux-mediated resistance.

One factor to be considered for all efflux-susceptible antifungals is that there can be a very rapid, reversible upregulation of efflux by fungi in response to such drugs. This is a stress response, as described above. Calcineurin mediates tolerance for several environmental stressors in fungal cells, and the use of calcineurin inhibitors has been suggested as a possible therapy in conjunction with azoles (342). Calcineurin inhibitors, such as FK506 (Fig. 4), render the normally fungistatic azole FLC fungicidal (363) and therefore would not only reduce the immediate upregulation of efflux and tolerance by negation of the calcineurin-mediated stress response, but would also act quickly enough to prevent the selection of mutants in the transporter regulatory pathway. Nonimmunosuppressive analogs of FK506, however, would have to be used for immuncompromised patients.

Finally, an alternative strategy for combating antifungal resistance due to efflux upregulation could be to increase uptake rather than decrease efflux of drugs (Fig. 5, image 4). This is a current strategy for combating human ABC transporter-mediated drug resistance; anticancer drugs can be more rapidly delivered to their intracellular targets by inclusion of multiple arginine residues, optimally eight (octaarginine [R8]) (83), thus overcoming the effects of MDR. R8 liposomes can be used to transport genes and drugs into cells by their rapid interaction with, and transport through, the plasma membrane and improved intracellular trafficking, avoiding lysosomal degradation (reviewed by Khalil et al. [164]). Fungal cell walls generally have an overall negative charge due to phosphate residues on mannoproteins. Although a positively charged triarginine motif prevents the uptake of peptides by yeast cells (229, 230), more than six arginines increase uptake (223, 229). The phosphomannan could, therefore, concentrate arginine-tagged drugs at the cell periphery, and provided the fungal transporter has a higher affinity for the drug than the high micromolar affinity shown by the cell wall site, the drug will be delivered to its target.

In summary, a number of ways to overcome antifungal resistance due to MDR can be envisaged. New antifungals that are fungicidal and not subject to efflux would be ideal. However, combination therapy with inhibitors of ABC transporters,

some of which have already been used in clinical trials, based either on direct inhibition of activity or on inhibition of their expression, shows promise for future successful treatment of fungal infections (151).

## CONCLUSION AND FUTURE PERSPECTIVES

IFIs are important diseases with high attributable morbidity and mortality that affect an ever-increasing population: the immunocompromised. Most life-threatening fungal infections could be treated more effectively if faster and more specific diagnostic technologies were available. For example, PCR amplification of rRNA intervening transcribed sequences followed by DNA pyrosequencing has the potential to halve the time needed for species-level fungal identification (33, 35). Translation of this technology into the clinic should allow the early identification of fungal species, including innately resistant species and those that are susceptible to the development of MDR. Pyrosequencing is already being applied in the laboratory to the detection of mutations responsible for fungal echinocandin resistance (381). This could be extended to detect transcriptional-regulator mutations responsible for efflux-mediated resistance, although multiple mutations of this type occur in CgPdr1p (93).

Molecular techniques could be adapted for clinical microbiology laboratories by automating aspects of the methodology, converting to a microfluidic format for PCR, and using robotic recovery of amplimers for DNA sequence analysis. DNA and RNA amplification systems that do not require PCR technology may assist DNA detection and automation. Finally, the hybridization of the amplified products to high-density oligonucleotide microarrays could be used to discriminate between matched sequences and their variants in a format suitable for rapid species identification by computer analysis. This could be achieved with a microfluidic technology that has been used to identify bacteria in complex microbial ecosystems (259).

Many fungal species, mostly *Candida* species, acquire azole resistance by the overexpression of efflux pumps, predominantly ABC transporters. This is not so for all fungi. In *Aspergillus* species, azole resistance appears to be dictated more by the drug target, Cyp51Ap (equivalent to Erg11p), than efflux pumps. Also, the contribution of efflux to the azole resistance of *C. neoformans* clinical isolates needs to be confirmed. The ability to functionally express individual fungal transporters in model organisms, such as *S. cerevisiae*, has enabled the analysis of pump function and screening for pump inhibitors. Although it may be difficult to identify broad-spectrum pump inhibitors with minimal toxicity, the structural resolution of fungal efflux pumps will make a major contribution to the understanding of these important eukaryotic membrane proteins and may help in rational drug design. As the structures for fungal transcription factors become available, it may even be possible to design multifunctional drugs that inhibit conventional targets, such as Erg11p, together with the transcription factors responsible for the overexpression of efflux pumps and the pumps themselves (228).

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## REFERENCES

1. **Abi-Said, D., E. Anaissie, O. Uzun, I. Raad, H. Pinzcowski, and S. Vartivarian.** 1997. The epidemiology of hematogenous candidiasis caused by different *Candida* species. Clin. Infect. Dis. **24:**1122–1128.
2. **Ahmad, A., M. A. Kabir, A. Kravets, E. Andaluz, G. Larriba, and E. Rustchenko.** 2008. Chromosome instability and unusual features of some widely used strains of *Candida albicans*. Yeast **25:**433–448.
3. **Akache, B., S. MacPherson, M. A. Sylvain, and B. Turcotte.** 2004. Complex interplay among regulators of drug resistance genes in *Saccharomyces cerevisiae*. J. Biol. Chem. **279:**27855–27860.
4. **Akins, R. A.** 2005. An update on antifungal targets and mechanisms of resistance in *Candida albicans*. Med. Mycol. **43:**285–318.
5. **Alarco, A. M., and M. Raymond.** 1999. The bZip transcription factor Cap1p is involved in multidrug resistance and oxidative stress response in *Candida albicans*. J. Bacteriol. **181:**700–708.
6. **Albertson, G. D., M. Niimi, R. D. Cannon, and H. F. Jenkinson.** 1996. Multiple efflux mechanisms are involved in *Candida albicans* fluconazole resistance. Antimicrob. Agents Chemother. **40:**2835–2841.
7. **Aller, A. I., R. Claro, C. Castro, C. Serrano, M. F. Colom, and E. Martin-Mazuelos.** 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. **Amoah, L. E., J. K. Lekostaj, and P. D. Roepe.** 2007. Heterologous expression and ATPase activity of mutant versus wild type PfMDR1 protein. Biochemistry **46:**6060–6073.
9. **Andes, D., A. Forrest, A. Lepak, J. Nett, K. Marchillo, and L. Lincoln.** 2006. Impact of antimicrobial dosing regimen on evolution of drug resistance in vivo: fluconazole and *Candida albicans*. Antimicrob. Agents Chemother. **50:**2374–2383.
10. **Andes, D., A. Lepak, J. Nett, L. Lincoln, and K. Marchillo.** 2006. In vivo fluconazole pharmacodynamics and resistance development in a previously susceptible *Candida albicans* population examined by microbiologic and transcriptional profiling. Antimicrob. Agents Chemother. **50:**2384–2394.
11. **Andrade, A. C., G. Del Sorbo, J. G. Van Nistelrooy, and M. A. Waard.** 2000. The ABC transporter AtrB from *Aspergillus nidulans* mediates resistance to all major classes of fungicides and some natural toxic compounds. Microbiology **146:**1987–1997.
12. **Andrade, A. C., J. G. Van Nistelrooy, R. B. Peery, P. L. Skatrud, and M. A. De Waard.** 2000. The role of ABC transporters from *Aspergillus nidulans* in protection against cytotoxic agents and in antibiotic production. Mol. Gen. Genet. **263:**966–977.
13. **Angermayr, K., W. Parson, G. Stoffler, and H. Haas.** 1999. Expression of *atrC*—encoding a novel member of the ATP binding cassette transporter family in *Aspergillus nidulans*—is sensitive to cycloheximide. Biochim. Biophys. Acta **1453:**304–310.
14. **Arnaud, M. B., M. C. Costanzo, M. S. Skrzypek, P. Shah, G. Binkley, C. Lane, S. R. Miyasato, and G. Sherlock.** 2007. Sequence resources at the *Candida* Genome Database. Nucleic Acids Res. **35:**D452–D456.
15. **Baccaglini, L., J. C. Atkinson, L. L. Patton, M. Glick, G. Ficarra, and D. E. Peterson.** 2007. Management of oral lesions in HIV-positive patients. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. **103**(Suppl. S50)**:**e1–e23.
16. **Bader, T., K. Schroppel, S. Bentink, N. Agabian, G. Kohler, and J. Morschhauser.** 2006. Role of calcineurin in stress resistance, morphogenesis, and virulence of a *Candida albicans* wild-type strain. Infect. Immun. **74:**4366–4369.
17. **Baixench, M. T., N. Aoun, M. Desnos-Ollivier, D. Garcia-Hermoso, S. Bretagne, S. Ramires, C. Piketty, and E. Dannaoui.** 2007. Acquired resistance to echinocandins in *Candida albicans*: case report and review. J. Antimicrob. Chemother. **59:**1076–1083.
18. **Bakos, E., I. Klein, E. Welker, K. Szabo, M. Muller, B. Sarkadi, and A. Varadi.** 1997. Characterization of the human multidrug resistance protein containing mutations in the ATP-binding cassette signature region. Biochem. J. **323:**777–783.
19. **Balan, I., A. M. Alarco, and M. Raymond.** 1997. The *Candida albicans CDR3* gene codes for an opaque-phase ABC transporter. J. Bacteriol. **179:**7210–7218.
20. **Balzi, E., M. Wang, S. Leterme, L. Van Dyck, and A. Goffeau.** 1994. *PDR5*, a novel yeast multidrug resistance conferring transporter controlled by the transcription regulator *PDR1*. J. Biol. Chem. **269:**2206–2214.
21. **Banerjee, D., G. Lelandais, S. Shukla, G. Mukhopadhyay, C. Jacq, F. Devaux, and R. Prasad.** 2008. Responses of pathogenic and nonpathogenic yeast species to steroids reveal the functioning and evolution of multidrug resistance transcriptional networks. Eukaryot. Cell **7:**68–77.
22. **Batova, M., S. Borecka-Melkusova, M. Simockova, V. Dzugasova, E. Goffa,**

**and J. Subik.** 2008. Functional characterization of the *CgPGS1* gene reveals a link between mitochondrial phospholipid homeostasis and drug resistance in *Candida glabrata*. Curr. Genet. **53:**313–322.
23. **Bauer, B. E., H. Wolfger, and K. Kuchler.** 1999. Inventory and function of yeast ABC proteins: about sex, stress, pleiotropic drug and heavy metal resistance. Biochim. Biophys. Acta **1461:**217–236.
24. **Bennett, J. E., K. Izumikawa, and K. A. Marr.** 2004. Mechanism of increased fluconazole resistance in *Candida glabrata* during prophylaxis. Antimicrob. Agents Chemother. **48:**1773–1777.
25. **Bhogal, N., and M. Balls.** 2008. Translation of new technologies: from basic research to drug discovery and development. Curr. Drug Discov. Technol. **5:**250–262.
26. **Bibi, E., P. Gros, and H. R. Kaback.** 1993. Functional expression of mouse mdr1 in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **90:**9209–9213.
27. **Bicanic, T., and T. S. Harrison.** 2004. Cryptococcal meningitis. Br. Med. Bull. **72:**99–118.
28. **Bii, C. C., K. Makimura, S. Abe, H. Taguchi, O. M. Mugasia, G. Revathi, N. C. Wamae, and S. Kamiya.** 2007. Antifungal drug susceptibility of *Cryptococcus neoformans* from clinical sources in Nairobi, Kenya. Mycoses **50:** 25–30.
29. **Bill, R. M.** 2001. Yeast—a panacea for the structure-function analysis of membrane proteins? Curr. Genet. **40:**157–171.
30. **Birley, H. D., E. M. Johnson, P. McDonald, C. Parry, P. B. Carey, and D. W. Warnock.** 1995. Azole drug resistance as a cause of clinical relapse in AIDS patients with cryptococcal meningitis. Int. J. STD AIDS **6:**353–355.
31. **Bissinger, P. H., and K. Kuchler.** 1994. Molecular cloning and expression of the *Saccharomyces cerevisiae STS1* gene product. A yeast ABC transporter conferring mycotoxin resistance. J. Biol. Chem. **269:**4180–4186.
32. **Blankenship, J. R., and J. Heitman.** 2005. Calcineurin is required for *Candida albicans* to survive calcium stress in serum. Infect. Immun. **73:** 5767–5774.
33. **Borman, A. M., C. J. Linton, S. J. Miles, and E. M. Johnson.** 2008. Molecular identification of pathogenic fungi. J. Antimicrob. Chemother. **61**(Suppl. 1)**:**i7–i12.
34. **Borst, A., M. T. Raimer, D. W. Warnock, C. J. Morrison, and B. A. Arthington-Skaggs.** 2005. Rapid acquisition of stable azole resistance by *Candida glabrata* isolates obtained before the clinical introduction of fluconazole. Antimicrob. Agents Chemother. **49:**783–787.
35. **Boyanton, B. L., Jr., R. A. Luna, L. R. Fasciano, K. G. Menne, and J. Versalovic.** 2008. DNA pyrosequencing-based identification of pathogenic *Candida* species by using the internal transcribed spacer 2 region. Arch. Pathol Lab. Med. **132:**667–674.
36. **Brandt, M. E., M. A. Pfaller, R. A. Hajjeh, R. J. Hamill, P. G. Pappas, A. L. Reingold, D. Rimland, and D. W. Warnock.** 2001. Trends in antifungal drug susceptibility of *Cryptococcus neoformans* isolates in the United States: 1992 to 1994 and 1996 to 1998. Antimicrob. Agents Chemother. **45:**3065–3069.
37. **Braun, B. R., M. van Het Hoog, C. d'Enfert, M. Martchenko, J. Dungan, A. Kuo, D. O. Inglis, M. A. Uhl, H. Hogues, M. Berriman, M. Lorenz, A. Levitin, U. Oberholzer, C. Bachewich, D. Harcus, A. Marcil, D. Dignard, T. Iouk, R. Zito, L. Frangeul, F. Tekaia, K. Rutherford, E. Wang, C. A. Munro, S. Bates, N. A. Gow, L. L. Hoyer, G. Kohler, J. Morschhauser, G. Newport, S. Znaidi, M. Raymond, B. Turcotte, G. Sherlock, M. Costanzo, J. Ihmels, J. Berman, D. Sanglard, N. Agabian, A. P. Mitchell, A. D. Johnson, M. Whiteway, and A. Nantel.** 2005. A human-curated annotation of the *Candida albicans* genome. PLoS Genet. **1:**36–57.
38. **Brun, S., T. Berges, P. Poupard, C. Vauzelle-Moreau, G. Renier, D. Chabasse, and J. P. Bouchara.** 2004. Mechanisms of azole resistance in petite mutants of *Candida glabrata*. Antimicrob. Agents Chemother. **48:**1788–1796.
39. **Bruno, V. M., and A. P. Mitchell.** 2005. Regulation of azole drug susceptibility by *Candida albicans* protein kinase CK2. Mol. Microbiol. **56:**559–573.
40. **Buckley, M.** 2008. The fungal kingdom—diverse and essential roles in earth's ecosystem. A report based on a colloquium held November 2–4, 2007. American Academy of Microbiology, Washington, DC.
41. **Cai, J., and P. Gros.** 2003. Overexpression, purification, and functional characterization of ATP-binding cassette transporters in the yeast, *Pichia pastoris*. Biochim. Biophys. Acta **1610:**63–76.
42. **Calabrese, D., J. Bille, and D. Sanglard.** 2000. A novel multidrug efflux transporter gene of the major facilitator superfamily from *Candida albicans* (*FLU1*) conferring resistance to fluconazole. Microbiology **146:**2743–2754.
43. **Cannon, R. D., A. R. Holmes, A. B. Mason, and B. C. Monk.** 1995. Oral *Candida*: clearance, colonization, or candidiasis? J. Dent. Res. **74:**1152–1161.
44. **Cannon, R. D., E. Lamping, A. R. Holmes, K. Niimi, K. Tanabe, M. Niimi, and B. C. Monk.** 2007. *Candida albicans* drug resistance another way to cope with stress. Microbiology **153:**3211–3217.
45. **Carvajal, E., H. B. van den Hazel, A. Cybularz-Kolaczkowska, E. Balzi, and A. Goffeau.** 1997. Molecular and phenotypic characterization of yeast *PDR1* mutants that show hyperactive transcription of various ABC multidrug transporter genes. Mol. Gen. Genet. **256:**406–415.
46. **Casadevall, A., and J. R. Perfect.** 1998. *Cryptococcus neoformans.* ASM Press, Washington, DC.
47. **Chamilos, G., and D. P. Kontoyiannis.** 2005. Update on antifungal drug resistance mechanisms of *Aspergillus fumigatus*. Drug Resist. Updat. **8:**344–358.
48. **Chapman, S. W., D. C. Sullivan, and J. D. Cleary.** 2008. In search of the holy grail of antifungal therapy. Trans Am. Clin. Climatol. Assoc. **119:**197–216.
49. **Chau, A. S., C. A. Mendrick, F. J. Sabatelli, D. Loebenberg, and P. M. McNicholas.** 2004. Application of real-time quantitative PCR to molecular analysis of *Candida albicans* strains exhibiting reduced susceptibility to azoles. Antimicrob. Agents Chemother. **48:**2124–2131.
50. **Chen, J., H. Li, R. Li, D. Bu, and Z. Wan.** 2005. Mutations in the *cyp51A* gene and susceptibility to itraconazole in *Aspergillus fumigatus* serially isolated from a patient with lung aspergilloma. J. Antimicrob. Chemother. **55:**31–37.
51. **Chloupkova, M., A. Pickert, J. Y. Lee, S. Souza, Y. T. Trinh, S. M. Connelly, M. E. Dumont, M. Dean, and I. L. Urbatsch.** 2007. Expression of 25 human ABC transporters in the yeast *Pichia pastoris* and characterization of the purified *ABCC3* ATPase activity. Biochemistry **46:**7992–8003.
52. **Clark, F. S., T. Parkinson, C. A. Hitchcock, and N. A. Gow.** 1996. Correlation between rhodamine 123 accumulation and azole sensitivity in *Candida* species: possible role for drug efflux in drug resistance. Antimicrob. Agents Chemother. **40:**419–425.
53. **Clark, T. A., and R. A. Hajjeh.** 2002. Recent trends in the epidemiology of invasive mycoses. Curr. Opin. Infect. Dis. **15:**569–574.
54. **Cogliati, M., M. C. Esposto, D. L. Clarke, B. L. Wickes, and M. A. Viviani.** 2001. Origin of *Cryptococcus neoformans* var. *neoformans* diploid strains. J. Clin. Microbiol. **39:**3889–3894.
55. **Coste, A., A. Selmecki, A. Forche, D. Diogo, M. E. Bougnoux, C. d'Enfert, J. Berman, and D. Sanglard.** 2007. Genotypic evolution of azole resistance mechanisms in sequential *Candida albicans* isolates. Eukaryot. Cell **6:**1889–1904.
56. **Coste, A., V. Turner, F. Ischer, J. Morschhauser, A. Forche, A. Selmecki, J. Berman, J. Bille, and D. Sanglard.** 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.
57. **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.
58. **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.
59. **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.
60. **Cowen, L. E., and S. Lindquist.** 2005. Hsp90 potentiates the rapid evolution of new traits: drug resistance in diverse fungi. Science **309:**2185–2189.
61. **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.
62. **Cruz, M. C., A. L. Goldstein, J. R. Blankenship, M. Del Poeta, D. Davis, M. E. Cardenas, J. R. Perfect, J. H. McCusker, and J. Heitman.** 2002. Calcineurin is essential for survival during membrane stress in *Candida albicans*. EMBO J. **21:**546–559.
63. **Cui, Z., D. Hirata, and T. Miyakawa.** 1999. Functional analysis of the promoter of the yeast *SNQ2* gene encoding a multidrug resistance transporter that confers the resistance to 4-nitroquinoline N-oxide. Biosci. Biotechnol. Biochem. **63:**162–167.
64. **da Silva Ferreira, M. E., J. L. Capellaro, E. dos Reis Marques, I. Malavazi, D. Perlin, S. Park, J. B. Anderson, A. L. Colombo, B. A. Arthington-Skaggs, M. H. Goldman, and G. H. Goldman.** 2004. In vitro evolution of itraconazole resistance in *Aspergillus fumigatus* involves multiple mechanisms of resistance. Antimicrob. Agents Chemother. **48:**4405–4413.
65. **Davey, K. G., E. M. Johnson, A. D. Holmes, A. Szekely, and D. W. Warnock.** 1998. In vitro susceptibility of *Cryptococcus neoformans* isolates to fluconazole and itraconazole. J. Antimicrob. Chemother. **42:**217–220.
66. **Davies, A. N., S. R. Brailsford, and D. Beighton.** 2006. Oral candidosis in patients with advanced cancer. Oral Oncol. **42:**698–702.
67. **Dawson, R. J., and K. P. Locher.** 2006. Structure of a bacterial multidrug ABC transporter. Nature **443:**180–185.
68. **Dawson, R. J., and K. P. Locher.** 2007. Structure of the multidrug ABC transporter Sav1866 from *Staphylococcus aureus* in complex with AMP-PNP. FEBS Lett. **581:**935–938.
69. **Dayan, G., H. Baubichon-Cortay, J. M. Jault, J. C. Cortay, G. Deleage, and A. Di Pietro.** 1996. Recombinant N-terminal nucleotide-binding domain from mouse P-glycoprotein. Overexpression, purification, and role of cysteine 430. J. Biol. Chem. **271:**11652–11658.
70. **De Backer, M. D., T. Ilyina, X. J. Ma, S. Vandoninck, W. H. Luyten, and H. Vanden Bossche.** 2001. Genomic profiling of the response of *Candida*

*albicans* to itraconazole treatment using a DNA microarray. Antimicrob. Agents Chemother. **45:**1660–1670.
71. **Decottignies, A., and A. Goffeau.** 1997. Complete inventory of the yeast ABC proteins. Nat. Genet. **15:**137–145.
72. **Decottignies, A., A. M. Grant, J. W. Nichols, H. de Wet, D. B. McIntosh, and A. Goffeau.** 1998. ATPase and multidrug transport activities of the overexpressed yeast ABC protein Yor1p. J. Biol. Chem. **273:**12612–12622.
73. **Del Sorbo, G., A. C. Andrade, J. G. Van Nistelrooy, J. A. Van Kan, E. Balzi, and M. A. De Waard.** 1997. Multidrug resistance in *Aspergillus nidulans* involves novel ATP-binding cassette transporters. Mol. Gen. Genet. **254:** 417–426.
74. **Del Sorbo, G., H. Schoonbeek, and M. A. De Waard.** 2000. Fungal transporters involved in efflux of natural toxic compounds and fungicides. Fungal Genet. Biol. **30:**1–15.
75. **Denning, D. W.** 2003. Echinocandin antifungal drugs. Lancet **362:**1142–1151.
76. **Denning, D. W., K. Venkateswarlu, K. L. Oakley, M. J. Anderson, N. J. Manning, D. A. Stevens, D. W. Warnock, and S. L. Kelly.** 1997. Itraconazole resistance in *Aspergillus fumigatus*. Antimicrob. Agents Chemother. **41:** 1364–1368.
77. **DeRisi, J., B. van den Hazel, P. Marc, E. Balzi, P. Brown, C. Jacq, and A. Goffeau.** 2000. Genome microarray analysis of transcriptional activation in multidrug resistance yeast mutants. FEBS Lett. **470:**156–160.
78. **De Rosa, M. F., C. Ackerley, B. Wang, S. Ito, D. M. Clarke, and C. Lingwood.** 2008. Inhibition of multidrug resistance by adamantylgb3, a globotriaosylceramide analog. J. Biol. Chem. **283:**4501–4511.
79. **Diaz-Guerra, T. M., E. Mellado, M. Cuenca-Estrella, and J. L. Rodriguez-Tudela.** 2003. A point mutation in the 14α-sterol demethylase gene *cyp51A* contributes to itraconazole resistance in *Aspergillus fumigatus*. Antimicrob. Agents Chemother. **47:**1120–1124.
80. **Dismukes, W. E.** 1988. Cryptococcal meningitis in patients with AIDS. J. Infect. Dis. **157:**624–628.
81. **Dodgson, A. R., K. J. Dodgson, C. Pujol, M. A. Pfaller, and D. R. Soll.** 2004. Clade-specific flucytosine resistance is due to a single nucleotide change in the *FUR1* gene of *Candida albicans*. Antimicrob. Agents Chemother. **48:** 2223–2227.
82. **Doring, F., T. Michel, A. Rosel, M. Nickolaus, and H. Daniel.** 1998. Expression of the mammalian renal peptide transporter *PEPT2* in the yeast *Pichia pastoris* and applications of the yeast system for functional analysis. Mol. Membr. Biol. **15:**79–88.
83. **Dubikovskaya, E. A., S. H. Thorne, T. H. Pillow, C. H. Contag, and P. A. Wender.** 2008. Overcoming multidrug resistance of small-molecule therapeutics through conjugation with releasable octaarginine transporters. Proc. Natl. Acad. Sci. USA **105:**10128–10133.
84. **Dujon, B., D. Sherman, G. Fischer, P. Durrens, S. Casaregola, I. Lafontaine, J. De Montigny, C. Marck, C. Neuveglise, E. Talla, N. Goffard, L. Frangeul, M. Aigle, V. Anthouard, A. Babour, V. Barbe, S. Barnay, S. Blanchin, J. M. Beckerich, E. Beyne, C. Bleykasten, A. Boisrame, J. Boyer, L. Cattolico, F. Confanioleri, A. De Daruvar, L. Despons, E. Fabre, C. Fairhead, H. Ferry-Dumazet, A. Groppi, F. Hantraye, C. Hennequin, N. Jauniaux, P. Joyet, R. Kachouri, A. Kerrest, R. Koszul, M. Lemaire, I. Lesur, L. Ma, H. Muller, J. M. Nicaud, M. Nikolski, S. Oztas, O. Ozier-Kalogeropoulos, S. Pellenz, S. Potier, G. F. Richard, M. L. Straub, A. Suleau, D. Swennen, F. Tekaia, M. Wesolowski-Louvel, E. Westhof, B. Wirth, M. Zeniou-Meyer, I. Zivanovic, M. Bolotin-Fukuhara, A. Thierry, C. Bouchier, B. Caudron, C. Scarpelli, C. Gaillardin, J. Weissenbach, P. Wincker, and J. L. Souciet.** 2004. Genome evolution in yeasts. Nature **430:**35–44.
85. **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.
86. **Edgar, R. C.** 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinform. **5:**113.
87. **Egner, R., B. E. Bauer, and K. Kuchler.** 2000. The transmembrane domain 10 of the yeast Pdr5p ABC antifungal efflux pump determines both substrate specificity and inhibitor susceptibility. Mol. Microbiol. **35:**1255–1263.
88. **Elamanchili, P., C. McEachern, and H. Burt.** 2009. Reversal of multidrug resistance by methoxypolyethylene glycol-block-polycaprolactone diblock copolymers through the inhibition of P-glycoprotein function. J. Pharm. Sci. **98:**945–958.
89. **Ernst, R., R. Klemm, L. Schmitt, and K. Kuchler.** 2005. Yeast ATP-binding cassette transporters: cellular cleaning pumps. Methods Enzymol. **400:**460–484.
90. **Ernst, R., P. Kueppers, C. M. Klein, T. Schwarzmueller, K. Kuchler, and L. Schmitt.** 2008. A mutation of the H-loop selectively affects rhodamine transport by the yeast multidrug ABC transporter Pdr5. Proc. Natl. Acad. Sci. USA **105:**5069–5074.
91. **Falcon-Perez, J. M., M. Martinez-Burgos, J. Molano, M. J. Mazon, and P. Eraso.** 2001. Domain interactions in the yeast ATP binding cassette transporter Ycf1p: intragenic suppressor analysis of mutations in the nucleotide binding domains. J. Bacteriol. **183:**4761–4770.
92. **Felsenstein, J.** 1989. PHYLIP: Phylogeny Inference Package (version 3.2). Cladistics **5:**164–166.
93. **Ferrari, S., F. Ischer, D. Calabrese, B. Posteraro, M. Sanguinetti, G. Fadda, B. Rohde, C. Bauser, O. Bader, and D. Sanglard.** 2009. Gain of function mutations in *CgPDR1* of *Candida glabrata* not only mediate antifungal resistance but also enhance virulence. PLoS Pathog **5:**e1000268.
94. **Ferreira, M. E., A. L. Colombo, I. Paulsen, Q. Ren, J. Wortman, J. Huang, M. H. Goldman, and G. H. Goldman.** 2005. The ergosterol biosynthesis pathway, transporter genes, and azole resistance in *Aspergillus fumigatus*. Med. Mycol. **43**(Suppl. 1)**:**S313–S319.
95. **Ferreira-Pereira, A., S. Marco, A. Decottignies, J. Nader, A. Goffeau, and J. L. Rigaud.** 2003. Three-dimensional reconstruction of the *Saccharomyces cerevisiae* multidrug resistance protein Pdr5p. J. Biol. Chem. **278:**11995–11999.
96. **Fidel, P. L., Jr., J. A. Vazquez, and J. D. Sobel.** 1999. *Candida glabrata*: review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. Clin. Microbiol. Rev. **12:**80–96.
97. **Fish, P. V., N. S. Barta, D. L. Gray, T. Ryckmans, A. Stobie, F. Wakenhut, and G. A. Whitlock.** 2008. Derivatives of (3*S*)-*N*-(biphenyl-2-ylmethyl)pyrrolidin-3-amine as selective noradrenaline reuptake inhibitors: reducing P-gp mediated efflux by modulation of H-bond acceptor capacity. Bioorg. Med. Chem. Lett. **18:**4355–4359.
98. **Fitzpatrick, D. A., M. E. Logue, J. E. Stajich, and G. Butler.** 2006. A fungal phylogeny based on 42 complete genomes derived from supertree and combined gene analysis. BMC Evol. Biol. **6:**99.
99. **Fling, M. E., J. Kopf, A. Tamarkin, J. A. Gorman, H. A. Smith, and Y. Koltin.** 1991. Analysis of a *Candida albicans* gene that encodes a novel mechanism for resistance to benomyl and methotrexate. Mol. Gen. Genet. **227:**318–329.
100. **Franz, R., S. Michel, and J. Morschhauser.** 1998. A fourth gene from the *Candida albicans* CDR family of ABC transporters. Gene **220:**91–98.
101. **Fraser, J. A., S. S. Giles, E. C. Wenink, S. G. Geunes-Boyer, J. R. Wright, S. Diezmann, A. Allen, J. E. Stajich, F. S. Dietrich, J. R. Perfect, and J. Heitman.** 2005. Same-sex mating and the origin of the Vancouver Island *Cryptococcus gattii* outbreak. Nature **437:**1360–1364.
102. **Fraser, J. A., R. L. Subaran, C. B. Nichols, and J. Heitman.** 2003. Recapitulation of the sexual cycle of the primary fungal pathogen *Cryptococcus neoformans* var. *gattii*: implications for an outbreak on Vancouver Island, Canada. Eukaryot. Cell **2:**1036–1045.
103. **Fukuoka, T., D. A. Johnston, C. A. Winslow, M. J. de Groot, C. Burt, C. A. Hitchcock, and S. G. Filler.** 2003. Genetic basis for differential activities of fluconazole and voriconazole against *Candida krusei*. Antimicrob. Agents Chemother. **47:**1213–1219.
104. **Galagan, J. E., S. E. Calvo, C. Cuomo, L. J. Ma, J. R. Wortman, S. Batzoglou, S. I. Lee, M. Basturkmen, C. C. Spevak, J. Clutterbuck, V. Kapitonov, J. Jurka, C. Scazzocchio, M. Farman, J. Butler, S. Purcell, S. Harris, G. H. Braus, O. Draht, S. Busch, C. D'Enfert, C. Bouchier, G. H. Goldman, D. Bell-Pedersen, S. Griffiths-Jones, J. H. Doonan, J. Yu, K. Vienken, A. Pain, M. Freitag, E. U. Selker, D. B. Archer, M. A. Penalva, B. R. Oakley, M. Momany, T. Tanaka, T. Kumagai, K. Asai, M. Machida, W. C. Nierman, D. W. Denning, M. Caddick, M. Hynes, M. Paoletti, R. Fischer, B. Miller, P. Dyer, M. S. Sachs, S. A. Osmani, and B. W. Birren.** 2005. Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. Nature **438:**1105–1115.
105. **Ganapathi, R., and D. Grabowski.** 1983. Enhancement of sensitivity to adriamycin in resistant P388 leukemia by the calmodulin inhibitor trifluoperazine. Cancer Res. **43:**3696–3699.
106. **Garcia-Effron, G., A. Dilger, L. Alcazar-Fuoli, S. Park, E. Mellado, and D. S. Perlin.** 2008. Rapid detection of triazole antifungal resistance in *Aspergillus fumigatus*. J. Clin. Microbiol. **46:**1200–1206.
107. **Garcia-Sanchez, S., S. Aubert, I. Iraqui, G. Janbon, J. M. Ghigo, and C. d'Enfert.** 2004. *Candida albicans* biofilms: a developmental state associated with specific and stable gene expression patterns. Eukaryot. Cell **3:**536–545.
108. **Gaur, M., D. Choudhury, and R. Prasad.** 2005. Complete inventory of ABC proteins in human pathogenic yeast, *Candida albicans*. J. Mol. Microbiol. Biotechnol. **9:**3–15.
109. **Gauthier, C., S. Weber, A. M. Alarco, O. Alqawi, R. Daoud, E. Georges, and M. Raymond.** 2003. Functional similarities and differences between *Candida albicans* Cdr1p and Cdr2p transporters. Antimicrob. Agents Chemother. **47:**1543–1554.
110. **Gbelska, Y., J. J. Krijger, and K. D. Breunig.** 2006. Evolution of gene families: the multidrug resistance transporter genes in five related yeast species. FEMS Yeast Res. **6:**345–355.
111. **Germann, U. A., M. C. Willingham, I. Pastan, and M. M. Gottesman.** 1990. Expression of the human multidrug transporter in insect cells by a recombinant baculovirus. Biochemistry **29:**2295–2303.
112. **Gerngross, T. U.** 2004. Advances in the production of human therapeutic proteins in yeasts and filamentous fungi. Nat. Biotechnol. **22:**1409–1414.
113. **Goffeau, A.** 2008. Drug resistance: the fight against fungi. Nature **452:**541–542.
114. **Goffeau, A., B. G. Barrell, H. Bussey, R. W. Davis, B. Dujon, H. Feldmann, F. Galibert, J. D. Hoheisel, C. Jacq, M. Johnston, E. J. Louis, H. W. Mewes,**

**Y. Murakami, P. Philippsen, H. Tettelin, and S. G. Oliver.** 1996. Life with 6000 genes. Science **274:**546, 563-567.
115. **Goldstein, N. E., E. Genden, and R. S. Morrison.** 2008. Palliative care for patients with head and neck cancer: "I would like a quick return to a normal lifestyle." JAMA **299:**1818–1825.
116. **Golin, J., S. V. Ambudkar, and L. May.** 2007. The yeast Pdr5p multidrug transporter: how does it recognize so many substrates? Biochem. Biophys. Res. Commun. **356:**1–5.
117. **Golin, J., Z. N. Kon, C. P. Wu, J. Martello, L. Hanson, S. Supernavage, S. V. Ambudkar, and Z. E. Sauna.** 2007. Complete inhibition of the Pdr5p multidrug efflux pump ATPase activity by its transport substrate clotrimazole suggests that GTP as well as ATP may be used as an energy source. Biochemistry **46:**13109–13119.
118. **Gomez-Lopez, A., G. Garcia-Effron, E. Mellado, A. Monzon, J. L. Rodriguez-Tudela, and M. Cuenca-Estrella.** 2003. In vitro activities of three licensed antifungal agents against Spanish clinical isolates of *Aspergillus* spp. Antimicrob. Agents Chemother. **47:**3085–3088.
119. **Griffith, D. A., C. Delipala, J. Leadsham, S. M. Jarvis, and D. Oesterhelt.** 2003. A novel yeast expression system for the overproduction of quality-controlled membrane proteins. FEBS Lett. **553:**45–50.
120. **Groll, A. H., and H. Kolve.** 2004. Antifungal agents: in vitro susceptibility testing, pharmacodynamics, and prospects for combination therapy. Eur. J. Clin. Microbiol. Infect. Dis. **23:**256–270.
121. **Gros, P., L. Beaudet, and I. L. Urbatsch.** 1998. Yeast as an expression system for the study of P-glycoprotein and other ABC transporters. Acta Physiol. Scand. Suppl. **643:**219–225.
122. **Gulshan, K., and W. S. Moye-Rowley.** 2007. Multidrug resistance in fungi. Eukaryot. Cell **6:**1933–1942.
123. **Hajjeh, R. A., A. N. Sofair, L. H. Harrison, G. M. Lyon, B. A. Arthington-Skaggs, S. A. Mirza, M. Phelan, J. Morgan, W. Lee-Yang, M. A. Ciblak, L. E. Benjamin, L. T. Sanza, S. Huie, S. F. Yeo, M. E. Brandt, and D. W. Warnock.** 2004. Incidence of bloodstream infections due to *Candida* species and in vitro susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. J. Clin. Microbiol. **42:**1519–1527.
124. **Halliday, C. L., and D. A. Carter.** 2003. Clonal reproduction and limited dispersal in an environmental population of *Cryptococcus neoformans* var. *gattii* isolates from Australia. J. Clin. Microbiol. **41:**703–711.
125. **Haque, A., V. Rai, B. S. Bahal, S. Shukla, A. A. Lattif, G. Mukhopadhyay, and R. Prasad.** 2007. Allelic variants of ABC drug transporter Cdr1p in clinical isolates of *Candida albicans*. Biochem. Biophys. Res. Commun. **352:**491–497.
126. **Helbok, R., S. Pongpakdee, S. Yenjun, W. Dent., R. Beer, P. Lackner, P. Bunyaratvej, B. Prasert, A. Vejjajiva, and E. Schmutzhard.** 2006. Chronic meningitis in Thailand. Clinical characteristics, laboratory data and outcome in patients with specific reference to tuberculosis and cryptococcosis. Neuroepidemiology **26:**37–44.
127. **Henry, C., I. Mouyna, and J. P. Latge.** 2007. Testing the efficacy of RNA interference constructs in *Aspergillus fumigatus*. Curr. Genet. **51:**277–284.
128. **Henry, K. W., J. T. Nickels, and T. D. Edlind.** 2000. Upregulation of *ERG* genes in *Candida* species by azoles and other sterol biosynthesis inhibitors. Antimicrob. Agents Chemother. **44:**2693–2700.
129. **Herbrecht, R., D. W. Denning, T. F. Patterson, J. E. Bennett, R. E. Greene, J. W. Oestmann, W. V. Kern, K. A. Marr, P. Ribaud, O. Lortholary, R. Sylvester, R. H. Rubin, J. R. Wingard, P. Stark, C. Durand, D. Caillot, E. Thiel, P. H. Chandrasekar, M. R. Hodges, H. T. Schlamm, P. F. Troke, and B. de Pauw.** 2002. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N. Engl. J. Med. **347:**408–415.
130. **Higgins, C. F.** 2001. ABC transporters: physiology, structure and mechanism—an overview. Res. Microbiol. **152:**205–210.
131. **Higgins, C. F.** 1994. Flip-flop: the transmembrane translocation of lipids. Cell **79:**393–395.
132. **Higgins, C. F.** 2007. Multiple molecular mechanisms for multidrug resistance transporters. Nature **446:**749–757.
133. **Hiller, D., D. Sanglard, and J. Morschhauser.** 2006. Overexpression of the *MDR1* gene is sufficient to confer increased resistance to toxic compounds in *Candida albicans*. Antimicrob. Agents Chemother. **50:**1365–1371.
134. **Hirata, D., K. Yano, K. Miyahara, and T. Miyakawa.** 1994. *Saccharomyces cerevisiae YDR1*, which encodes a member of the ATP-binding cassette (ABC) superfamily, is required for multidrug resistance. Curr. Genet. **26:**285–294.
135. **Holmes, A. R., Y. H. Lin, K. Niimi, E. Lamping, M. Keniya, M. Niimi, K. Tanabe, B. C. Monk, and R. D. Cannon.** 2008. ABC transporter Cdr1p contributes more than Cdr2p does to fluconazole efflux in fluconazole-resistant *Candida albicans* clinical isolates. Antimicrob. Agents Chemother. **52:**3851–3862.
136. **Holmes, A. R., S. Tsao, E. Lamping, K. Niimi, B. C. Monk, K. Tanabe, M. Niimi, and R. D. Cannon.** 2006. Amino acid residues affecting drug pump function in *Candida albicans*—*C. albicans* drug pump function. Nippon Ishinkin Gakkai Zasshi **47:**275–281.
137. **Holmes, A. R., S. Tsao, S. W. Ong, E. Lamping, K. Niimi, B. C. Monk, M. Niimi, A. Kaneko, B. R. Holland, J. Schmid, and R. D. Cannon.** 2006. Heterozygosity and functional allelic variation in the *Candida albicans* efflux pump genes *CDR1* and *CDR2*. Mol. Microbiol. **62:**170–186.
138. **Holmes, C. B., E. Losina, R. P. Walensky, Y. Yazdanpanah, and K. A. Freedberg.** 2003. Review of human immunodeficiency virus type 1-related opportunistic infections in sub-Saharan Africa. Clin. Infect. Dis. **36:**652–662.
139. **Hsueh, P. R., Y. J. Lau, Y. C. Chuang, J. H. Wan, W. K. Huang, J. M. Shyr, J. J. Yan, K. W. Yu, J. J. Wu, W. C. Ko, Y. C. Yang, Y. C. Liu, L. J. Teng, C. Y. Liu, and K. T. Luh.** 2005. Antifungal susceptibilities of clinical isolates of *Candida* species, *Cryptococcus neoformans*, and *Aspergillus* species from Taiwan: surveillance of multicenter antimicrobial resistance in Taiwan program data from 2003. Antimicrob. Agents Chemother. **49:**512–517.
140. **Hu, W., S. Sillaots, S. Lemieux, J. Davison, S. Kauffman, A. Breton, A. Linteau, C. Xin, J. Bowman, J. Becker, B. Jiang, and T. Roemer.** 2007. Essential gene identification and drug target prioritization in *Aspergillus fumigatus*. PLoS Pathog. **3:**e24.
141. **Hughes, T. R., M. J. Marton, A. R. Jones, C. J. Roberts, R. Stoughton, C. D. Armour, H. A. Bennett, E. Coffey, H. Dai, Y. D. He, M. J. Kidd, A. M. King, M. R. Meyer, D. Slade, P. Y. Lum, S. B. Stepaniants, D. D. Shoemaker, D. Gachotte, K. Chakrabortty, J. Simon, M. Bard, and S. H. Friend.** 2000. Functional discovery via a compendium of expression profiles. Cell **102:**109–126.
142. **Hughes, T. R., C. J. Roberts, H. Dai, A. R. Jones, M. R. Meyer, D. Slade, J. Burchard, S. Dow, T. R. Ward, M. J. Kidd, S. H. Friend, and M. J. Marton.** 2000. Widespread aneuploidy revealed by DNA microarray expression profiling. Nat. Genet. **25:**333–337.
143. **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.
144. **Huson, D. H., D. C. Richter, C. Rausch, T. Dezulian, M. Franz, and R. Rupp.** 2007. Dendroscope: an interactive viewer for large phylogenetic trees. BMC Bioinform. **8:**460.
145. **Idnurm, A., Y. S. Bahn, K. Nielsen, X. Lin, J. A. Fraser, and J. Heitman.** 2005. Deciphering the model pathogenic fungus *Cryptococcus neoformans*. Nat. Rev. Microbiol. **3:**753–764.
146. **Izumikawa, K., H. Kakeya, H. F. Tsai, B. Grimberg, and J. E. Bennett.** 2003. Function of *Candida glabrata* ABC transporter gene, *PDH1*. Yeast **20:**249–261.
147. **Jacobsen, M. D., N. A. Gow, M. C. Maiden, D. J. Shaw, and F. C. Odds.** 2007. Strain typing and determination of population structure of *Candida krusei* by multilocus sequence typing. J. Clin. Microbiol. **45:**317–323.
148. **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.
149. **Janbon, G., F. Sherman, and E. Rustchenko.** 1998. Monosomy of a specific chromosome determines L-sorbose utilization: a novel regulatory mechanism in *Candida albicans*. Proc. Natl. Acad. Sci. USA **95:**5150–5155.
150. **Jidenko, M., R. C. Nielsen, T. L. Sorensen, J. V. Moller, M. le Maire, P. Nissen, and C. Jaxel.** 2005. Crystallization of a mammalian membrane protein overexpressed in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **102:**11687–11691.
151. **Johnson, M. D., and J. R. Perfect.** 2007. Combination antifungal therapy: what can and should we expect? Bone Marrow Transplant. **40:**297–306.
152. **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.
153. **Juliano, R. L., and V. Ling.** 1976. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim. Biophys. Acta **455:**152–162.
154. **Kanafani, Z. A., and J. R. Perfect.** 2008. Antimicrobial resistance: resistance to antifungal agents: mechanisms and clinical impact. Clin. Infect. Dis. **46:**120–128.
155. **Karababa, M., A. T. Coste, B. Rognon, J. Bille, and D. Sanglard.** 2004. Comparison of gene expression profiles of *Candida albicans* azole-resistant clinical isolates and laboratory strains exposed to drugs inducing multidrug transporters. Antimicrob. Agents Chemother. **48:**3064–3079.
156. **Karababa, M., E. Valentino, G. Pardini, A. T. Coste, J. Bille, and D. Sanglard.** 2006. *CRZ1*, a target of the calcineurin pathway in *Candida albicans*. Mol. Microbiol. **59:**1429–1451.
157. **Karthaus, M., and O. A. Cornely.** 2007. Treatment options in candidaemia. Mycoses **50**(Suppl. 1)**:**44–49.
158. **Katiyar, S. K., and T. D. Edlind.** 2001. Identification and expression of multidrug resistance-related ABC transporter genes in *Candida krusei*. Med. Mycol. **39:**109–116.
159. **Katzmann, D. J., E. A. Epping, and W. S. Moye-Rowley.** 1999. Mutational disruption of plasma membrane trafficking of *Saccharomyces cerevisiae* Yor1p, a homologue of mammalian multidrug resistance protein. Mol. Cell. Biol. **19:**2998–3009.
160. **Katzmann, D. J., T. C. Hallstrom, Y. Mahe, and W. S. Moye-Rowley.** 1996. Multiple Pdr1p/Pdr3p binding sites are essential for normal expression of

the ATP binding cassette transporter protein-encoding gene *PDR5*. J. Biol. Chem. **271:**23049–23054.

161. **Kelly, S. L., D. C. Lamb, D. E. Kelly, N. J. Manning, J. Loeffler, H. Hebart, U. Schumacher, and H. Einsele.** 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.
162. **Kelly, S. L., D. C. Lamb, M. Taylor, A. J. Corran, B. C. Baldwin, and W. G. Powderly.** 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.
163. **Kerr, K. M., Z. E. Sauna, and S. V. Ambudkar.** 2001. Correlation between steady-state ATP hydrolysis and vanadate-induced ADP trapping in human P-glycoprotein. Evidence for ADP release as the rate-limiting step in the catalytic cycle and its modulation by substrates. J. Biol. Chem. **276:**8657–8664.
164. **Khalil, I. A., K. Kogure, S. Futaki, and H. Harashima.** 2008. Octaarginine-modified liposomes: enhanced cellular uptake and controlled intracellular trafficking. Int. J. Pharm. **354:**39–48.
165. **Kidd, S. E., F. Hagen, R. L. Tscharke, M. Huynh, K. H. Bartlett, M. Fyfe, L. Macdougall, T. Boekhout, K. J. Kwon-Chung, and W. Meyer.** 2004. A rare genotype of *Cryptococcus gattii* caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). Proc. Natl. Acad. Sci. USA **101:**17258–17263.
166. **Klingspor, L., and S. Jalal.** 2006. Molecular detection and identification of *Candida* and *Aspergillus* spp. from clinical samples using real-time PCR. Clin. Microbiol. Infect. **12:**745–753.
167. **Kohli, A., V. Gupta, S. Krishnamurthy, S. E. Hasnain, and R. Prasad.** 2001. Specificity of drug transport mediated by *CaMDR1*: a major facilitator of *Candida albicans*. J. Biosci. **26:**333–339.
168. **Kolaczkowska, A., M. Kolaczkowski, A. Goffeau, and W. S. Moye-Rowley.** 2008. Compensatory activation of the multidrug transporters Pdr5p, Snq2p, and Yor1p by Pdr1p in *Saccharomyces cerevisiae*. FEBS Lett. **582:**977–983.
169. **Kolaczkowski, M., and A. Goffeau.** 1997. Active efflux by multidrug transporters as one of the strategies to evade chemotherapy and novel practical implications of yeast pleiotropic drug resistance. Pharmacol. Ther. **76:**219–242.
170. **Kontoyiannis, D. P., and G. P. Bodey.** 2002. Invasive aspergillosis in 2002: an update. Eur. J. Clin. Microbiol. Infect. Dis. **21:**161–172.
171. **Kontoyiannis, D. P., and R. E. Lewis.** 2002. Antifungal drug resistance of pathogenic fungi. Lancet **359:**1135–1144.
172. **Kontoyiannis, D. P., M. S. Lionakis, R. E. Lewis, G. Chamilos, M. Healy, C. Perego, A. Safdar, H. Kantarjian, R. Champlin, T. J. Walsh, and I. I. Raad.** 2005. Zygomycosis in a tertiary-care cancer center in the era of *Aspergillus*-active antifungal therapy: a case-control observational study of 27 recent cases. J. Infect. Dis. **191:**1350–1360.
173. **Kralli, A., S. P. Bohen, and K. R. Yamamoto.** 1995. LEM1, an ATP-binding-cassette transporter, selectively modulates the biological potency of steroid hormones. Proc. Natl. Acad. Sci. USA **92:**4701–4705.
174. **Kruppa, M., and R. Calderone.** 2006. Two-component signal transduction in human fungal pathogens. FEMS Yeast Res. **6:**149–159.
175. **Kuchler, K., and J. Thorner.** 1992. Functional expression of human mdr1 in the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **89:**2302–2306.
176. **Kumamoto, C. A.** 2008. Niche-specific gene expression during *C. albicans* infection. Curr. Opin. Microbiol. **11:**325–330.
177. **Kumarasamy, N., S. Vallabhaneni, T. P. Flanigan, K. H. Mayer, and S. Solomon.** 2005. Clinical profile of HIV in India. Indian J. Med. Res. **121:**377–394.
178. **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.
179. **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.
180. **Lamb, D. C., A. Corran, B. C. Baldwin, J. Kwon-Chung, and S. L. Kelly.** 1995. Resistant P45051A1 activity in azole antifungal tolerant *Cryptococcus neoformans* from AIDS patients. FEBS Lett. **368:**326–330.
181. **Lamping, E., B. C. Monk, K. Niimi, A. R. Holmes, S. Tsao, K. Tanabe, M. Niimi, Y. Uehara, and R. D. Cannon.** 2007. Characterization of three classes of membrane proteins involved in fungal azole resistance by functional hyperexpression in *Saccharomyces cerevisiae*. Eukaryot. Cell **6:**1150–1165.
182. **Lamping, E., A. Ranchod, K. Nakamura, J. D. A. Tyndall, K. Niimi, A. Holmes, M. Niimi, and R. D. Cannon.** 2009. Abc1p is a multidrug efflux transporter that tips the balance in favour of innate azole resistance in *Candida krusei*. Antimicrob. Agents Chemother. **53:**334–369.
183. **Lamping, E., K. Tanabe, M. Niimi, Y. Uehara, B. C. Monk, and R. D. Cannon.** 2005. Characterization of the *Saccharomyces cerevisiae sec6-4* mutation and tools to create *S. cerevisiae* strains containing the *sec6-4* allele. Gene **361:**57–66.
184. **Langfelder, K., S. Gattung, and A. A. Brakhage.** 2002. A novel method used to delete a new *Aspergillus fumigatus* ABC transporter-encoding gene. Curr. Genet. **41:**268–274.
185. **Larsen, B., S. Anderson, A. Brockman, M. Essmann, and M. Schmidt.** 2006. Key physiological differences in *Candida albicans CDR1* induction by steroid hormones and antifungal drugs. Yeast **23:**795–802.
186. **Le Crom, S., F. Devaux, P. Marc, X. Zhang, W. S. Moye-Rowley, and C. Jacq.** 2002. New insights into the pleiotropic drug resistance network from genome-wide characterization of the *YRR1* transcription factor regulation system. Mol. Cell. Biol. **22:**2642–2649.
187. **Legrand, M., A. Forche, A. Selmecki, C. Chan, D. T. Kirkpatrick, and J. Berman.** 2008. Haplotype mapping of a diploid non-meiotic organism using existing and induced aneuploidies. PLoS Genet. **4:**e1.
188. **Leonard, G. D., O. Polgar, and S. E. Bates.** 2002. ABC transporters and inhibitors: new targets, new agents. Curr. Opin. Investig. Drugs **3:**1652–1659.
189. **Lerner-Marmarosh, N., K. Gimi, I. L. Urbatsch, P. Gros, and A. E. Senior.** 1999. Large scale purification of detergent-soluble P-glycoprotein from *Pichia pastoris* cells and characterization of nucleotide binding properties of wild-type, Walker A, and Walker B mutant proteins. J. Biol. Chem. **274:**34711–34718.
190. **Li, Y., and W. A. Prinz.** 2004. ATP-binding cassette (ABC) transporters mediate nonvesicular, raft-modulated sterol movement from the plasma membrane to the endoplasmic reticulum. J. Biol. Chem. **279:**45226–45234.
191. **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.
192. **Lin, X., and J. Heitman.** 2006. The biology of the *Cryptococcus neoformans* species complex. Annu. Rev. Microbiol. **60:**69–105.
193. **Lin, X., K. Nielsen, S. Patel, and J. Heitman.** 2008. Impact of mating type, serotype, and ploidy on the virulence of *Cryptococcus neoformans*. Infect. Immun. **76:**2923–2938.
194. **Liu, H., T. R. Cottrell, L. M. Pierini, W. E. Goldman, and T. L. Doering.** 2002. RNA interference in the pathogenic fungus *Cryptococcus neoformans*. Genetics **160:**463–470.
195. **Liu, T. T., S. Znaidi, K. S. Barker, L. Xu, R. Homayouni, S. Saidane, J. Morschhauser, A. Nantel, M. Raymond, and P. D. Rogers.** 2007. Genome-wide expression and location analyses of the *Candida albicans* Tac1p regulon. Eukaryot. Cell **6:**2122–2138.
196. **Loeffler, J., and D. A. Stevens.** 2003. Antifungal drug resistance. Clin. Infect. Dis. **36:**S31–S41.
197. **Loftus, B. J., E. Fung, P. Roncaglia, D. Rowley, P. Amedeo, D. Bruno, J. Vamathevan, M. Miranda, I. J. Anderson, J. A. Fraser, J. E. Allen, I. E. Bosdet, M. R. Brent, R. Chiu, T. L. Doering, M. J. Donlin, C. A. D'Souza, D. S. Fox, V. Grinberg, J. Fu, M. Fukushima, B. J. Haas, J. C. Huang, G. Janbon, S. J. Jones, H. L. Koo, M. I. Krzywinski, J. K. Kwon-Chung, K. B. Lengeler, R. Maiti, M. A. Marra, R. E. Marra, C. A. Mathewson, T. G. Mitchell, M. Pertea, F. R. Riggs, S. L. Salzberg, J. E. Schein, A. Shvartsbeyn, H. Shin, M. Shumway, C. A. Specht, B. B. Suh, A. Tenney, T. R. Utterback, B. L. Wickes, J. R. Wortman, N. H. Wye, J. W. Kronstad, J. K. Lodge, J. Heitman, R. W. Davis, C. M. Fraser, and R. W. Hyman.** 2005. The genome of the basidiomycetous yeast and human pathogen *Cryptococcus neoformans*. Science **307:**1321–1324.
198. **Loo, T. W., and D. M. Clarke.** 2005. Recent progress in understanding the mechanism of P-glycoprotein-mediated drug efflux. J. Membr. Biol. **206:**173–185.
199. **Lucau-Danila, A., G. Lelandais, Z. Kozovska, V. Tanty, T. Delaveau, F. Devaux, and C. Jacq.** 2005. Early expression of yeast genes affected by chemical stress. Mol. Cell. Biol. **25:**1860–1868.
200. **Macauley-Patrick, S., M. L. Fazenda, B. McNeil, and L. M. Harvey.** 2005. Heterologous protein production using the *Pichia pastoris* expression system. Yeast **22:**249–270.
201. **Maebashi, K., M. Niimi, M. Kudoh, F. J. Fischer, K. Makimura, K. Niimi, R. J. Piper, K. Uchida, M. Arisawa, R. D. Cannon, and H. Yamaguchi.** 2001. Mechanisms of fluconazole resistance in *Candida albicans* isolates from Japanese AIDS patients. J. Antimicrob. Chemother. **47:**527–536.
202. **Maertens, J., I. Raad, G. Petrikkos, M. Boogaerts, D. Selleslag, F. B. Petersen, C. A. Sable, N. A. Kartsonis, A. Ngai, A. Taylor, T. F. Patterson, D. W. Denning, and T. J. Walsh.** 2004. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. Clin. Infect. Dis. **39:**1563–1571.
203. **Magee, B. B., and P. T. Magee.** 2000. Induction of mating in *Candida albicans* by construction of MTLa and MTLα strains. Science **289:**310–313.
204. **Maki, N., K. Moitra, C. Silver, P. Ghosh, A. Chattopadhyay, and S. Dey.** 2006. Modulator-induced interference in functional cross talk between the substrate and the ATP sites of human P-glycoprotein. Biochemistry **45:**2739–2751.
205. **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.
206. **Mamnun, Y. M., R. Pandjaitan, Y. Mahe, A. Delahodde, and K. Kuchler.** 2002. The yeast zinc finger regulators Pdr1p and Pdr3p control pleiotropic drug resistance (PDR) as homo- and heterodimers in vivo. Mol. Microbiol. **46:**1429–1440.

207. **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.
208. **Manfredi, R., L. Calza, and F. Chiodo.** 2003. AIDS-associated *Cryptococcus* infection before and after the highly active antiretroviral therapy era: emerging management problems. Int. J. Antimicrob. Agents **22:**449–452.
209. **Manfredi, R., L. Calza, and F. Chiodo.** 2001. Lack of change in the distribution of AIDS-defining opportunistic diseases and the related degree of immunodeficiency during the periods before and after the introduction of highly active antiretroviral therapy. Eur. J. Clin. Microbiol. Infect. Dis. **20:**410–413.
210. **Mann, P. A., R. M. Parmegiani, S. Q. Wei, C. A. Mendrick, X. Li, D. Loebenberg, B. DiDomenico, R. S. Hare, S. S. Walker, and P. M. McNicholas.** 2003. Mutations in *Aspergillus fumigatus* resulting in reduced susceptibility to posaconazole appear to be restricted to a single amino acid in the cytochrome P450 14α-demethylase. Antimicrob. Agents Chemother. **47:**577–581.
211. **Mao, Q., G. Conseil, A. Gupta, S. P. Cole, and J. D. Unadkat.** 2004. Functional expression of the human breast cancer resistance protein in *Pichia pastoris*. Biochem. Biophys. Res. Commun. **320:**730–737.
212. **Marger, M. D., and M. H. Saier, Jr.** 1993. A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport. Trends Biochem. Sci. **18:**13–20.
213. **Marichal, P., J. Gorrens, M. C. Coene, L. Le Jeune, and H. Vanden Bossche.** 1995. Origin of differences in susceptibility of *Candida krusei* to azole antifungal agents. Mycoses **38:**111–117.
214. **Marichal, P., L. Koymans, S. Willemsens, D. Bellens, P. Verhasselt, W. Luyten, M. Borgers, F. C. Ramaekers, F. C. Odds, and H. V. Bossche.** 1999. Contribution of mutations in the cytochrome P450 14α-demethylase (Erg11p, Cyp51p) to azole resistance in *Candida albicans*. Microbiology **145:**2701–2713.
215. **Marr, K. A.** 2008. Fungal infections in hematopoietic stem cell transplant recipients. Med. Mycol. **46:**293–302.
216. **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.
217. **McDevitt, C. A., and R. Callaghan.** 2007. How can we best use structural information on P-glycoprotein to design inhibitors? Pharmacol. Ther. **113:**429–441.
218. **Mellado, E., T. M. Diaz-Guerra, M. Cuenca-Estrella, and J. L. Rodriguez-Tudela.** 2001. Identification of two different 14-alpha sterol demethylase-related genes (*cyp51A* and *cyp51B*) in *Aspergillus fumigatus* and other *Aspergillus* species. J. Clin. Microbiol. **39:**2431–2438.
219. **Mellado, E., G. Garcia-Effron, L. Alcazar-Fuoli, M. Cuenca-Estrella, and J. L. Rodriguez-Tudela.** 2004. Substitutions at methionine 220 in the 14α-sterol demethylase (*Cyp51A*) of *Aspergillus fumigatus* are responsible for resistance in vitro to azole antifungal drugs. Antimicrob. Agents Chemother. **48:**2747–2750.
220. **Mellado, E., G. Garcia-Effron, L. Alcazar-Fuoli, W. J. Melchers, P. E. Verweij, M. Cuenca-Estrella, and J. L. Rodriguez-Tudela.** 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.
221. **Mellado, E., G. Garcia-Effron, M. J. Buitrago, L. Alcazar-Fuoli, M. Cuenca-Estrella, and J. L. Rodriguez-Tudela.** 2005. Targeted gene disruption of the 14-alpha sterol demethylase (*cyp51A*) in *Aspergillus fumigatus* and its role in azole drug susceptibility. Antimicrob. Agents Chemother. **49:**2536–2538.
222. **Mirza, S. A., M. Phelan, D. Rimland, E. Graviss, R. Hamill, M. E. Brandt, T. Gardner, M. Sattah, G. P. de Leon, W. Baughman, and R. A. Hajjeh.** 2003. The changing epidemiology of cryptococcosis: an update from population-based active surveillance in 2 large metropolitan areas, 1992–2000. Clin. Infect. Dis. **36:**789–794.
223. **Mitchell, D. J., D. T. Kim, L. Steinman, C. G. Fathman, and J. B. Rothbard.** 2000. Polyarginine enters cells more efficiently than other polycationic homopolymers. J. Pept. Res. **56:**318–325.
224. **Miyazaki, H., Y. Miyazaki, A. Geber, T. Parkinson, C. Hitchcock, D. J. Falconer, D. J. Ward, K. Marsden, and J. E. Bennett.** 1998. Fluconazole resistance associated with drug efflux and increased transcription of a drug transporter gene, *PDH1*, in *Candida glabrata*. Antimicrob. Agents Chemother. **42:**1695–1701.
225. **Mnaimneh, S., A. P. Davierwala, J. Haynes, J. Moffat, W. T. Peng, W. Zhang, X. Yang, J. Pootoolal, G. Chua, A. Lopez, M. Trochesset, D. Morse, N. J. Krogan, S. L. Hiley, Z. Li, Q. Morris, J. Grigull, N. Mitsakakis, C. J. Roberts, J. F. Greenblatt, C. Boone, C. A. Kaiser, B. J. Andrews, and T. R. Hughes.** 2004. Exploration of essential gene functions via titratable promoter alleles. Cell **118:**31–44.
226. **Mondon, P., R. Petter, G. Amalfitano, R. Luzzati, E. Concia, I. Polacheck, and K. J. Kwon-Chung.** 1999. Heteroresistance to fluconazole and voriconazole in *Cryptococcus neoformans*. Antimicrob. Agents Chemother. **43:**1856–1861.
227. **Monge, R. A., E. Roman, C. Nombela, and J. Pla.** 2006. The MAP kinase signal transduction network in *Candida albicans*. Microbiology **152:**905–912.
228. **Monk, B. C., and A. Goffeau.** 2008. Outwitting multidrug resistance to antifungals. Science **321:**367–369.
229. **Monk, B. C., and D. R. Harding.** 2005. Peptide motifs for cell-surface intervention: application to anti-infective and biopharmaceutical development. BioDrugs **19:**261–278.
230. **Monk, B. C., K. Niimi, S. Lin, A. Knight, T. B. Kardos, R. D. Cannon, R. Parshot, A. King, D. Lun, and D. R. Harding.** 2005. Surface-active fungicidal D-peptide inhibitors of the plasma membrane proton pump that block azole resistance. Antimicrob. Agents Chemother. **49:**57–70.
231. **Moore, C. B., N. Sayers, J. Mosquera, J. Slaven, and D. W. Denning.** 2000. Antifungal drug resistance in *Aspergillus*. J. Infect. **41:**203–220.
232. **Morschhauser, J., K. S. Barker, T. T. Liu, B. W. J. Bla, R. Homayouni, and P. D. Rogers.** 2007. The transcription factor Mrr1p controls expression of the *MDR1* efflux pump and mediates multidrug resistance in *Candida albicans*. PLoS Pathog. **3:**e164.
233. **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.
234. **Mukherjee, P. K., D. J. Sheehan, C. A. Hitchcock, and M. A. Ghannoum.** 2005. Combination treatment of invasive fungal infections. Clin. Microbiol. Rev. **18:**163–194.
235. **Mukherjee, P. K., G. Zhou, R. Munyon, and M. A. Ghannoum.** 2005. Candida biofilm: a well-designed protected environment. Med. Mycol. **43:**191–208.
236. **Murray, C. K., F. L. Loo, D. R. Hospenthal, L. C. Cancio, J. A. Jones, S. H. Kim, J. B. Holcomb, C. E. Wade, and S. E. Wolf.** 2008. Incidence of systemic fungal infection and related mortality following severe burns. Burns **34:**1108–1112.
237. **Nakamura, K., M. Niimi, K. Niimi, A. R. Holmes, J. E. Yates, A. Decottignies, B. C. Monk, A. Goffeau, and R. D. Cannon.** 2001. Functional expression of *Candida albicans* drug efflux pump Cdr1p in a *Saccharomyces cerevisiae* strain deficient in membrane transporters. Antimicrob. Agents Chemother. **45:**3366–3374.
238. **Nakayama, H., M. Izuta, N. Nakayama, M. Arisawa, and Y. Aoki.** 2000. Depletion of the squalene synthase (*ERG9*) gene does not impair growth of *Candida glabrata* in mice. Antimicrob. Agents Chemother. **44:**2411–2418.
239. **Nakayama, H., K. Tanabe, M. Bard, W. Hodgson, S. Wu, D. Takemori, T. Aoyama, N. S. Kumaraswami, L. Metzler, Y. Takano, H. Chibana, and M. Niimi.** 2007. The *Candida glabrata* putative sterol transporter gene *CgAUS1* protects cells against azoles in the presence of serum. J. Antimicrob. Chemother. **60:**1264–1272.
240. **Nascimento, A. M., G. H. Goldman, S. Park, S. A. Marras, G. Delmas, U. Oza, K. Lolans, M. N. Dudley, P. A. Mann, and D. S. Perlin.** 2003. Multiple resistance mechanisms among *Aspergillus fumigatus* mutants with high-level resistance to itraconazole. Antimicrob. Agents Chemother. **47:**1719–1726.
241. **Nierman, W. C., A. Pain, M. J. Anderson, J. R. Wortman, H. S. Kim, J. Arroyo, M. Berriman, K. Abe, D. B. Archer, C. Bermejo, J. Bennett, P. Bowyer, D. Chen, M. Collins, R. Coulsen, R. Davies, P. S. Dyer, M. Farman, N. Fedorova, N. Fedorova, T. V. Feldblyum, R. Fischer, N. Fosker, A. Fraser, J. L. Garcia, M. J. Garcia, A. Goble, G. H. Goldman, K. Gomi, S. Griffith-Jones, R. Gwilliam, B. Haas, H. Haas, D. Harris, H. Horiuchi, J. Huang, S. Humphray, J. Jimenez, N. Keller, H. Khouri, K. Kitamoto, T. Kobayashi, S. Konzack, R. Kulkarni, T. Kumagai, A. Lafon, J. P. Latge, W. Li, A. Lord, C. Lu, W. H. Majoros, G. S. May, B. L. Miller, Y. Mohamoud, M. Molina, M. Monod, I. Mouyna, S. Mulligan, L. Murphy, S. O'Neil, I. Paulsen, M. A. Penalva, M. Pertea, C. Price, B. L. Pritchard, M. A. Quail, E. Rabbinowitsch, N. Rawlins, M. A. Rajandream, U. Reichard, H. Renauld, G. D. Robson, S. Rodriguez de Cordoba, J. M. Rodriguez-Pena, C. M. Ronning, S. Rutter, S. L. Salzberg, M. Sanchez, J. C. Sanchez-Ferrero, D. Saunders, K. Seeger, R. Squares, S. Squares, M. Takeuchi, F. Tekaia, G. Turner, C. R. Vazquez de Aldana, J. Weidman, O. White, J. Woodward, J. H. Yu, C. Fraser, J. E. Galagan, K. Asai, M. Machida, N. Hall, B. Barrell, and D. W. Denning.** 2005. Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. Nature **438:**1151–1156.
242. **Niimi, K., D. R. Harding, R. Parshot, A. King, D. J. Lun, A. Decottignies, M. Niimi, S. Lin, R. D. Cannon, A. Goffeau, and B. C. Monk.** 2004. Chemosensitization of fluconazole resistance in *Saccharomyces cerevisiae* and pathogenic fungi by a D-octapeptide derivative. Antimicrob. Agents Chemother. **48:**1256–1271.
243. **Niimi, K., K. Maki, F. Ikeda, A. R. Holmes, E. Lamping, M. Niimi, B. C. Monk, and R. D. Cannon.** 2006. Overexpression of *Candida albicans CDR1*, *CDR2*, or *MDR1* does not produce significant changes in echinocandin susceptibility. Antimicrob. Agents Chemother. **50:**1148–1155.
244. **Niimi, M., Y. Nagai, K. Niimi, S. Wada, R. D. Cannon, Y. Uehara, and B. C. Monk.** 2002. Identification of two proteins induced by exposure of the pathogenic fungus *Candida glabrata* to fluconazole. J. Chromatogr. B **782:**245–252.

245. **Niimi, M., K. Niimi, Y. Takano, A. R. Holmes, F. J. Fischer, Y. Uehara, and R. D. Cannon.** 2004. Regulated overexpression of *CDR1* in *Candida albicans* confers multidrug resistance. J. Antimicrob. Chemother. **54:**999–1006.
246. **Noble, S. M., and A. D. Johnson.** 2007. Genetics of *Candida albicans*, a diploid human fungal pathogen. Annu. Rev. Genet. **41:**193–211.
247. **Nomura, S. M., S. Kondoh, W. Asayama, A. Asada, S. Nishikawa, and K. Akiyoshi.** 2008. Direct preparation of giant proteo-liposomes by in vitro membrane protein synthesis. J. Biotechnol. **133:**190–195.
248. **Nourani, A., D. Papajova, A. Delahodde, C. Jacq, and J. Subik.** 1997. Clustered amino acid substitutions in the yeast transcription regulator Pdr3p increase pleiotropic drug resistance and identify a new central regulatory domain. Mol. Gen. Genet. **256:**397–405.
249. **Odds, F. C., and R. Bernaerts.** 1994. CHROMagar *Candida*, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J. Clin. Microbiol. **32:**1923–1929.
250. **Odds, F. C., M. E. Bougnoux, D. J. Shaw, J. M. Bain, A. D. Davidson, D. Diogo, M. D. Jacobsen, M. Lecomte, S. Y. Li, A. Tavanti, M. C. Maiden, N. A. Gow, and C. d'Enfert.** 2007. Molecular phylogenetics of *Candida albicans*. Eukaryot. Cell **6:**1041–1052.
251. **Odds, F. C., A. J. Brown, and N. A. Gow.** 2003. Antifungal agents: mechanisms of action. Trends Microbiol. **11:**272–279.
252. **Odds, F. C., A. D. Davidson, M. D. Jacobsen, A. Tavanti, J. A. Whyte, C. C. Kibbler, D. H. Ellis, M. C. Maiden, D. J. Shaw, and N. A. Gow.** 2006. *Candida albicans* strain maintenance, replacement, and microvariation demonstrated by multilocus sequence typing. J. Clin. Microbiol. **44:**3647–3658.
253. **Odds, F. C., and M. D. Jacobsen.** 2008. Multilocus sequence typing of pathogenic *Candida* species. Eukaryot. Cell **7:**1075–1084.
254. **Ogawa, A., T. Hashida-Okado, M. Endo, H. Yoshioka, T. Tsuruo, K. Takesako, and I. Kato.** 1998. Role of ABC transporters in aureobasidin A resistance. Antimicrob. Agents Chemother. **42:**755–761.
255. **O'Gorman, C. M., H. T. Fuller, and P. S. Dyer.** 2009. Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. Nature **457:**471–474.
256. **Oliveira, K., G. Haase, C. Kurtzman, J. J. Hyldig-Nielsen, and H. Stender.** 2001. Differentiation of *Candida albicans* and *Candida dubliniensis* by fluorescent in situ hybridization with peptide nucleic acid probes. J. Clin. Microbiol. **39:**4138–4141.
257. **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.
258. **Orozco, A. S., L. M. Higginbotham, C. A. Hitchcock, T. Parkinson, D. Falconer, A. S. Ibrahim, M. A. Ghannoum, and S. G. Filler.** 1998. Mechanism of fluconazole resistance in *Candida krusei*. Antimicrob. Agents Chemother. **42:**2645–2649.
259. **Ottesen, E. A., J. W. Hong, S. R. Quake, and J. R. Leadbetter.** 2006. Microfluidic digital PCR enables multigene analysis of individual environmental bacteria. Science **314:**1464–1467.
260. **Panwar, S. L., R. Pasrija, and R. Prasad.** 2008. Membrane homoeostasis and multidrug resistance in yeast. Biosci. Rep. **28:**217–228.
261. **Paoletti, M., C. Rydholm, E. U. Schwier, M. J. Anderson, G. Szakacs, F. Lutzoni, J. P. Debeaupuis, J. P. Latge, D. W. Denning, and P. S. Dyer.** 2005. Evidence for sexuality in the opportunistic fungal pathogen *Aspergillus fumigatus*. Curr. Biol. **15:**1242–1248.
262. **Pappas, P. G., J. H. Rex, J. Lee, R. J. Hamill, R. A. Larsen, W. Powderly, C. A. Kauffman, N. Hyslop, J. E. Mangino, S. Chapman, H. W. Horowitz, J. E. Edwards, and W. E. Dismukes.** 2003. A prospective observational study of candidemia: epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. Clin. Infect. Dis. **37:**634–643.
263. **Pasqualotto, A. C., and D. W. Denning.** 2008. New and emerging treatments for fungal infections. J. Antimicrob. Chemother. **61**(Suppl. 1)**:**i19–i30.
264. **Pasrija, R., D. Banerjee, and R. Prasad.** 2007. Structure and function analysis of CaMdr1p, a major facilitator superfamily antifungal efflux transporter protein of *Candida albicans*: identification of amino acid residues critical for drug/$H^+$ transport. Eukaryot. Cell **6:**443–453.
265. **Paterson, D. L., and N. Singh.** 1999. Invasive aspergillosis in transplant recipients. Medicine (Baltimore) **78:**123–138.
266. **Paugam, A., J. Dupouy-Camet, P. Blanche, J. P. Gangneux, C. Tourte-Schaefer, and D. Sicard.** 1994. Increased fluconazole resistance of *Cryptococcus neoformans* isolated from a patient with AIDS and recurrent meningitis. Clin. Infect. Dis. **19:**975–976.
267. **Paulsen, I. T., M. H. Brown, and R. A. Skurray.** 1996. Proton-dependent multidrug efflux systems. Microbiol. Rev. **60:**575–608.
268. **Peetermans, W., H. Bobbaers, J. Verhaegen, and J. Vandepitte.** 1993. Fluconazole-resistant *Cryptococcus neoformans* var. *gattii* in an AIDS patient. Acta Clin. Belg. **48:**405–409.
269. **Perea, S., J. L. Lopez-Ribot, W. R. Kirkpatrick, R. K. McAtee, R. A. Santillan, M. Martinez, D. Calabrese, D. Sanglard, and T. F. Patterson.** 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.
270. **Perepnikhatka, V., F. J. Fischer, M. Niimi, R. A. Baker, R. D. Cannon, Y. K. Wang, F. Sherman, and E. Rustchenko.** 1999. Specific chromosome alterations in fluconazole-resistant mutants of *Candida albicans*. J. Bacteriol. **181:**4041–4049.
271. **Perkins, A., A. Gomez-Lopez, E. Mellado, J. L. Rodriguez-Tudela, and M. Cuenca-Estrella.** 2005. Rates of antifungal resistance among Spanish clinical isolates of *Cryptococcus neoformans* var. *neoformans.* J. Antimicrob. Chemother. **56:**1144–1147.
272. **Perlin, D. S.** 2007. Resistance to echinocandin-class antifungal drugs. Drug Resist. Updat. **10:**121–130.
273. **Perumal, P., S. Mekala, and W. L. Chaffin.** 2007. Role for cell density in antifungal drug resistance in *Candida albicans* biofilms. Antimicrob. Agents Chemother. **51:**2454–2463.
274. **Pfaller, M. A.** 1996. Nosocomial candidiasis: emerging species, reservoirs, and modes of transmission. Clin. Infect. Dis. **22**(Suppl. 2)**:**S89–S94.
275. **Pfaller, M. A., and D. J. Diekema.** 2007. Epidemiology of invasive candidiasis: a persistent public health problem. Clin. Microbiol. Rev. **20:**133–163.
276. **Pfaller, M. A., and D. J. Diekema.** 2002. Role of sentinel surveillance of candidemia: trends in species distribution and antifungal susceptibility. J. Clin. Microbiol. **40:**3551–3557.
277. **Pfaller, M. A., and D. J. Diekema.** 2004. Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates of *Candida*. Clin. Microbiol. Infect. **10**(Suppl. 1)**:**11–23.
278. **Pfaller, M. A., D. J. Diekema, D. L. Gibbs, V. A. Newell, E. Nagy, S. Dobiasova, M. Rinaldi, R. Barton, and A. Veselov.** 2008. *Candida krusei*, a multidrug-resistant opportunistic fungal pathogen: geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. J. Clin. Microbiol. **46:**515–521.
279. **Pfaller, M. A., S. A. Messer, L. Boyken, C. Rice, S. Tendolkar, R. J. Hollis, G. V. Doern, and D. J. Diekema.** 2005. Global trends in the antifungal susceptibility of *Cryptococcus neoformans* (1990 to 2004). J. Clin. Microbiol. **43:**2163–2167.
280. **Pfaller, M. A., S. A. Messer, L. Boyken, S. Tendolkar, R. J. Hollis, and D. J. Diekema.** 2008. Selection of a surrogate agent (fluconazole or voriconazole) for initial susceptibility testing of posaconazole against *Candida* spp.: results from a global antifungal surveillance program. J. Clin. Microbiol. **46:**551–559.
281. **Pfaller, M. A., P. G. Pappas, and J. R. Wingard.** 2006. Invasive fungal pathogens: current epidemiological trends. Clin. Infect. Dis. **43:**S3–S14.
282. **Piper, P., Y. Mahe, S. Thompson, R. Pandjaitan, C. Holyoak, R. Egner, M. Muhlbauer, P. Coote, and K. Kuchler.** 1998. The pdr12 ABC transporter is required for the development of weak organic acid resistance in yeast. EMBO J. **17:**4257–4265.
283. **Pontón, J., M. D. Moragues, and G. Quindós.** 2002. Non-culture-based diagnostics, p. 395–425. *In* R. Calderone (ed.), *Candida* and candidiasis. ASM Press, Washington, DC.
284. **Posteraro, B., M. Sanguinetti, D. Sanglard, M. La Sorda, S. Boccia, L. Romano, G. Morace, and G. Fadda.** 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.
285. **Powderly, W. G.** 1993. Cryptococcal meningitis and AIDS. Clin. Infect. Dis. **17:**837–842.
286. **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.
287. **Prasad, R., N. A. Gaur, M. Gaur, and S. S. Komath.** 2006. Efflux pumps in drug resistance of *Candida*. Infect. Disord. Drug Target. **6:**69–83.
288. **Pujol, C., M. A. Pfaller, and D. R. Soll.** 2004. Flucytosine resistance is restricted to a single genetic clade of *Candida albicans*. Antimicrob. Agents Chemother. **48:**262–266.
289. **Pusztai, L., P. Wagner, N. Ibrahim, E. Rivera, R. Theriault, D. Booser, F. W. Symmans, F. Wong, G. Blumenschein, D. R. Fleming, R. Rouzier, G. Boniface, and G. N. Hortobagyi.** 2005. Phase II study of tariquidar, a selective P-glycoprotein inhibitor, in patients with chemotherapy-resistant, advanced breast carcinoma. Cancer **104:**682–691.
290. **Qiao, J., W. Liu, and R. Li.** 2008. Antifungal resistance mechanisms of *Aspergillus*. Nippon Ishinkin Gakkai Zasshi **49:**157–163.
291. **Quinn, J., and A. J. P. Brown.** 2007. Stress responses in *Candida albicans*, p. 217–261. *In* C. d'Enfert and B. Hube (ed.), *Candida*: comparative and functional genomics. Caister Adademic Press, Norwich, United Kingdom.
292. **Ramachandra, M., S. V. Ambudkar, D. Chen, C. A. Hrycyna, S. Dey, M. M. Gottesman, and I. Pastan.** 1998. Human P-glycoprotein exhibits reduced affinity for substrates during a catalytic transition state. Biochemistry **37:**5010–5019.
293. **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.
294. **Raymond, M., S. Ruetz, D. Y. Thomas, and P. Gros.** 1994. Functional

expression of P-glycoprotein in *Saccharomyces cerevisiae* confers cellular resistance to the immunosuppressive and antifungal agent FK520. Mol. Cell. Biol. **14:**277–286.
295. **Rester, U.** 2008. From virtuality to reality—virtual screening in lead discovery and lead optimization: a medicinal chemistry perspective. Curr. Opin. Drug Discov. Dev. **11:**559–568.
296. **Rex, J. H., M. G. Rinaldi, and M. A. Pfaller.** 1995. Resistance of *Candida* species to fluconazole. Antimicrob. Agents Chemother. **39:**1–8.
297. **Richardson, M. D., and P. Carlson.** 2002. Culture- and non-culture-based diagnostics for *Candida* species, p. 387–394. *In* R. Calderone (ed.), *Candida* and candidiasis. ASM Press, Washington, DC.
298. **Rodero, L., E. Mellado, A. C. Rodriguez, A. Salve, L. Guelfand, P. Cahn, M. Cuenca-Estrella, G. Davel, and J. L. Rodriguez-Tudela.** 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.
299. **Rogers, B., A. Decottignies, M. Kolaczkowski, E. Carvajal, E. Balzi, and A. Goffeau.** 2001. The pleitropic drug ABC transporters from *Saccharomyces cerevisiae*. J. Mol. Microbiol. Biotechnol. **3:**207–214.
300. **Rogers, P. D., and K. S. Barker.** 2003. Genome-wide expression profile analysis reveals coordinately regulated genes associated with stepwise acquisition of azole resistance in *Candida albicans* clinical isolates. Antimicrob. Agents Chemother. **47:**1220–1227.
301. **Rogers, P. D., J. P. Vermitsky, T. D. Edlind, and G. M. Hilliard.** 2006. Proteomic analysis of experimentally induced azole resistance in *Candida glabrata*. J. Antimicrob. Chemother. **58:**434–438.
302. **Rognon, B., Z. Kozovska, A. T. Coste, G. Pardini, and D. Sanglard.** 2006. Identification of promoter elements responsible for the regulation of *MDR1* from *Candida albicans*, a major facilitator transporter involved in azole resistance. Microbiology **152:**3701–3722.
303. **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.
304. **Rustchenko, E.** 2007. Chromosome instability in *Candida albicans*. FEMS Yeast Res. **7:**2–11.
305. **Saag, M. S., R. J. Graybill, R. A. Larsen, P. G. Pappas, J. R. Perfect, W. G. Powderly, J. D. Sobel, W. E. Dismukes, et al.** 2000. Practice guidelines for the management of cryptococcal disease. Clin. Infect. Dis. **30:**710–718.
306. **Saini, P., T. Prasad, N. A. Gaur, S. Shukla, S. Jha, S. S. Komath, L. A. Khan, Q. M. Haq, and R. Prasad.** 2005. Alanine scanning of transmembrane helix 11 of Cdr1p ABC antifungal efflux pump of *Candida albicans*: identification of amino acid residues critical for drug efflux. J. Antimicrob. Chemother. **56:**77–86.
307. **Sanglard, D., and J. Bille.** 2002. Current understanding of the modes of action of and resistance mechanisms to conventional and emerging antifungal agents for treatment of *Candida* infections, p. 349–383. *In* R. A. Calderone (ed.), *Candida* and candidiasis. ASM Press, Washington, DC.
308. **Sanglard, D., F. Ischer, and J. Bille.** 2001. Role of ATP-binding-cassette transporter genes in high-frequency acquisition of resistance to azole antifungals in *Candida glabrata*. Antimicrob. Agents Chemother. **45:**1174–1183.
309. **Sanglard, D., F. Ischer, D. Calabrese, P. A. Majcherczyk, and J. Bille.** 1999. The ATP binding cassette transporter gene *CgCDR1* from *Candida glabrata* is involved in the resistance of clinical isolates to azole antifungal agents. Antimicrob. Agents Chemother. **43:**2753–2765.
310. **Sanglard, D., F. Ischer, L. Koymans, and J. Bille.** 1998. Amino acid substitutions in the cytochrome P-450 lanosterol 14α-demethylase (*CYP51A1*) from azole-resistant *Candida albicans* clinical isolates contribute to resistance to azole antifungal agents. Antimicrob. Agents Chemother. **42:**241–253.
311. **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.
312. **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:**405–416.
313. **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.
314. **Sanglard, D., K. Kuchler, F. Ischer, J. L. Pagani, M. Monod, and J. Bille.** 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.
315. **Sanguinetti, M., B. Posteraro, B. Fiori, S. Ranno, R. Torelli, and G. Fadda.** 2005. Mechanisms of azole resistance in clinical isolates of *Candida glabrata* collected during a hospital survey of antifungal resistance. Antimicrob. Agents Chemother. **49:**668–679.
316. **Sanguinetti, M., B. Posteraro, M. La Sorda, R. Torelli, B. Fiori, R. Santangelo, G. Delogu, and G. Fadda.** 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.
317. **Sar, B., D. Monchy, M. Vann, C. Keo, J. L. Sarthou, and Y. Buisson.** 2004. Increasing in vitro resistance to fluconazole in *Cryptococcus neoformans* Cambodian isolates: April 2000 to March 2002. J. Antimicrob. Chemother. **54:**563–565.
318. **Sauna, Z. E., S. S. Bohn, R. Rutledge, M. P. Dougherty, S. Cronin, L. May, D. Xia, S. V. Ambudkar, and J. Golin.** 2008. Mutations define cross-talk between the N-terminal nucleotide-binding domain and transmembrane helix-2 of the yeast multidrug transporter Pdr5: possible conservation of a signaling interface for coupling ATP hydrolysis to drug transport. J. Biol. Chem. **283:**35010–35022.
319. **Schmid, J., S. Herd, P. R. Hunter, R. D. Cannon, M. S. Yasin, S. Samad, M. Carr, D. Parr, W. McKinney, M. Schousboe, B. Harris, R. Ikram, M. Harris, A. Restrepo, G. Hoyos, and K. P. Singh.** 1999. Evidence for a general-purpose genotype in *Candida albicans*, highly prevalent in multiple geographical regions, patient types and types of infection. Microbiology **145:**2405–2413.
320. **Schuetzer-Muehlbauer, M., B. Willinger, G. Krapf, S. Enzinger, E. Presterl, and K. Kuchler.** 2003. The *Candida albicans* Cdr2p ATP-binding cassette (ABC) transporter confers resistance to caspofungin. Mol. Microbiol. **48:**225–235.
321. **Segal, B. H., N. G. Almyroudis, M. Battiwalla, R. Herbrecht, J. R. Perfect, T. J. Walsh, and J. R. Wingard.** 2007. Prevention and early treatment of invasive fungal infection in patients with cancer and neutropenia and in stem cell transplant recipients in the era of newer broad-spectrum antifungal agents and diagnostic adjuncts. Clin. Infect. Dis. **44:**402–409.
322. **Selmecki, A., S. Bergmann, and J. Berman.** 2005. Comparative genome hybridization reveals widespread aneuploidy in *Candida albicans* laboratory strains. Mol. Microbiol. **55:**1553–1565.
323. **Selmecki, A., A. Forche, and J. Berman.** 2006. Aneuploidy and isochromosome formation in drug-resistant *Candida albicans*. Science **313:**367–370.
324. **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.
325. **Shao, P. L., L. M. Huang, and P. R. Hsueh.** 2007. Recent advances and challenges in the treatment of invasive fungal infections. Int. J. Antimicrob. Agents **30:**487–495.
326. **Sheehan, D. J., C. A. Hitchcock, and C. M. Sibley.** 1999. Current and emerging azole antifungal agents. Clin. Microbiol. Rev. **12:**40–79.
327. **Ship, J. A., A. Vissink, and S. J. Challacombe.** 2007. Use of prophylactic antifungals in the immunocompromised host. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. **103**(Suppl. S6)**:**e1–e14.
328. **Shukla, S., S. V. Ambudkar, and R. Prasad.** 2004. Substitution of threonine-1351 in the multidrug transporter Cdr1p of *Candida albicans* results in hypersusceptibility to antifungal agents and threonine-1351 is essential for synergic effects of calcineurin inhibitor FK520. J. Antimicrob. Chemother. **54:**38–45.
329. **Shukla, S., P. Saini, Smriti, S. Jha, S. V. Ambudkar, and R. Prasad.** 2003. Functional characterization of *Candida albicans* ABC transporter Cdr1p. Eukaryot. Cell **2:**1361–1375.
330. **Silveira, F. P., and S. Husain.** 2007. Fungal infections in solid organ transplantation. Med. Mycol. **45:**305–320.
331. **Singh, N., A. P. Limaye, G. Forrest, N. Safdar, P. Munoz, K. Pursell, S. Houston, F. Rosso, J. G. Montoya, P. Patton, R. Del Busto, J. M. Aguado, R. A. Fisher, G. B. Klintmalm, R. Miller, M. M. Wagener, R. E. Lewis, D. P. Kontoyiannis, and S. Husain.** 2006. Combination of voriconazole and caspofungin as primary therapy for invasive aspergillosis in solid organ transplant recipients: a prospective, multicenter, observational study. Transplantation **81:**320–326.
332. **Singh, N., and D. L. Paterson.** 2005. *Aspergillus* infections in transplant recipients. Clin. Microbiol. Rev. **18:**44–69.
333. **Reference deleted.**
334. **Sipos, G., and K. Kuchler.** 2006. Fungal ATP-binding cassette (ABC) transporters in drug resistance and detoxification. Curr. Drug Targets **7:**471–481.
335. **Slaven, J. W., M. J. Anderson, D. Sanglard, G. K. Dixon, J. Bille, I. S. Roberts, and D. W. Denning.** 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.
336. **Smriti, S. Krishnamurthy, B. L. Dixit, C. M. Gupta, S. Milewski, and R. Prasad.** 2002. ABC transporters Cdr1p, Cdr2p and Cdr3p of a human pathogen *Candida albicans* are general phospholipid translocators. Yeast **19:**303–318.
337. **Soll, D. R., and C. Pujol.** 2003. *Candida albicans* clades. FEMS Immunol. Med. Microbiol. **39:**1–7.
338. **Soysa, N. S., L. P. Samaranayake, and A. N. Ellepola.** 2008. Antimicrobials as a contributory factor in oral candidosis—a brief overview. Oral Dis. **14:**138–143.
339. **Stein, U., W. Walther, A. Stege, A. Kaszubiak, I. Fichtner, and H. Lage.** 2008. Complete in vivo reversal of the multidrug resistance phenotype by jet-injection of anti-*MDR1* short hairpin RNA-encoding plasmid DNA. Mol. Ther. **16:**178–186.

340. **Steinbach, W. J., D. K. Benjamin, Jr., D. P. Kontoyiannis, J. R. Perfect, I. Lutsar, K. A. Marr, M. S. Lionakis, H. A. Torres, H. Jafri, and T. J. Walsh.** 2004. Infections due to *Aspergillus terreus*: a multicenter retrospective analysis of 83 cases. Clin. Infect. Dis. **39:**192–198.
341. **Steinbach, W. J., J. R. Perfect, W. A. Schell, T. J. Walsh, and D. K. Benjamin, Jr.** 2004. In vitro analyses, animal models, and 60 clinical cases of invasive *Aspergillus terreus* infection. Antimicrob. Agents Chemother. **48:**3217–3225.
342. **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.
343. **Steinbach, W. J., and D. A. Stevens.** 2003. Review of newer antifungal and immunomodulatory strategies for invasive aspergillosis. Clin. Infect. Dis. **37**(Suppl. 3)**:**S157–S187.
344. **Sullivan, D., and D. Coleman.** 1998. *Candida dubliniensis*: characteristics and identification. J. Clin. Microbiol. **36:**329–334.
345. **Sullivan, D. J., G. P. Moran, E. Pinjon, A. Al-Mosaid, C. Stokes, C. Vaughan, and D. C. Coleman.** 2004. Comparison of the epidemiology, drug resistance mechanisms, and virulence of *Candida dubliniensis* and *Candida albicans*. FEMS Yeast Res. **4:**369–376.
346. **Tanabe, K., E. Lamping, K. Adachi, Y. Takano, K. Kawabata, Y. Shizuri, M. Niimi, and Y. Uehara.** 2007. Inhibition of fungal ABC transporters by unnarmicin A and unnarmicin C, novel cyclic peptides from marine bacterium. Biochem. Biophys. Res. Commun. **364:**990–995.
347. **Tanaka, S., S. J. Currier, E. P. Bruggemann, K. Ueda, U. A. Germann, I. Pastan, and M. M. Gottesman.** 1990. Use of recombinant P-glycoprotein fragments to produce antibodies to the multidrug transporter. Biochem. Biophys. Res. Commun. **166:**180–186.
348. **Tekaia, F., and J. P. Latge.** 2005. *Aspergillus fumigatus*: saprophyte or pathogen? Curr. Opin. Microbiol. **8:**385–392.
349. **Thakur, J. K., H. Arthanari, F. Yang, S. J. Pan, X. Fan, J. Breger, D. P. Frueh, K. Gulshan, D. K. Li, E. Mylonakis, K. Struhl, W. S. Moye-Rowley, B. P. Cormack, G. Wagner, and A. M. Naar.** 2008. A nuclear receptor-like pathway regulating multidrug resistance in fungi. Nature **452:**604–609.
350. **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.
351. **Tibayrenc, M.** 1997. Are *Candida albicans* natural populations subdivided? Trends Microbiol. **5:**253–257.
352. **Tobin, M. B., R. B. Peery, and P. L. Skatrud.** 1997. Genes encoding multiple drug resistance-like proteins in *Aspergillus fumigatus* and *Aspergillus flavus*. Gene **200:**11–23.
353. **Tommasini, R., R. Evers, E. Vogt, C. Mornet, G. J. Zaman, A. H. Schinkel, P. Borst, and E. Martinoia.** 1996. The human multidrug resistance-associated protein functionally complements the yeast cadmium resistance factor 1. Proc. Natl. Acad. Sci. USA **93:**6743–6748.
354. **Torelli, R., B. Posteraro, S. Ferrari, M. La Sorda, G. Fadda, D. Sanglard, and M. Sanguinetti.** 2008. The ATP-binding cassette transporter-encoding gene *CgSNQ2* is contributing to the *CgPDR1*-dependent azole resistance of *Candida glabrata*. Mol. Microbiol. **68:**186–201.
355. **Torres, E. M., T. Sokolsky, C. M. Tucker, L. Y. Chan, M. Boselli, M. J. Dunham, and A. Amon.** 2007. Effects of aneuploidy on cellular physiology and cell division in haploid yeast. Science **317:**916–924.
356. **Trtkova, J., and V. Raclavsky.** 2006. Molecular-genetic approaches to identification and typing of pathogenic *Candida* yeasts. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub. **150:**51–61.
357. **Tsai, H. F., A. A. Krol, K. E. Sarti, and J. E. Bennett.** 2006. *Candida glabrata PDR1*, a transcriptional regulator of a pleiotropic drug resistance network, mediates azole resistance in clinical isolates and petite mutants. Antimicrob. Agents Chemother. **50:**1384–1392.
358. **Tschopp, J. F., P. F. Brust, J. M. Cregg, C. A. Stillman, and T. R. Gingeras.** 1987. Expression of the *lacZ* gene from two methanol-regulated promoters in *Pichia pastoris*. Nucleic Acids Res. **15:**3859–3876.
359. **Tsujimura, S., K. Saito, M. Nawata, S. Nakayamada, and Y. Tanaka.** 2008. Overcoming drug resistance induced by P-glycoprotein on lymphocytes in patients with refractory rheumatoid arthritis. Ann. Rheum Dis. **67:**380–388.
360. **Tsuruo, T., H. Iida, Y. Kitatani, K. Yokota, S. Tsukagoshi, and Y. Sakurai.** 1984. Effects of quinidine and related compounds on cytotoxicity and cellular accumulation of vincristine and adriamycin in drug-resistant tumor cells. Cancer Res. **44:**4303–4307.
361. **Tsuruo, T., H. Iida, S. Tsukagoshi, and Y. Sakurai.** 1981. Overcoming of vincristine resistance in P388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. Cancer Res. **41:**1967–1972.
362. **Tutulan-Cunita, A. C., M. Mikoshi, M. Mizunuma, D. Hirata, and T. Miyakawa.** 2005. Mutational analysis of the yeast multidrug resistance ABC transporter Pdr5p with altered drug specificity. Genes Cells **10:**409–420.
363. **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.
364. **vanden Bossche, H., P. Marichal, F. C. Odds, L. Le Jeune, and M. C. Coene.** 1992. Characterization of an azole-resistant *Candida glabrata* isolate. Antimicrob. Agents Chemother. **36:**2602–2610.
365. **Venkateswarlu, K., D. W. Denning, and S. L. Kelly.** 1997. Inhibition and interaction of cytochrome P450 of *Candida krusei* with azole antifungal drugs. J. Med. Vet. Mycol. **35:**19–25.
366. **Venkateswarlu, K., D. W. Denning, N. J. Manning, and S. L. Kelly.** 1996. Reduced accumulation of drug in *Candida krusei* accounts for itraconazole resistance. Antimicrob. Agents Chemother. **40:**2443–2446.
367. **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.
368. **Vermitsky, J. P., K. D. Earhart, W. L. Smith, R. Homayouni, T. D. Edlind, and P. D. Rogers.** 2006. Pdr1 regulates multidrug resistance in *Candida glabrata*: gene disruption and genome-wide expression studies. Mol. Microbiol. **61:**704–722.
369. **Vermitsky, J. P., and T. D. Edlind.** 2004. Azole resistance in *Candida glabrata*: coordinate upregulation of multidrug transporters and evidence for a Pdr1-like transcription factor. Antimicrob. Agents Chemother. **48:** 3773–3781.
370. **Verweij, P. E., E. Mellado, and W. J. Melchers.** 2007. Multiple-triazole-resistant aspergillosis. N. Engl. J. Med. **356:**1481–1483.
371. **Viscoli, C., C. Girmenia, A. Marinus, L. Collette, P. Martino, B. Vandercam, C. Doyen, B. Lebeau, D. Spence, V. Krcmery, B. De Pauw, and F. Meunier.** 1999. Candidemia in cancer patients: a prospective, multicenter surveillance study by the Invasive Fungal Infection Group (IFIG) of the European Organization for Research and Treatment of Cancer (EORTC). Clin. Infect. Dis. **28:**1071–1079.
372. **Wada, S., M. Niimi, K. Niimi, A. R. Holmes, B. C. Monk, R. D. Cannon, and Y. Uehara.** 2002. *Candida glabrata* ATP-binding cassette transporters Cdr1p and Pdh1p expressed in a *Saccharomyces cerevisiae* strain deficient in membrane transporters show phosphorylation-dependent pumping properties. J. Biol. Chem. **277:**46809–46821.
373. **Wada, S., K. Tanabe, A. Yamazaki, M. Niimi, Y. Uehara, K. Niimi, E. Lamping, R. D. Cannon, and B. C. Monk.** 2005. Phosphorylation of *Candida glabrata* ATP-binding cassette transporter Cdr1p regulates drug efflux activity and ATPase stability. J. Biol. Chem. **280:**94–103.
374. **Walsh, T. J., V. Petraitis, R. Petraitiene, A. Field-Ridley, D. Sutton, M. Ghannoum, T. Sein, R. Schaufele, J. Peter, J. Bacher, H. Casler, D. Armstrong, A. Espinel-Ingroff, M. G. Rinaldi, and C. A. Lyman.** 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.
375. **Walworth, N. C., and P. J. Novick.** 1987. Purification and characterization of constitutive secretory vesicles from yeast. J. Cell Biol. **105:**163–174.
376. **Werle, M.** 2008. Natural and synthetic polymers as inhibitors of drug efflux pumps. Pharm. Res. **25:**500–511.
377. **Wheat, L. J.** 2006. Antigen detection, serology, and molecular diagnosis of invasive mycoses in the immunocompromised host. Transpl. Infect. Dis. **8:**128–139.
378. **White, T. C.** 1997. Increased mRNA levels of *ERG16*, CDR, and *MDR1* correlate with increases in azole resistance in *Candida albicans* isolates from a patient infected with human immunodeficiency virus. Antimicrob. Agents Chemother. **41:**1482–1487.
379. **White, T. C., S. Holleman, F. Dy, L. F. Mirels, and D. A. Stevens.** 2002. Resistance mechanisms in clinical isolates of *Candida albicans*. Antimicrob. Agents Chemother. **46:**1704–1713.
380. **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.
381. **Wiederhold, N. P., J. L. Grabinski, G. Garcia-Effron, D. S. Perlin, and S. A. Lee.** 2008. Pyrosequencing to detect mutations in *FKS1* that confer reduced echinocandin susceptibility in *Candida albicans*. Antimicrob. Agents Chemother. **52:**4145–4148.
382. **Wilson, L. S., C. M. Reyes, M. Stolpman, J. Speckman, K. Allen, and J. Beney.** 2002. The direct cost and incidence of systemic fungal infections. Value Health **5:**26–34.
383. **Wingard, J. R.** 1995. Importance of *Candida* species other than *C. albicans* as pathogens in oncology patients. Clin. Infect. Dis. **20:**115–125.
384. **Wingard, J. R., W. G. Merz, M. G. Rinaldi, C. B. Miller, J. E. Karp, and R. Saral.** 1993. Association of *Torulopsis glabrata* infections with fluconazole prophylaxis in neutropenic bone marrow transplant patients. Antimicrob. Agents Chemother. **37:**1847–1849.
385. **Winzeler, E. A., D. D. Shoemaker, A. Astromoff, H. Liang, K. Anderson, B. Andre, R. Bangham, R. Benito, J. D. Boeke, H. Bussey, A. M. Chu, C. Connelly, K. Davis, F. Dietrich, S. W. Dow, M. El Bakkoury, F. Foury, S. H. Friend, E. Gentalen, G. Giaever, J. H. Hegemann, T. Jones, M. Laub, H. Liao, N. Liebundguth, D. J. Lockhart, A. Lucau-Danila, M. Lussier, N. M'Rabet, P. Menard, M. Mittmann, C. Pai, C. Rebischung, J. L. Revuelta, L. Riles, C. J. Roberts, P. Ross-MacDonald, B. Scherens, M. Snyder, S. Sookhai-Mahadeo, R. K. Storms, S. Veronneau, M. Voet, G. Volckaert, T. R. Ward, R. Wysocki, G. S. Yen, K. Yu, K. Zimmermann, P. Philippsen, M. Johnston, and R. W. Davis.** 1999. Functional characterization of the *S.*

*cerevisiae* genome by gene deletion and parallel analysis. Science **285:**901–906.
386. **Wirsching, S., S. Michel, and J. Morschhauser.** 2000. Targeted gene disruption in *Candida albicans* wild-type strains: the role of the *MDR1* gene in fluconazole resistance of clinical *Candida albicans* isolates. Mol. Microbiol. **36:**856–865.
387. **Wolfger, H., Y. M. Mamnun, and K. Kuchler.** 2001. Fungal ABC proteins: pleiotropic drug resistance, stress response and cellular detoxification. Res. Microbiol. **152:**375–389.
388. **Xiao, L., V. Madison, A. S. Chau, D. Loebenberg, R. E. Palermo, and P. M. McNicholas.** 2004. Three-dimensional models of wild-type and mutated forms of cytochrome P450 14α-sterol demethylases from *Aspergillus fumigatus* and *Candida albicans* provide insights into posaconazole binding. Antimicrob. Agents Chemother. **48:**568–574.
389. **Xu, D., B. Jiang, T. Ketela, S. Lemieux, K. Veillette, N. Martel, J. Davison, S. Sillaots, S. Trosok, C. Bachewich, H. Bussey, P. Youngman, and T. Roemer.** 2007. Genome-wide fitness test and mechanism-of-action studies of inhibitory compounds in *Candida albicans*. PLoS Pathog. **3:**e92.
390. **Xu, Z., L. X. Zhang, J. D. Zhang, Y. B. Cao, Y. Y. Yu, D. J. Wang, K. Ying, W. S. Chen, and Y. Y. Jiang.** 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.
391. **Yamazumi, T., M. A. Pfaller, S. A. Messer, A. K. Houston, L. Boyken, R. J. Hollis, I. Furuta, and R. N. Jones.** 2003. Characterization of heteroresistance to fluconazole among clinical isolates of *Cryptococcus neoformans*. J. Clin. Microbiol. **41:**267–272.
392. **Zastre, J. A., J. K. Jackson, W. Wong, and H. M. Burt.** 2008. P-glycoprotein efflux inhibition by amphiphilic diblock copolymers: relationship between copolymer concentration and substrate hydrophobicity. Mol. Pharm. **5:**643–653.
393. **Zhang, H., M. Paguio, and P. D. Roepe.** 2004. The antimalarial drug resistance protein *Plasmodium falciparum* chloroquine resistance transporter binds chloroquine. Biochemistry **43:**8290–8296.
394. **Zhao, K. Q., R. Hurst, M. R. Slater, and R. F. Bulleit.** 2007. Functional protein expression from a DNA based wheat germ cell-free system. J. Struct. Funct. Genomics **8:**199–208.
395. **Znaidi, S., X. De Deken, S. Weber, T. Rigby, A. Nantel, and M. Raymond.** 2007. The zinc cluster transcription factor Tac1p regulates *PDR16* expression in *Candida albicans*. Mol. Microbiol. **66:**440–452.

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