Antifungals: mechanism of action and resistance, established and novel drugs

Nafsika H Georgopapadakou

Serious fungal infections, caused mostly by opportunistic species, are increasingly common in immunocompromised and other vulnerable patients. The use of antifungal drugs, primarily azoles and polyenes, has increased in parallel. Yet, established agents do not satisfy the medical need completely: azoles are fungistatic and vulnerable to resistance, whereas polyenes cause serious host toxicity. Drugs in clinical development include echinocandins, pneumocandins, and improved azoles. Promising novel agents in preclinical development include several inhibitors of fungal protein, lipid and cell wall syntheses. Recent advances in fungal genomics, combinatorial chemistry, and high-throughput screening may accelerate the antifungal discovery process.

Addresses
DuPont Pharmaceuticals Research Laboratories, Experimental Station, E400/3442, Rt 141 & Henry Clay Road, PO Box 80400, Wilmington, DE 19880-0400, USA; e-mail: nafsikag@aol.com

Current Opinion in Microbiology 1998, **1**:547–557

http://biomednet.com/elecref/1369527400100547

Abbreviations

ABC	ATP-binding cassette
CoA	coenzyme A
EF	elongation factor
5-FC	5-fluorocytosine
5-FU	5-fluorouracil
GlcNAc	*N*-acetylglucosamine
GPI	glycosyl phosphatidylinositol
IPC	inositolphosphorylceramide
MF	major facilitator
NMT	*N*-myristoyltransferase
PMN	polymorphonuclear leukocyte

Introduction

Fungal infections in humans range from the superficial and common, such as dermatophytoses and onychomycoses, to deeply invasive and disseminated, such as candidiasis and aspergillosis. In the past 20 years, the frequency of systemic fungal infections has increased dramatically along with the number of invasive, mostly opportunistic, species [1]. The main factor for the increase is the proliferation of severely immunocompromised patients either with AIDS, undergoing cancer chemotherapy, or immunosuppressive therapy for organ transplantation [2–4]. Additional factors include treatment with broad-spectrum antibacterial drugs or glucocorticosteroids; invasive procedures such as surgery, in-dwelling catheters or prosthetic devices; and parenteral nutrition or dialysis.

The major pathogen has been *Candida albicans*, normally a commensal of the oral cavity and gastrointestinal tract of humans. Non-*albicans Candida* species (e.g. *C. glabrata*, *C. tropicalis*, *C. krusei*), however, are also found with increasing frequency [5], as is *Aspergillus* (e.g. *A. niger*; *A. flavus*), *Histoplasma capsulatum* and *Cryptococcus neoformans*. Emerging opportunistic pathogens include *Fusarium* and *Trichosporon* (both significant in neutropenic patients and commonly associated with disseminated infection), *Rhizopus*, *Mucor*, dematiaceous fungi, and others. Fungal infections are important causes of morbidity and mortality in hospitalized patients: candidiasis is the fourth most common blood culture isolate in US hospitals [6], pulmonary aspergillosis is the leading cause of death in bone marrow transplant recipients [7], and *Pneumocystis carinii* (formerly thought to be a protozoal parasite) pneumonia is the leading cause of death in AIDS patients in North America and Europe [8].

The increase in life-threatening fungal infections has brought about an increased use of antifungal drugs and a pressing need for new, broad-spectrum, fungicidal agents that can be used empirically in immunocompromised patients [9,10]. Empirical treatment of suspected fungal infections is necessitated by problems in diagnosis and susceptibility testing and may be more common than treatment of confirmed infections. None of the existing systemic antifungals satisfies the medical need completely; there are weaknesses in spectrum, potency, safety, and pharmacokinetic properties. Some of the weaknesses are addressed by antifungal agents recently introduced or in development. This review discusses the mechanisms of action and resistance of established and new antifungal agents, highlighting areas of recent growth.

Established agents

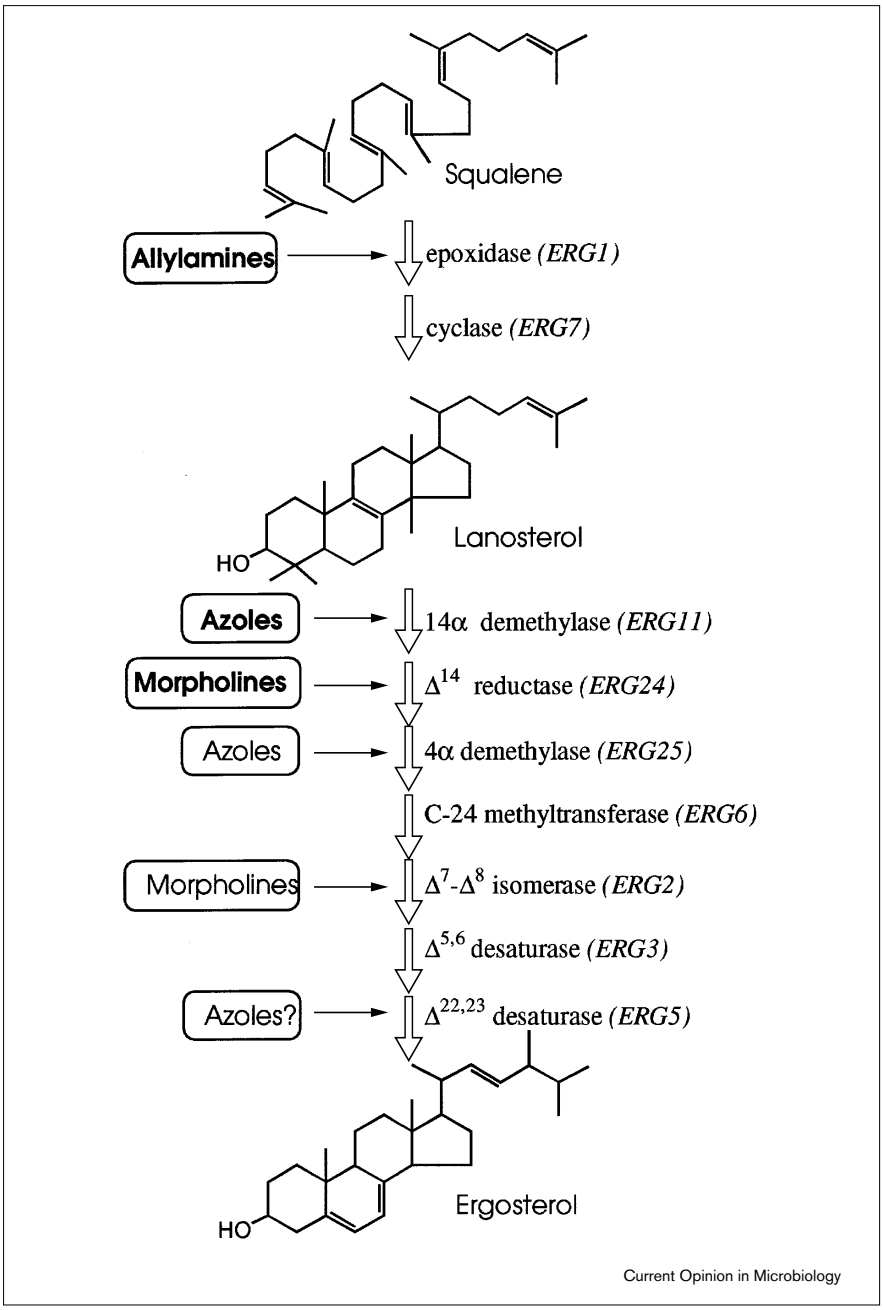
There are four classes of systemic antifungal compounds currently in clinical use: the polyene antibiotics, the azole derivatives, the allylamines/thiocarbamates, and the fluoropyrimidines. The first three classes target ergosterol, the major sterol in the fungal plasma membrane. They are thus ineffective against *P. carinii*, which has cholesterol instead of ergosterol, possibly acquired from its mammalian host [11].

Although selective towards ergosterol, systemic antifungals nevertheless also affect the function (polyenes) or biosynthesis (azoles, allylamines) of mammalian sterols, and thereby the host's immune response [12]. For example, amphotericin (a polyene) increases the aggregation, adherence and fungicidal activity of polymorphonuclear leukocytes (PMNs) [13]; allylamines slightly increase the fungicidal activity of PMNs [14]; whereas azoles inhibit chemotaxis and superoxide production of PMNs [15].

Polyenes

The polyene antibiotics, discovered in the late 1950s, are produced by *Streptomyces* species. They are fungicidal and

Figure 1



The biosynthesis of the fungal sterol ergosterol and the sites of inhibition for some antifungal agents.

have the broadest activity spectrum of any clinically useful antifungal. They complex with ergosterol in the fungal plasma membrane and thereby compromise its barrier function. In addition, they cause oxidative damage which may contribute to their fungicidal action [3,16]. The only systemic polyene in clinical use is amphotericin B. It has a higher affinity for ergosterol than the mammalian counterpart, cholesterol, and is thus less toxic to mammalian cells. In patients, however, it has acute and chronic side effects, notably nephrotoxicity. The side effects are significantly reduced when amphotericin B is used in costly lipid formulations, such as liposomes (Ambisome), lipid complexes (Abelcet), and colloidal dispersions (Amphocil/Amphotech) [17,18]. Interestingly, phospholipases, virulent factors of *C. albicans* [19], have been suggested to release amphotericin B from Abelcet *in vivo*, thereby targeting it to the site of infection [20]. A fourth formulation, polyethylene glycol-phospholipid liposomes (PEG-AmB), where the PEG moiety increases the circulation of intact liposomes and thereby decreases toxicity, is in preclinical development [21].

Fungal resistance to polyenes is associated with altered membrane lipids, particularly sterols [22,23•]. Other

resistance mechanisms may involve altered phospholipids and increased catalase activity with decreased susceptibility to oxidative damage [24]. Intrinsic clinical resistance to amphotericin B is still rare in *Candida* species other than *C. lusitaniae*, but is common in emerging pathogens, such as *Fusarium* and *Trichosporon* species [25,26]. Secondary clinical resistance, following amphotericin B treatment, has been reported for both *Candida* and *Cryptococcus* species but is uncommon.

Azoles

The azole antifungals, discovered in the late 1960s, are totally synthetic and are the most rapidly expanding group of antifungal agents [27,28]. They are classified as imidazoles or triazoles, on the basis of whether they have two or three nitrogens in the 5-membered azole ring. Two of the three systemic azoles in clinical use, and almost all azoles in development, are triazoles. Systemic azoles have fungistatic, broad-spectrum activity that includes most yeasts and filamentous fungi and some emerging pathogens such as *Trichosporon* species.

Azoles act on ergosterol biosynthesis at the C-14 demethylation stage, a three-step, oxidative reaction catalyzed by the cytochrome P-450 enzyme 14α-sterol demethylase (P-450_{DM}) (Figure 1). The resulting ergosterol depletion and accumulation of lanosterol and other 14-methylated sterols interferes with the 'bulk' functions of ergosterol as a membrane component: it disrupts the structure of the plasma membrane, making it more vulnerable to further damage, and alters the activity of several membrane-bound enzymes, such as those associated with nutrient transport and chitin synthesis [16,29,30]. Severe ergosterol depletion (>99%) may additionally interfere with the hormone-like ('sparking') functions of ergosterol, affecting cell growth and proliferation [31]. By inhibiting P-450_{DM}, azoles may, in addition, sensitize fungal cells to oxidative metabolites produced by phagocytes [32]. Although systemic azoles are generally free of serious host toxicity, they may produce endocrine side effects, such as decrease in testosterone and glucocorticoids, stemming from their ability to interact with mammalian cytochrome P-450.

Resistance to azoles, particularly fluconazole, is emerging in *C. albicans* after long-term suppressive therapy for oropharyngeal candidiasis in HIV-infected patients [33]. Resistance to fluconazole has also been reported in other *Candida* species, particularly *C. glabrata* and *C. krusei* and *C. neoformans* [34,35]. This resistance is due to decreased membrane permeability resulting from changes in membrane sterols, active efflux, altered or overproduced P-450_{DM}, and compensatory mutations in $\Delta^{5,6}$ desaturase [36,37••,38,39••]. Suppressor mutations in $\Delta^{5,6}$ desaturase also occur after sterol 14α-sterol demethylase gene disruption [40], suggesting that accumulation of 14-methyl-3,6-diol, rather than accumulation of 14-methylated sterols in general or ergosterol depletion, is the cause of growth inhibition. Transport-associated resistance is associated with increased efflux. There are two types of efflux pumps in yeast, ATP-binding cassette (ABC) transporters and major facilitators (MFs). Thirty ABC transporter genes and 28 MF genes have been identified in *S. cerevisiae*, following the recent sequencing of its genome [41,42,43••]. A comparable number has been described for *C. albicans*. Of the ABC transporters, azole resistance has been correlated with CDR in *C. albicans* [44–46]. In a very recent report, CDR expression was shown to be induced by azoles [47•]. Of the MFs, azole resistance has been correlated with overexpression of the *MDR1* (also known as Ben^R) gene and found to be specific for fluconazole [48].

Allylamines and thiocarbamates

This class of compounds, discovered in the 1970s, are also totally synthetic. The only systemic allylamine antifungal in clinical use is terbinafine [49]. It is a reversible, noncompetitive inhibitor of squalene epoxidase [50], an enzyme which, together with (2,3)-oxidosqualene cyclase, is responsible for the cyclization of squalene to lanosterol (Figure 1). The resulting ergosterol depletion and squalene accumulation affect membrane structure and function, such as nutrient uptake [51].

Pharmacokinetics limit the clinical efficacy of terbinafine (and other compounds of this class) to skin and nail infections, despite broad-spectrum, *in vitro* activity. Resistance has not been reported for human pathogens but has been described in the corn pathogen *Ustilago maydis* [52], where it involves decreased affinity for the target enzyme and decreased drug accumulation in the fungal cell.

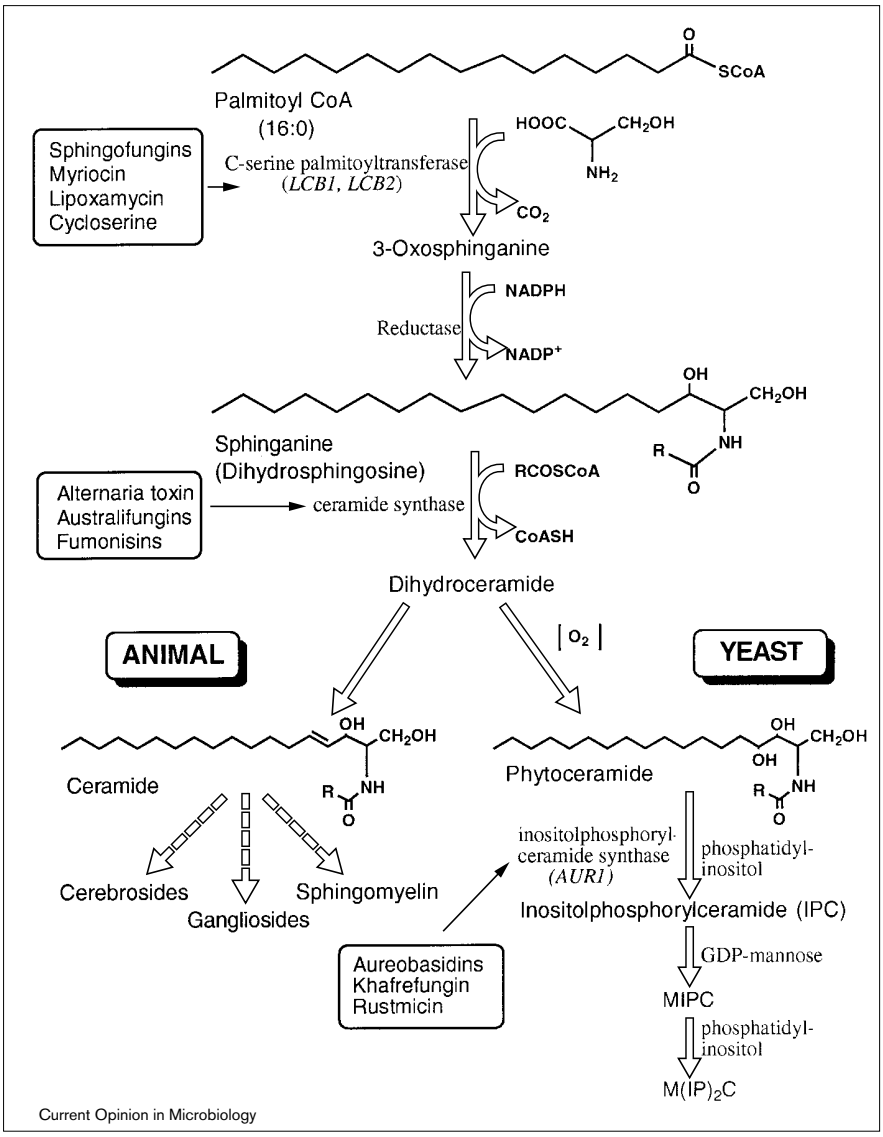
5-Fluorocytosine

The fluoropyrimidine 5-fluorocytosine (5-FC), though fungicidal, has a limited activity spectrum. It is mainly used in combination with amphotericin B in cryptococcal meningitis and in cases of disseminated candidiasis [53]. It is also used as a single agent for the treatment of chromoblastomycosis and mycoses in the urinary tract where 5-FC achieves concentrations up to 100-fold higher than in the serum [54].

5-FC is taken up into fungal cells by a cytosine permease, deaminated to 5-fluorouracil (5-FU), converted to the nucleoside triphosphate, and incorporated into RNA where it causes miscoding [55]. In addition, 5-FU is converted to deoxynucleoside, which inhibits thymidylate synthase and thereby DNA biosynthesis. 5-FC is relatively nontoxic to mammalian cells due to the absence or very low activity of cytosine deaminase; 5-FU, on the other hand, is a potent and widely used anticancer agent. In the US a parenteral formulation of 5-FC has been associated with host toxicity, stemming from its conversion to 5-FU by intestinal bacteria. Toxicity is exacerbated by amphotericin B-induced renal insufficiency [54].

Primary resistance to 5-FC is usually due to impaired cytosine deaminase [16,55]. Secondary resistance, common

Figure 2



Sphingolipid synthesis and sites of inhibitor action. The early biosynthetic steps of this pathway are the same in mammalian and fungal cells. Inhibitors of these early biosynthetic steps have both broad-spectrum antifungal activity and host toxicity. MIPC, mannose-IPC; $M(IP)_2C$, mannose-inositolphosphoryl-IPC.

when 5-FC is used alone, results from mutations in any of the enzymes necessary for 5-FC action, particularly uracil phosphoribosyltransferase [16]. The frequency of 5-FC resistance is highest in *Aspergillus* species, followed by *C. neoformans* and *Candida* species [16].

Novel agents

Novel antifungal agents have originated from random or target-based screening of natural products and synthetic compounds followed by lead optimization. The process has picked up considerable speed with the introduction of high-throughput screening, combinatorial chemistry, and fungal genomics. The latter is particularly valuable for target validation and characterization, including differentiation from the mammalian counterpart. Rational design has been limited to very few, well characterized targets and mechanistically understood reactions, mainly in ergosterol synthesis.

Echinocandins, pneumocandins and papulacandins

Echinocandins, the related pneumocandins, and papulacandins are natural products discovered in the 1970s. Echinocandins are fatty acid derivatives of cyclic hexapeptides, whereas papulacandins are fatty acid derivatives of the disaccharide β-(1,4)-galactosylglucose [56]. Both classes inhibit β-(1,3)-glucan synthesis noncompetitively, with K_is of 0.2–2 μM, though they may bind to different sites on glucan synthase since some yeast strains resistant to the echinocandins remain susceptible to papulacandins [57]. Glucans are glucose homopolymers of β-(1,3)-linked residues with occasional sidechains involving β-(1,6)-linkages and are major components of the fungal cell wall [56]. Polymerization is catalyzed by β-(1,3)-glucan synthase which has at least two functional components: a catalytic component that acts on the UDP-glucose substrate, and a regulatory 21 kDa GTP-binding protein (Rho1p) that is

activated by cell wall defects [58] and may link glucan synthesis to the cell cycle via a phosphorylation/dephosphorylation relay system [59–65]. There are two glucan synthase systems in *S. cerevisiae*, and most likely in pathogenic fungi [66,67].

Papulacandins are no longer being pursued as antifungals, because their *in vitro* activity is limited to *Candida* species and, most importantly, does not translate to *in vivo* activity [56,68–70]. Echinocandins, on the other hand, have fungicidal activity both *in vitro* and in animal models. Two water-soluble semisynthetic derivatives of echinocandin B (LY-303366) and pneumocandin B_0 (L-743872) have promising *in vitro* and *in vivo* activity against *Candida* species, *Aspergillus* species and *P. carinii*, though not *C. neoformans* or *Trichosporon* [71–73,74•]. They are currently in late clinical development. Interestingly, very recent studies with a photoaffinity analog of LY303366 labeled a protein other than glucan synthase [75•]. Recently, echinocandin-type compounds, the cyclopeptamines, have been produced by combinatorial chemistry [76–78].

Polyoxins and nikkomycins

Polyoxins and the related nikkomycins are nucleoside-peptide antibiotics produced by *Streptomycetes* and discovered in the 1960s and 1970s. They inhibit chitin synthesis competitively, acting as analogs of the substrate UDP–*N*-acetylglucosamine, with K_is of 0.1–1 μM [79••]. Chitin is a linear homopolymer of β-(1,4)-linked *N*-acetylglucosamine (GlcNAc) and is a major component of the fungal cell wall. Unlike bacterial cell wall synthesis — where polymerization of cell wall units occurs on the outer surface of the plasma membrane and is catalyzed by multiple, individually essential transpeptidases — GlcNAc polymerization occurs on the cytoplasmic surface of the plasma membrane and is catalyzed by multiple, partially redundant, chitin synthases. In *C. albicans* and the nonpathogenic but extensively studied *Saccharomyces cerevisiae*, there are three chitin synthases (Chs) [80]. The major *in vitro* activity, Chs2 in *C. albicans* (Chs1 in *S. cerevisiae*), is a nonessential, repair enzyme *in vivo*. Of the other two activities, Chs1 in *C. albicans* (Chs2 in *S. cerevisiae*) is involved in septum formation, while Chs3 is involved in cell wall maturation and bud ring formation.

Different isozymes of chitin synthase may be inhibited to different degrees by inhibitors. For example, in *S. cerevisiae*, Chs1 and Chs3 are more sensitive than Chs2 to nikkomycin derivatives [81,82]. This may have chemotherapeutic implications, especially in view of the functional redundancy of the enzymes. Thus, the aromatic natural product xanthofulvin, which selectively inhibits Chs2 of *S. cerevisiae* at micromolar concentrations, has apparently no significant antifungal activity [P1]. On the other hand, Chs3 might be a good chemotherapeutic target since Chs3-deficient mutants of *C. albicans* are less virulent than the parent strain in mice [83].

Polyoxins and nikkomycins have modest activity against human pathogens due to transport limitations; they are taken up by a dipeptide permease and thus peptides in body fluids antagonize their uptake. Nikkomycin Z is the only compound of that class which has shown some activity in animal models as a single agent and synergy with glucan and ergosterol synthesis inhibitors [79••]. Nikkomycin Z progressed to clinical development, but stalled in Phase I last year (R Hector, personal communication).

Pradimicins and benanomycins

Pradimicins and the related benanomycins are benzonaphthacene quinones conjugated with a D-amino acid and a disaccharide sidechain [84,85]. They are produced by *Actinomadura* species and were discovered in the 1980s. Their mechanism of antifungal action involves calcium-dependent complexing of their free carboxyl group with the saccharide portion of cell-surface mannoproteins followed by disruption of the plasma membrane and leakage of intracellular potassium [86].

Pradimicins are active in animal models of cryptococcosis, candidiasis and aspergillosis with potencies intermediate of that of ketoconazole and amphotericin B [84]. Water-soluble pradimicin derivatives such as BMS 181184 have been synthesized, but their development was halted because of animal toxicity findings [87].

Ergosterol synthesis inhibitors

Ergosterol synthesis has been an attractive area for rational drug design. Fungal sterols are structurally distinct from their mammalian counterparts and there is extensive knowledge of their biosynthesis [88,89]. Three azoles, voriconazole (UK-109,496), SCH 56592 and BMS 20714 (ER 30346) are in clinical development [90]. Apart from 14α-sterol demethylase ($P\text{-}450_{DM}$) and squalene epoxidase, targets actively pursued in the post-squalene synthesis segment are oxidosqualene cyclase and C-24 methyltransferase [91–93] (Figure 1). The latter has no mammalian counterpart (cholesterol is not methylated at C-24) and thus is a particularly attractive target. Interestingly, inhibitors of Δ^{24} methyltransferase (such as 22, 26 azasterol) are also active against *P carinii* [93], because, although the organism imports sterols from the infected host, it still needs to methylate them to produce 24-alkyl sterols. With both oxidosqualene cyclase and Δ^{24} methyltransferase, research has focused on designing high energy intermediates or transition state analogs [88]. Inhibitors of the post-squalene steps need not be selective, unless they cause accumulation of compounds toxic to mammalian cells, because mammalian cells can take up dietary cholesterol via the low density lipoprotein (LDL) pathway whereas fungi have no uptake system for exogenous sterols.

Targets in the pre-squalene segment of the ergosterol pathway are less attractive, not so much because the reactions are the same in fungi and mammalian cells (as are the targets for the clinically useful allylamines and azoles), but because

inhibitors may affect the synthesis of other essential terpenoids in mammalian cells. Nevertheless, inhibitors of hydroxymethylglutaryl coenzyme A (CoA) and mevalonic acid synthesis are potential or commercial cholesterol-lowering agents, suggesting that enzymes at the branch-points of the sterol pathway may have different affinities for substrates, sparing critical but quantitative minor pathways during depletion of key intermediates. Inhibitors of squalene synthase, the squalestatins/zaragosic acids, have also been reported [94,95], though none has promising antifungal activity, probably due to membrane permeability constraints.

Protein synthesis inhibitors

Both fungal and mammalian cells require two soluble protein factors, elongation factor 1 (EF-1) and 2 (EF-2) for the polypeptide chain elongation reactions of protein synthesis. Fungi, however, require an additional factor, elongation factor 3 (EF-3) that is absent from mammalian cells [96]. This 120–125 kDa protein, initially discovered in *S. cerevisiae* over 20 years ago, is present in most fungi, including *C. albicans* and *P. carinii* [97,98], and is essential for cell viability since disruption of its gene is lethal to the organism [99]. EF-3 contains two ABC domains, has ATPase activity, and is specifically required by the yeast 40S ribosomal subunit. Though the exact function of EF-3 in the elongation cycle is unclear, it stimulates the function of EF-1α in binding aminoacyl-tRNA to the ribosome, plays a role in translational accuracy and mediates release of deacylated tRNA from the ribosome E site when the A site is occupied [100]. Very recently, a homolog of EF-3, EF-3B, was identified in the *S. cerevisiae* genome database, but apparently is not expressed during growth and thus can not rescue deletion mutants of EF-3 [101•]. So far, structures of EF-3 inhibitors have not been reported. Surprisingly, a selective inhibitor of EF-2, GM 237354, was reported recently [102–106]. It is a synthetic tetrahydrofuran derivative belonging to the sordarin family of natural products and has both *in vitro* and *in vivo* activity against susceptible fungi (*Candida* species, *Cryptococcus*, *Coccidioides immitis* and *H. capsulatum*) and is well tolerated in laboratory animals. A related compound, GR135402, was also reported to inhibit protein synthesis [107].

Fungi synthesize a small number of *N*-myristoylated proteins, the most prominent being the 20 kDa ADP-ribosylation factors (Arfs). Myristoylation involves the co-translational transfer of myristate, a 14-carbon saturated fatty acid, from CoA to the amino-terminal glycine of proteins [108]. The reaction is essential in *C. neoformans, C. albicans* and other fungi [109–111], and is catalyzed by myristoyl-CoA:protein *N*-myristoyltransferase (NMT) that has different peptide substrate specificity from its mammalian counterpart [112–114]. A crystal structure of the enzyme has recently been published [115••]. Selective inhibitors of fungal NMT, some having antifungal activity, have been synthesized [116,117•]. Another target in the post-translational modification of proteins is the enzyme that transfers glycosyl phosphatidylinositol (GPI)-mannoproteins from their GPI membrane anchors to β-(1,3)-glucan [118,119•]. This reaction differs somewhat from its mammalian counterpart and may thus be an attractive target.

Sphingolipid synthesis inhibitors

Several inhibitors of fungal sphingolipid synthesis (Figure 2), all natural products and most of them toxic to mammalian cells, have been reported in the past ten years. Sphingolipids are essential components of the cytoplasmic membrane in both mammalian and fungal cells but differ in their structure and biosynthesis [120].

The early biosynthetic steps that lead to ceramide (phytoceramide in fungi) are shared in mammalian and fungal cells and inhibitors of these biosynthetics steps (such as the sphingofungins, fumonisins and australifungins) have both broad-spectrum antifungal activity and host toxicity [121,122]. Steps subsequent to ceramide formation are unique to fungi and involve the sequential addition of phosphorylated sugars to form inositolphosphorylceramide (IPC), mannose-IPC (MIPC) and mannose-inositolphosphoryl-IPC ($M(IP)_2C$). The first addition of inositol phosphate, catalyzed by IPC synthase, is inhibited by nanomolar concentrations of aureobasidins, cyclic depsipeptides produced by the black yeast *Aureobasidium pullulans* R106 [123••,124,125]. Aureobasidin itself has fungicidal, moderate-spectrum activity, which includes *C. albicans*, *C. neoformans* and some *Aspergillus* species, is orally active in a murine infection model of candidiasis and has low animal toxicity. Inhibition of IPC synthase causes both sphingolipid depletion and ceramide accumulation, the latter being probably responsible for the fungicidal effect. Other nanomolar inhibitors of IPC synthase from natural products are the lipid khafrefungin and the macrolide galbonolide/rustmicin [126,127,128•].

Emerging targets

Microtubules are dynamic polymers of α- and β-tubulin dimers whose aggregation/ disaggregation plays a key role in cell morphology and growth. Microtubule aggregation is inhibited by griseofulvin, the agricultural fungicide benomyl, and the anticancer drugs vincristine and vinblastine; disaggregation is inhibited by taxol [129]. This is an area of intense research for anticancer agents that has the potential of spilling over to antifungal research.

Topoisomerases I and II control the topological state of DNA so that it can undergo replication, transcription, repair, and chromosomal segregation. They act by introducing transient, enzyme-bridged DNA breaks (single strand breaks for type I and double strand breaks for type II) that allow the passing of DNA strands. The enormous success of topoisomerase inhibitors in antibacterial and anticancer chemotherapy has underscored the potential of fungal topoisomerases as drug targets. Recent evidence suggests that fungal topoisomerase I and II can be inhibited selectively [130–132], the latter by cationic aromatic compounds that bind to the minor groove of DNA.

The success of trimethoprim/sulfomethoxazole in treating *P. carinii* pneumonia has validated their sites of action in the folate pathway as drug targets for this organism [133], though not for other fungi. More recently, phosphoribosylaminoimidazole carboxylase, an enzyme of the purine pathway, has been suggested as a target for *C. neoformans*, due to the low levels of free adenine in the cerebrospinal fluid [134]. Dehydroorotate dehydrogenase (DHO-DH), an enzyme of the pyrimidine pathway, has been explored for over a decade as a target both in infectious diseases and cancer chemotherapy [135,136]. A series of 4-phenoxy-quinoline agricultural fungicides target this enzyme in mitochondria.

The discovery of the amino acid analog cispentacin, an antifungal with excellent *in vivo* activity and multiple cellular targets [137–139], raised the possibility of interfering with amino acid synthesis. Other amino acid analogs with antifungal activity are RI-331, which inhibits homoserine dehydrogenase [140], a particularly attractive target since it is absent in mammalian cells, and azoxybacillin, which inhibits the biosynthesis of sulfur-containing amino acids [141].

Ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine synthesis [142] and the target for suicide substrate analogs such as eflornithine, may also be an antifungal target. *P. carinii* ODC is far less sensitive to DFMO than the mammalian enzyme, suggesting differences in the active sites, though the available information is not yet sufficient for rational drug design. An inhibitor of S-adenosylmethionine decarboxylase recently showed promising activity against *P. carinii in vivo* [143].

Proton ATPases and efflux pumps

The plasma membrane H^+-ATPase is an integral, abundant membrane protein involved in the maintenance of electrochemical proton gradients and the regulation of intracellular pH. Plasma membrane H^+-ATPases are known in sufficient molecular detail to be targets for rational drug design, provided there are exploitable differences between the fungal and mammalian enzymes. The vesicular H^+-ATPase (V-ATPase) is inhibited specifically by folimycin, an antifungal agent structurally related to bafilomycins [144]. These compounds block acidification of intracellular organelles and thereby affect intracelllular protein trafficking and translocation to the cell surface. As with the plasma membrane ATPase, the selectivity between fungal and mammalian enzymes is presently unclear.

Proteins with pump function have been reported in *Candida* species [145,146,147••] and may be responsible for the observed broad resistance of these organisms to azoles and perhaps to other antifungal agents. Although they are functionally similar to multidrug resistance proteins reported in bacteria, parasites, and mammalian cells, their encoding genes may be different from the multiple-drug resistance (MDR) genes of the P-glycoprotein family. The lack of structural similarity to mammalian P-glycoproteins may be exploited in designing specific inhibitors of the fungal efflux pumps. Recent studies have shown that deletion of a multidrug resistance gene in *C. albicans* results in a marked attenuation of virulence of the organism, suggesting the possibility of simultaneously potentiating antifungal activity and attenuating virulence.

Conclusions

The recent surge in the use of antifungals, particularly azoles, is selecting resistant strains of susceptible species and is shifting the population of fungal pathogens toward species that are intrinsically resistant, such as non-*albicans Candida* species, *C. krusei* and *C. glabrata*. Since the vast majority of life-threatening mycoses occur in immunocompromised patients, the importance of broad-spectrum, fungicidal agents of acceptable toxicity can not be overemphasized. The continued broad use of amphotericin B, despite the advent of less toxic agents, underscores the critical need for potent, fungicidal agents.

One class of selective fungicidal agents, the echinocandins and pneumocandins, is currently in clinical development. Other leads with selective fungal toxicity have emerged, mainly from natural product screening (aureobasidin A, sordaricins, cispentacin) though as yet none is in clinical development. New technologies are accelerating the discovery process: genomics, combinatorial chemistry, high-throughput screening. As technologies mature, their track record points to their limitations. Genomics, although useful in discovering essential genes and spotting motifs (isozymes, homologs), has been less useful in defining functions; the gene-to-screen road can be a tremendous challenge, as the example of IPC synthase has shown. Combinatorial chemistry, while useful in lead optimization (cyclopeptamines), maybe less useful in lead discovery. High-throughput screening presupposes a robust assay (relatively stable reagents, linear kinetics), which is not always possible. Good quality compound libraries are, of course, essential for lead discovery. As are creative assays (biochemical and whole cell) and a 'prepared mind'. An example of the latter is the recent report on the mechanism of action of galbonolide/rustmicin [128•].

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Anaissie EJ: **Opportunistic mycoses in the immunocompromised host: experience at a cancer center and review.** *Clin Infect Dis* 1992, **14**:43-53.

2. Walsh TJ: **Invasive fungal infections: problems and challenges in developing new antifungal compounds.** In *Emerging Targets in Antibacterial and Antifungal Chemotherapy.* Edited by Sutcliffe J, Georgopapadakou NH. New York: Chapman and Hall; 1992:349-373.

3. Georgopapadakou NH, Walsh TJ: **Human mycoses: drugs and targets for emerging pathogens.** *Science* 1994, **264**:371-373.

4. Beck-Sague CM, Jarvis WR: **Secular trends in the epidemiology of nosocomial fungal infections in the United States, 1980-1990.** *J Infect Dis* 1993, **167**:1247-1251.

5. Nguyen MH, Peacock JE, Morris AJ, Tanner DC, Nguyen ML, Snydman DR, Wagener MM, Rinaldi MG, Yu VL: **The changing face of candidemia – emergence of non-*Candida albicans* species and fungal resistance.** *Am J Med* 1996, **100**:617-623.

6. Pfaller MA, Jones RN, Messer SA, Edmond MB, Wenzel RP: **National surveillance of nosocomial blood stream infection due to *Candida albicans*: frequency of occurrence and antifungal susceptibility in the SCOPE program.** *Diagn Microbiol Infect Dis* 1998, **31**:327-332.

7. Panutti CS, Gingrich R, Pfaller MA, Kao C, Wenzel RP: **Nosocomial pneumonia in patients having bone marrow transplant.** *Cancer* 1992, **69**:2653-2662.

8. Hughes WT: ***Pneumocystis carinii* pneumonia: new approaches to diagnosis, treatment and prevention.** *Pediatr Infect Dis J* 1991, **10**:391-399.

9. Walsh TJ, Lyman CA, Pizzo PA: **Laboratory diagnosis of invasive fungal infections in patients with neoplastic diseases.** In *Bailliere's Clinical Infectious Diseases International Practice and Research. Invasive Fungal Infections in Cancer Patients,* vol 2. Edited by Meunier F. Philadelphia: Bailliere-Tidall; 1995:25-70.

10. Working Party of the British Society for Antimicrobial Chemotherapy: **Therapy of deep fungal infection in haematological malignancy.** *J Antimicrob Chemother* 1997, **40**:779-788.

11. Fishman JA: **The treatment of infection due to *Pneumocystis carinii.*** *Antimicrob Agents Chemother* 1998, **42**:1309-1314.

12. Roilides E, Walsh TJ, Rubin M, Venzon D, Pizzo PA: **Effects of antifungal agents on the function of human neutrophils *in vitro*.** *Antimicrob Agents Chemother* 1990, **34**:196-201.

13. Martin E, Stuben A, Gorz A, Weller U, Bhakdi S: **Novel aspect of amphotericin action: accumulation in human monocytes potentiates killing of phagocytosed *Candida albicans*.** *Antimicrob Agents Chemother* 1994, **38**:13-22.

14. Vago T, Baldi G, Colombo D, Barbareschi M, Norbiato G, Dallegri F, Bevilaqua M: **Effects of naftifine and terbinafine, two allylamine antifungal drugs, on selected functions of human polymorphonuclear leukocytes.** *Antimicrob Agents Chemother* 1994, **38**:2605-2611.

15. Johnson EM, Warnock DW, Richardson MD, Douglas CJ: ***In vitro* effect of itraconazole, ketoconazole and amphotericin B on the phagocytic and candidacidal function of human neutrophils.** *J Antimicrob Chemother* 1986, **18**:83-92.

16. Vanden Bossche H, Marichal P, Odds FC: **Molecular mechanisms of drug resistance in fungi.** *Trends Microbiol* 1994, **2**:393-400.

17. Graybill JR: **Lipid formulations for amphotericin B: does the emperor need new clothes?** *Ann Int Med* 1996, **124**:921-923.

18. Hartsel S, Bolard J: **Amphotericin B: new life for an old drug.** *Trends Pharmac Sci* 1996, **17**:445-449.

19. Ibrahim AS, Mirbod F, Filler SG, Banno Y, Cole GT, Kitajima Y, Edwards JE, Nozawa EY, Ghannoum MA: **Evidence implicating phospholipase as a virulence factor of *Candida albicans*.** *Infect Immun* 1995, **63**:1993-1998.

20. Swenson CE, Perkins WR, Roberts P, Ahmad I, Stevens R, Stevens DA, Janoff DS: **Evidence for a role for phospholipases in the *in vitro* and *in vivo* antifungal activity of amphotericin B lipid complex (ABLC) [Abstract F91].** In *Abstracts of the 37th Interscience Conference Antimicrobial Agents and Chemotherapy (ICAAC): 1997 Sept 28–Oct 1; Toronto.* Washington DC: American Society for Microbiology; 1997:161.

21. VanEtten EWM, Tenkate MT, Bakker-Woudenberg IAJM: **Efficacy of a new type of liposomal amphotericin B in severe invasive pulmonary aspergillosis in leukopenic rats [Abstract B15].** In *Abstracts of the 37th Interscience Conference Antimicrobial Agents and Chemotherapy (ICAAC): 1997 Sept 28–Oct 1; Toronto.* Washington DC: American Society for Microbiology; 1997:29.

22. Kelly SL, Lamb DC, Kelly DE, Manning NJ, Loeffer J, Einsele H: **Resistance to fluconazole and amphotericin in *Candida albicans* from AIDS patients.** *Lancet* 1996, **348**:1523-1524.

23. • Kelly SL, Lamb DC, Kelly DE, Manning NJ, Loeffer J, Hebart H, Schumacher U, Einsele H: **Resistance to fluconazole and cross-resistance to amphotericin B in *Candida albicans* from AIDS patients caused by defective sterol $\Delta^{5,6}$ desaturation.** *FEBS Lett* 1997, **400**:80-82.

This paper describes the basis for the cross-resistance between fluconazole and amphotericin observed in some clinical isolates.

24. Sokol-Anderson M, Sligh JE, Elberg S, Brajtburg J, Kobayashi GS, Medoff G: **Role of cell defense against oxidative damage in the resistance of *Candida albicans* to the killing effect of amphotericin B.** *Antimicrob Agents Chemother* 1988, **32**:702-705.

25. Anaissie E, Nelson P, Beremand M, Kontoyiannis D, Rinaldi M: ***Fusarium*-caused hyalohyphomycosis: an overview.** *Curr Top Med Mycol* 1992, **4**:231-249.

26. Walsh TJ, Melcher G, Rinaldi M, Lecciones J, McGough D, Lee J, Callender D, Rubin M, Pizzo PA: ***Trichosporon beigelii*: an emerging pathogen resistant to amphotericin B.** *J Clin Microbiol* 1990, **28**:1616-1622.

27. Fromtling R: **Overview of medically important azole derivatives.** *Clin Microbiol Rev* 1988, **1**:187-217.

28. Georgopapadakou NH, Walsh TJ: **Antifungal agents: chemotherapeutic targets and immunologic strategies.** *Antimicrob Agents Chemother* 1996, **40**:279-291.

29. Georgopapadakou NH, Dix BA, Smith SA, Freudenberger J, Funke PT: **Effect of antifungal agents on lipid biosynthesis and membrane integrity in *Candida albicans*.** *Antimicrob Agents Chemother* 1987, **31**:46-51.

30. Georgopapadakou NH, Bertasso A: **Effects of ergosterol inhibitors on chitin synthesis *in vitro* and *in vivo*.** In *Recent Advances in Chemotherapy: Antimicrobial Section II. Proc Intl Congr Chemother.* Edited by Adam A, Lode H, Rubinstein E. Munich: Futuramed Verlag; 1992:2208-2209.

31. Parks LW, Lorenz RT, Casey WM. **Functions for sterols in yeast membranes.** In *Emerging Targets in Antibacterial and Antifungal Chemotherapy.* Edited by Sutcliffe J, Georgopapadakou NH. New York: Chapman and Hall; 1992:393-409.

32. Shimokawa O, Nakayama H: **Increased sensitivity of *Candida albicans* cells accumulating 14α-methylated sterols to active oxygen: possible relevance to *in vivo* efficacies of azole antifungal agents.** *Antimicrob Agents Chemother* 1992, **36**:1626-1629.

33. Johnson EM, Warnock DW, Luker J, Porter SR, Scully C: **Emergence of azole drug resistance in *Candida* species from HIV-infected patients receiving prolonged fluconazole therapy for oral candidosis.** *J Antimicrob Chemother* 1995, **35**:103-114.

34. Rex HJ, Rinaldi MG, Pfaller MA: **Resistance of *Candida* species to fluconazole.** *Antimicrob Agents Chemother* 1995, **39**:1-8.

35. Coker RJ, Harris JRW: **Failure of fluconazole treatment in cryptococcal meningitis despite adequate CSF levels.** *J Infect* 1991, **23**:101-103.

36. Kelly SL, Rowe J, Watson PF: **Molecular genetic studies on the mode of action of azole antifungal agents.** *Biochem Soc Trans* 1991, **19**:796-798.

37. •• White TC, Marr KA, Bowden RA: **Clinical, cellular, and molecular factors that contribute to antifungal drug resistance.** *Clin Microbiol Rev* 1998, **11**:382-402.

A thoughtful, up-to-date review of resistance mechanisms for major antifungal classes.

38. Hitchcock CA: **Resistance of *Candida albicans* to azole antifungal agents.** *Biochem Soc Trans* 1993, **21**:1039-1047.

39. •• Sanglard D, Ischer F, Calabrese D, de Micheli M, Bille J: **Multiple resistance mechanisms to azole antifungal agents in yeast clinical isolates.** *Drug Resist Updates* 1998, **1**:255-265.

A superb review of mechanisms of clinical resistance to azole antifungals that incorporates some unpublished work from the authors' laboratory.

40. Kelly SL, Lamb DC, Corran AJ, Baldwin BC, Kelly DE: **Mode of action and resistance to azole antifungals associated with the formation of 14α-methylergosta-8,24(28)-dien-3β,6α-diol.** *Biochem Biophys Res Commun* 1995, **207**:910-915.

41. Taglicht D, Michaelis S: ***Saccharomyces cerevisiae* ABC proteins and their relevance to human health and disease.** *Methods Enzymol* 1998, **292**: in press.

42. Goffeau A, Park J, Paulsen IT, Jonniaux JL, Dinh T, Mordant P, Saier MH: **Multidrug-resistant transport proteins in yeast: complete inventory and phylogenetic characterization of yeast**

open reading frames within the major facilitator superfamily. *Yeast* 1997, **13**:43-54.

43. *Saccharomyces* Genome Database on World Wide Web URL:
•• http://genome-www.stanford.edu/Saccharomyces
A very useful web site for the genome of this model, nonpathogenic yeast. It serves as a reference for less well studied genomes, such as *Candida albicans*, with which it shares some homology.

44. Prasad R, De WP, Goffeau A, Balzi E: **Molecular cloning and characterization of a novel gene of *Candida albicans*, *CDR1*, conferring multiple resistance to drugs and antifungals.** *Curr Genet* 1997, **27**:320-329.

45. Sanglard D, Ischer F, Monod M, Bille J: **Cloning of *Candida albicans* genes conferring resistance to azole antifungal agents: characterization of *CDR2*, a new multidrug ABC transporter gene.** *Microbiol* 1997, **143**:405-416.

46. Balan I, Alarco AM, Raymond M: **The *Candida albicans CDR3* gene codes for an opaque-phase ABC transporter.** *J Bacteriol* 1997, **179**:7210-7218.

47. Hernaez ML, Gil C, Pla J, Nombela C: **Induced expression of the
• *Candida albicans* multidrug resistance gene *CDR1* in response to fluconazole and other antifungals.** *Yeast* 1998, **14**:517-526.
This is the first description of azole-induced expression of an efflux pump.

48. Sanglard D, Kuchler K, Ischer F, Pagani JL, Monod M, Bille J: **Mechanisms of resistance to azole antifungal agents in *Candida albicans* isolates from AIDS patients involve specific multidrug transporters.** *Antimicrob Agents Chemother* 1995, **39**:2378-2386.

49. Ryder NS: **Mechanism of action and biochemical selectivity of allylamine antimycotic agents.** *Ann NY Acad Sci* 1988, **544**:208-220.

50. Ryder NS: **Squalene epoxidase as a target for the allylamines.** *Biochem Soc Trans* 1991, **19**:774-777.

51. Georgopapadakou NH, Bertasso A: **Effects of squalene epoxidase inhibitors in *Candida albicans.*** *Antimicrob Agents Chemother* 1992, **36**:1779-1781.

52. Orth AB, Henry MJ, Sisler HD: **Mechanism of resistance to terbinafine in two isolates of *Ustilago maydis.*** *Pestic Biochem Physiol* 1990, **37**:182-191.

53. Francis P, Walsh TJ: **Evolving role of flucytosine in immunocompromised patients: new insights into safety, pharmacokinetics, and antifungal therapy.** *Rev Infect Dis* 1992, **15**:1003-1018.

54. Viviani MA: **Flucytosine – what is its future?** *J Antimicrob Chemother* 1995, **35**:241-244.

55. Polak A: **Mode of action studies**. In *Handbook of Experimental Pharmacology,* vol 96. *Chemotherapy of Fungal Diseases.* Edited by Ryley JF. Heidelberg: Springer-Verlag; 1990:153-182.

56. Tkacz JS. **Glucan biosynthesis in fungi and its inhibition.** In *Emerging Targets in Antibacterial and Antifungal Chemotherapy*. Edited by Sutcliffe J, Georgopapadakou NH. New York: Chapman and Hall; 1992:495-523.

57. Douglas CM, Marrinan JA, Li W, Kurtz MB: **A *Saccharomyces cerevisiae* mutant with echinocandin-resistant 1,3-–D-glucan synthase.** *J Bacteriol* 1994, **176**:5686-5696.

58. Bickle M, Delley P-A, Schmidt A, Hall MN: **Cell wall integrity modulates RHO1 activity via the exchange factor ROM2.** *EMBO J* 1998, **17**:2235-2245.

59. Mol PC, Park H-M, Mullins JT, Cabib EA: **GTP-binding protein regulates the activity of (1,3)-–glucan synthase, an enzyme directly involved in yeast cell wall morphogenesis.** *J Biol Chem* 1994, **269**:31267-31274.

60. Drgonova J, Drgon T, Tanaka K, Kollar R, Chen G-C, Ford RA, Chan CSM, Takai Y, Cabib E: **Rho1p, a yeast protein at the interface between cell polarization and morphogenesis.** *Science* 1996**, 272**:277-279.

61. Qadota H, Python CP, Inoue SB, Arisawa M, Anraku Y, Zheng Y, Watanabe T, Levin DE, Ohya Y: **Identification of yeast Rho1p GTPase as a regulatory subunit of 1,3-–glucan synthase.** *Science* 1996, **272**:279-281.

62. Errede B, Levin DE: **Yeast and signal transduction.** *Curr Opin Cell Biol* 1993, **5**:254-260.

63. Nonaka H, Tanaka K, Hirano H, Fujiwara T, Kohno H, M. Umikawa M, Mino A, Takai Y: **A downstream target of RHO1 small GTP-binding protein is PKC1, a homolog of protein kinase C, which leads to activation of the MAP kinase cascade *in Saccharomyces cerevisiae.*** *EMBO J* 1995, **14**:5931.

64. Levin DE, Bartlett-Heubusch E: **Mutants in the *Saccharomyces cerevisiae* PKC1 gene display a cell cycle-specific osmotic instability defect.** *J Cell Biol* 1992, **116**:1221-1229.

65. Cid VJ, Duran A, Del Rey F, Snyder MP, Nombela C, Sanchez M: **Molecular basis of cell integrity and morphogenesis in *Saccharomyces cerevisiae.*** *Microbiol Rev* 1995, **59**:345-386.

66. Douglas CM, Foor F, Marrinan JA, Morin N, Nielsen JB, Dahl AM, Mazur P, Baginsky W, Li WL, El Sherbeini M *et al.*: **The *Saccharomyces cerevisiae FKS1* (*ETG1*) gene encodes an integral membrane protein which is a subunit of 1,3-–D-glucan synthase.** *Proc Natl Acad Sci USA* 1994, **91**:1207-1211.

67. Mazur P, Morin N, Baginsky W, El-Sherbeini M, Clemas JA, Nielsen JB, Foor F: **Differential expression and function of two homologous subunits of yeast 1,3-β-D-glucan synthase.** *Mol Cell Biol* 1995, **15**:5671-5681.

68. Georgopapadakou NH, Tkacz JS: **The fungal cell wall as a drug target.** *Trends Microbiol* 1995, **3**:98-104.

69. Gozalbo D, Elorza MV, Sanjuan R, Marcilla A, Valentin E, Sentandreu R: **Critical steps in fungal cell wall synthesis: strategies for their inhibition.** *Pharmac Ther* 1993, **60**:337-345.

70. Debono N, Gordee RS: **Antibiotics that inhibit fungal cell wall development.** *Annu Rev Microbiol* 1994, **48**:471-497.

71. Current WL, Tang J, Boylan C, Watson P, Zeckner D, Turner W, Rodriguez M, Dixon C, Ma D, Radding JA: **Glucan biosynthesis as a target for antifungals: the echinocandin class of antifungal agents.** In *Antifungal Agents: Discovery and Mode of Action.* Edited by Dixon GK, Copping LG, Hollomon DW. Oxford: Bios Scientific Publishers; 1995:143-160.

72. Abruzzo GK, Flattery AM, Gill CJ, Kong L, Smith JG, Krupa D, Pikounis VB, Kropp H, Bartizal K: **Evaluation of water-soluble pneumocandin analogs L-733560, L-705589, L-731373 with mouse models of disseminated aspergillosis, candidiasis, and cryptococcosis.** *Antimicrob Agents Chemother* 1995, **39**:1077-1081.

73. Bouffard FA, Dropinski JF, Balkovec JM, Black RM, Hammond ML, Nollstadt KH, Dreikorn S: **L-743,872, a novel antifungal lipopeptide: synthesis and structure-activity relationships of new aza-substituted pneumocandins [Abstract F27].** In *Abstracts of the 36th Interscience Conference Antimicrobial Agents and Chemotherapy (ICAAC): 1996 Sept 15–18, New Orleans.* Washington DC: American Society for Microbiology; 1996:104.

74. Denning DW: **Echinocandins and pneumocandins – a new
• antifungal class with a novel mode of action.** *J Antimicrob Chemother* 1997, **40**:611-614.
A readable review of this class of β-(1,3)-glucan synthase inhibitors, focusing on the two compounds that are currently in clinical development.

75. Radding JA, Heidler SA, Turner WW: **Photoaffinity analog of the
• semisynthetic echinocandin LY303366: identification of echinocandin targets in *Candida albicans.*** *Antimicrob Agents Chemother* 1998, **42**:1187-1194.
A surprising finding suggesting that echinocandins/pneumocandins may interact with proteins other than their target, β-(1,3)-glucan synthase.

76. Li L, Thomas S, Grampovnik DJ, Klein LL, Degoey D, Chen HJ, Yeung CM, Leone C, Curty C, Frost D, Nilius A, Meulbroek J, Lartey PA, Plattner JJ: **Synthesis and antifungal activities of cyclopeptamines. Novel cell wall synthesis inhibitors [Abstract F238].** In *Abstracts of the 37th Interscience Conference Antimicrobial Agents and Chemotherapy (ICAAC): 1997 Sept 28–Oct 1; Toronto.* Washington DC: American Society for Microbiology; 1997:186.

77. Meulbroek JA, Pleksijew A, Tovcimak A, Alder JD, Tanaka SK, Degoey D, Klein LL, Li L, Lartey P: **Efficacy of A-175800.0, an inhibitor of fungal cell wall synthesis, against experimental systemic candidiasis [Abstract F82].** In *Abstracts of the 37th Interscience Conference Antimicrobial Agents and Chemotherapy (ICAAC): 1997 Sept 28–Oct 1; Toronto.* Washington DC: American Society for Microbiology; 1997:159.

78. Capobianco JO, Zakula D, Frost JD, Goldman RC, Li L, Klein L, Lartey P: **Cellular uptake, localization and activity of A172013, a synthetic cyclopeptamine, in fungi [Abstract F83].** In *Abstracts of the 37th Interscience Conference Antimicrobial Agents and*

Chemotherapy (ICAAC): 1997 Sept 28–Oct 1; Toronto. Washington DC: American Society for Microbiology; 1997:160.

79. •• Georgopapadakou NH: **Cell-wall active antifungals and emerging targets.** In *Antiinfectives: Recent Advances in Chemistry and Structure-Activity Relationships.* Edited by Bentley PH, O-Hanlon PJ. Cambridge: The Royal Society of Chemistry; 1997:163-175.
A useful summary of cell fungal cell wall biosynthesis and its inhibition.

80. Bulawa CE: **Genetics and molecular biology of chitin synthesis in fungi.** *Annu Rev Microbiol* 1993, **47**:505-534.

81. Cabib E: **Differential inhibition of chitin synthases 1 and 2 from *Saccharomyces cerevisiae* by polyoxin D and nikkomycins.** *Antimicrob Agents Chemother* 1991, **35**:170-173.

82. Gaughran JP, Lai MH, Kirsch DR, Silverman SJ: **Nikkomycin Z is a specific inhibitor of *Saccharomyces cerevisiae* chitin synthase isozyme Chs3 *in vitro* and *in vivo*.** *J Bacteriol* 1994, **176**:5857-5860.

83. Bulawa CE, Miller DW, Henry LK, Becker JM: **Attenuated virulence of chitin-deficient mutants of *Candida albicans*.** *Proc Natl Acad Sci* USA 1995, **92**:10570-10574.

84. Oki T, Kakushima M, Hirano M, Takahashi A, Ohta A, Masuyoshi S, Hatori M, Kamei H: ***In vitro* and *in vivo* antifungal activities of BMS-181184.** *J Antibiot* 1992, **45**:1512-1517.

85. Fungtomc JC, Minassian B, Huczko E, Kolek B, Bonner DP, Kessler RE: ***In vitro* antifungal and fungicidal spectra of a new pradimicin derivative, BMS-181184.** *Antimicrob Agents Chemother* 1995, **39**:295-300.

86. Ueki T, Numata K-I, Sawada Y, Nishio M, Ohkuma H, Toda S, Kamachi, Fukagawa Y, Oki T: **Studies on the mode of antifungal action of pradimicin antibiotics. II. D-Mannopyranoside-binding site and calcium-binding site.** *J Antibiot* 1993, **46**:455-469.

87. Georgopapadakou NH: **Antifungals targeted to the cell wall.** *Exp Opin Invest Drugs* 1997, **6**:147-150.

88. Oehlschlager AC, Czyzewska E: **Rationally designed inhibitors for sterol biosynthesis.** In *Emerging Targets in Antibacterial and Antifungal Chemotherapy*. Edited by Sutcliffe J, Georgopapadakou NH. New York: Chapman and Hall; 1992: 437-475.

89. Mercer EI: **Inhibitors of sterol biosynthesis and their applications.** *Prog Lipid Res* 1993, **2**:357-416.

90. Koltin Y, Hitchcock CA: **The search for new triazole antifungal agents.** *Curr Opin Chem Biol* 1997, **1**:176-182.

91. Dodd DS, Oehlschlager AC, Georgopapadakou NH, Polak AM, Hartman PG: **Synthesis of inhibitors of 2,3-oxidosqualene lanosterol cyclase. Part II: cyclocondensation of γ,δ-unsaturated β-ketoesters with imines.** *J Org Chem* 1992, **57**:7226-7234.

92. Zheng YF, Oehlschlager AC, Georgopapadakou NH, Hartman PG, Scheliga P: **Synthesis of the sulfur- and sulfoxide-substituted 2,3-oxidosqualenes and their evaluation as inhibitors of 2,3-oxidosqualene-lanosterol cyclase.** *J Amer Chem Soc* 1995, **117**:670-680.

93. Urbina JA, Visbal G, Contreras LM, McLaughlin GL, Docampo R: **Inhibitors of $\Delta^{24(25)}$ sterol methyltransferase block sterol synthesis and cell proliferation in *Pneumocystis carinii* [Abstract F95].** In *Abstracts of the 37th Interscience Conference Antimicrobial Agents and Chemotherapy (ICAAC): 1997 Sept 28–Oct 1; Toronto.* Washington: American Society for Microbiology; 1997:162.

94. Dawson MJ, Farthing JE, Marshall PS, Middleton RF, O'Neill MJ, Shuttleworth A, Stylli C, Tait RM, Taylor PM, Wildman HG *et al.*: **The squalestatins, novel inhibitors of squalene synthase produced by a species of *Phoma*. I. Taxonomy, fermentation, isolation, physicochemical properties and biological activity.** *J Antibiot* 1992, **45**:639-647.

95. Robinson GW, Tsay YH, Kienzle BK, Smith-Monroy CA, Bishop RW: **Conservation between human and fungal squalene synthetases: similarities in structure, function, and regulation.** *Mol Cell Biol* 1993, **13**:2706-2717.

96. Belfield GP, Tuite MF: **Translation elongation factor 3: a fungus-specific translation factor?** *Mol Microbiol* 1993, **8**:411-418.

97. Ypma-Wong MF, Fonzi WA, Sypherd PS: **Fungus-specific translation elongation factor 3 gene present in *Pneumocystis carinii*.** *Infect Immun* 1992, **60**:4140-4145.

98. Colthurst DR, Santos M, Grant CM, Tuite MF: ***Candida albicans* and three other *Candida* species contain an elongation factor structurally and functionally analogous to elongation factor 3.** *FEMS Lett* 1991, **80**:45-50.

99. Belfield GP, Ross-Smith NJ, Tuite MF: **Translation elongation factor 3 (EF-3): an evolving eukaryotic ribosomal protein?** *J Mol Evol* 1995, **41**:376-387.

100. Triana Alonso FJ, Chakraburtty K, Nierhaus KH: **The elongation factor 3 unique in higher fungi and essential for protein biosynthesis is an E site factor.** *J Biol Chem* 1995, **270**:20473-20478.

101. • Sarthy AV, McGonigal T, Capobianco JO, Schmidt M, Green SR, Moehle CM, Goldman RC: **Identification and kinetic analysis of a functional homolog of elongation factor 3, YEF3 in *Saccharomyces cerevisiae*.** *Yeast* 1998, **14**:239-253.
This paper brings up the possibility that there may be more than a single elongation factor 3 in fungi.

102. Bueno JM, Chicharro J, Huss SI, Fiandor JM, Gomez de las Heras F: **Synthesis of the antifungal GM237354 [Abstract F55].** In *Abstracts of the 37th Interscience Conference Antimicrobial Agents and Chemotherapy (ICAAC): 1997 Sept 28–Oct 1; Toronto.* Washington DC: American Society for Microbiology; 1997:155.

103. Dominguez JM, Capa L, Serramia MJ, Mendoza A, Viana JM, Garcia-Bustos JF, Martin JJ: **Translation elongation factor 2 (EF2) is the target for sordarin-derived antifungals [Abstract F59].** In *Abstracts of the 37th Interscience Conference Antimicrobial Agents and Chemotherapy (ICAAC): 1997 Sept 28–Oct 1; Toronto.* Washington DC: American Society for Microbiology; 1997:155.

104. Herreros E, Martinez A, Almela MJ, Martinez CM, Lozano S, Jimenez E, Gomez de las Heras F, Gargallo D: **GM 237254: in vitro selectivity and tolerance in rodents [Abstract F56].** In *Abstracts of the 37th Interscience Conference Antimicrobial Agents and Chemotherapy (ICAAC): 1997 Sept 28–Oct 1; Toronto.* Washington DC: American Society for Microbiology; 1997:156.

105. Sanchez-Sousa A, Alvarez ME, Parra C, Baquero F: **Activity on clinical yeast isolates of a new antifungal agent, GM 237354 [Abstract F57].** In *Abstracts of the 37th Interscience Conference Antimicrobial Agents and Chemotherapy (ICAAC): 1997 Sept 28–Oct 1; Toronto.* Washington DC: American Society for Microbiology; 1997:155.

106. Herreros E, Martinez CM, Almela MJ, Lozano S, Gomez de las Heras F, Gargallo D: ***In vitro* activity of GM 237354 against a broad range of fungi [Abstract F57].** In *Abstracts of the 37th Interscience Conference Antimicrobial Agents and Chemotherapy (ICAAC): 1997 Sept 28–Oct 1; Toronto.* Washington DC: American Society for Microbiology; 1997:155.

107. Kinsman OS, Chalk PA, Jackson HC, Middleton RF, Shuttleworth A, Rudd BAM, Jones CA, Noble HM, Wildman HG, Dawson MJ, Stylli C *et al.*: **Isolation and characterization of an antifungal antibiotic (GR135402) with protein synthesis inhibition.** *J Antibiot* 1998, **51**:41-49.

108. Johnson DR, Bhatnagar RS, Knoll LJ, Gordon JI: **Genetics and biochemical studies of protein *N*-myristoylation.** *Annu Rev Biochem* 1994, **63**:869-914.

109. Langner CA, Lodge JK, Travis SJ, Caldwell JE, Lu T, Li Q, Bryant ML, Devadas B, Gokel GW, Kobayashi GS, Gordon JI: **4-Oxatetradecanoic acid is fungicidal for *Cryptococcus neoformans* and inhibits replication of human immunodeficiency virus I.** *J Biol Chem* 1992, **267**:17159-17169.

110. Lodge JK, Jackson-Machelski E, Toffaletti DL, Perfect JR, Gordon JI: **Targeted gene replacement demonstrates that myristoyl-CoA:protein *N*-myristoyltransferase is essential for viability of *Cryptococcus neoformans*.** *Proc Nat Acad Sci USA* 1994, **91**:12008-12012.

111. Weinberg RA, McWherter CA, Freeman SK, Wood DC, Gordon JI, Lee SC: **Genetic studies reveal that myristoylCoA:protein *N*-myristoyltransferase is an essential enzyme in *Candida albicans*.** *Mol Microbiol* 1995, **6**:241-250.

112. Lodge JK, Johnson RL, Weinberg RA, Gordon JI**. I. Comparison of myristoyl CoA:protein *N*-myristoyltransferases from three pathogenic fungi — *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Candida albicans*.** *J Biol Chem* 1994, **269**:2996-3009.

113. Kishore NS, Wood DC, Mehta PP, Wade AC, Liu T, Gokel GW, Gordon JI: **A comparison of the acyl chain specificities of human**

myristoyl-CoA synthetase and human myristoyl-CoA protein *N*-myristoyltransferase. *J Biol Chem* 1993, **268**:4889-4902.

114. Rocque WJ, McWherter CA, Wood DC, Gordon JI: **A comparative analysis of the kinetic mechanism and peptide substrate specificity of human and *Saccharomyces cerevisiae* myristoyl-CoA:protein *N*-myristoyltransferase.** *J Biol Chem* 1993, **268**:9964-9971.

115. •• Weston SA, Camble R, Colls J, Rosenbrock G, Taylor I, Egerton M, Tucker AD, Tunnicliffe A, Mistry A, Mancia F *et al.*: **Crystal structure of the anti-fungal target *N*-myristoyl transferase.** *Nat Struct Biol* 1998, **5**:213-221.

This excellent paper on the crystal structure of *N*-myristoyl transferase will undoubtedly aid in rational drug design.

116. Devadas B, Freeman SK, Zupec ME, Lu HF, Nagarajan SR, Kishore NS, Lodge JK, Kuneman DW, McWherter CA, Vinjamoori DV *et al.*: **Design and synthesis of novel imidazole-substituted dipeptide amides as potent and selective inhibitors of *Candida albicans* myristoylCoA:protein *N*-myristoyltransferase and identification of related tripeptide inhibitors with mechanism-based antifungal activity.** *J Med Chem* 1997, **40**:2609-2625.

117. • Devadas B, Freeman SK, McWherter CA, Kishore NS, Lodge JK, Jackson-Machelski E, Gordon JI, Sikorski JA: **Novel biologically active nonpeptidic inhibitors of myristoylCoA:protein *N*-myristoyltransferase.** *J Med Chem* 1998, **41**:996-1000.

This latest report on *N*-myristoyltransferase inhibitors moves away from peptides and towards compounds with antifungal activity.

118. deNobel H, Lipke PN: **Is there a role for GPIs in yeast cell-wall assembly?** *Trends Cell Biol* 1994, **4**:42-45.

119. • Caro LHP, Tettelin H, Vossen JH, Ram AFJ, van den Ende H, Klis FM: ***In silicio* identification of glycosyl phosphatidylinositol-anchored plasma membrane and cell wall proteins of *Saccharomyces cerevisiae*.** *Yeast* 1997, **13**:1477-1489.

This interesting report suggests that the synthesis of glycosyl phosphatidylinositol-anchored proteins may be an antifungal target.

120. Lester RL, Dickson RC: **Sphingolipids with inositolphosphate-containing head groups.** *Adv Lipid Res* 1993, **26**:253-273.

121. Merrill AH, Liotta DC, Riley RT: **Fumonisins: fungal toxins that shed light on sphingolipid function.** *Trends Cell Biol* 1996, **6**:218-223.

122. Mandala S, Thornton R, Frommer B, Curotto J, Rozdilsky W, Kurtz M, Giacobbe R, Bills G, Cabello M, Martin I: **The discovery of australifungin, a novel inhibitor of sphinganine *N*-acyltransferase from *Sporormiella australis*. Producing organism, fermentation, isolation, and biological activity.** *J Antibiot* 1995, **48**:349-356.

123. •• Nagiek MM, Nagiec EE, Baltisberger JA, Wells GB, Lester RL, Dickson RC: **Sphingolipid synthesis as a target for antifungal drugs.** *J Biol Chem* 1997, **272**:9809-9817.

This excellent paper links the aureobasidin-resistance gene with inositolphosphorylceramide synthase and describes an activity assay for the enzyme.

124. Heidler SA, Radding JA: **The *AUR1* gene in *Saccharomyces cerevisiae* encodes dominant resistance to the antifungal agent aureobasidin A (LY295337).** *Antimicrob Agents Chemother* 1995, **39**:2765-2769.

125. Ikai K, Takesako K, Shiomi K, Moriguchi M, Umeda Y, Yamamoto J, Kato I, Naganawa H: **Structure of aureobasidin A.** *J Antibiot* 1991, **44**:925-933.

126. Mandala SM, Thornton RA, Rosenbach M, Milligan J, Garcia-Calvo M, Bull HG, Kurtz MB: **Khafrefungin, a novel inhibitor of sphingolipid synthesis.** *J Biol Chem* 1997, **272**:32709-32714.

127. Achenbach H, Muhlenfeld A, Fauth U, Zahner H: **The galbonolides: novel, powerful antifungal macrolides from *Streptomyces galbus* spp. *Eurythermus*.** *Ann NY Acad Sci* 1988, **544**:128-140.

128. • Mandala SM, Thornton RA, Milligan J, Rosenbach M, Garcia Calvo M, Bull HG, Harris G, Abruzzo GK, Flattery AM, Gill CJ *et al.*: **Rustmicin, a potent antifungal agent, inhibits sphingolipid synthesis at inositol phosphoceramide synthase.** *J Biol Chem* 1998, **273**:14942-14949.

This paper identifies the target of the macrolide galbonolide/rustmicin as inositolphosphorylceramide synthase.

129. Iwasaki S: **Antimitotic agents: chemistry and recognition of tubulin molecule.** *Med Res Rev* 1993, **13**:183-198.

130. Shen LL, Baranowski J, Fostel J, Montgomery DA, Lartey PA: **DNA topoisomerases from pathogenic fungi: targets for the discovery of antifungal drugs.** *Antimicrob Agents Chemother* 1992, **36**:2778-2784.

131. Dykstra CC, McClernon DR, Elwell LP, Tidwell RR: **Selective inhibition of topoisomerases from *Pneumocystis carinii* compared with that of topoisomerases from mammalian cells.** *Antimicrob Agents Chemother* 1994, **38**:1890-1898.

132. Fostel J, Montgomery D: **Identification of the aminocatechol A-3253 as an *in vitro* poison of DNA topoisomerase I from *Candida albicans*.** *Antimicrob Agents Chemother* 1995, **39**:586-592.

133. Rosowsky A, Hynes JB, Queener SF: **Structure-activity and structure-selectivity studies on diaminoquinazolines and other inhibitors of *Pneumocystis carinii* and *Toxoplasma gondii* dihydrofolate reductase.** *Antimicrob Agents Chemother* 1995, **39**:79-86.

134. Perfect JR, Toffaletti DL, Rude TH: **The gene encoding phosphoribosyl-aminoimidazole carboxylase (ADE2) is essential for growth of *Cryptococcus neoformans* in cerebrospinal fluid.** *Infect Immun* 1993, **61**:4446-4451.

135. Gustafson G, Davis G, Waldron C, Smith A, Henry M: **Identification of a new antifungal target site through a dual biochemical and molecular-genetics approach.** *Curr Genet* 1996, **30**:159-165.

136. Chen SF, Perrella FW, Behrens DL, Papp LM: **Inhibition of dihydroorotate dehydrogenase activity by brequinar sodium.** *Cancer Res* 1992, **52**:3521-3527.

137. Konishi M, Nishio M, Saitoh K, Miyaki T, Oki T, Kawaguchi H: **Cispentacin, a new antifungal antibiotic. I. Production, isolation, physicochemical properties, and structure.** *J Antibiot* 1989, **42**:1749-1755.

138. Oki T, Hirano M, Tomatsu K, Numata K-I, Kamei H: **Cispentacin, a new antifungal antibiotic. II. *In vitro* and *in vivo* antifungal activities.** *J Antibiot* 1989; **42**:1756-1760.

139. Capobianco JO, Zakula D, Coen ML, Goldman RC: **Anticandidal activity of cispentacin. The active transport by amino acid permease and possible mechanisms of action.** *Biochem Biophys Res Commun* 1993, **190**:1037-1044.

140. Yamaki H, Yamaguchi M, Imamura H, Suzuki H, Nishimura T, Saito H, Yamaguchi H: **The mechanism of antifungal action of (S)-2-amino-4-oxo-5-hydroxypentanoic acid, RI 331: the inhibition of homoserine dehydrogenase in *Saccharomyces cerevisiae*.** *Biochem Biophys Res Commun* 1990, **168**:837-843.

141. Aoki Y, Kondoh M, Nakamura M, Fujii T, Yamazaki T, Shimada H, Arisawa M: **A new methionine antagonist that has antifungal activity: mode of action.** *J Antibiot* 1994, **47**:909-916.

142. McCann PP, Pegg AE: **Ornithine decarboxylase as an enzyme target for therapy.** *Pharmac Ther* 1992, **54**:195-215.

143. Saric M, Clarkson AB Jr: **Ornithine decarboxylase in *Pneumocystis carinii* and implications for therapy.** *Antimicrob Agents Chemother* 1994, **38**:2545-2552.

144. Monk BC, Perlin DS: **Fungal plasma membrane protein pumps as promising new antifungal targets.** *Crit Rev Microbiol* 1994, **20**:209-223.

145. Ben-Yaacov R., Becker JM, Oppenheim JM, Oppenheim A, Goldway M, Schmidt R, Jiang W, Clifford J, Koltin Y: **Multidrug resistance in *Candida albicans*.** *J Cell Biochem* 1995, **B4**:146.

146. Balzi E, Goffeau A: **Genetics and biochemistry of yeast multidrug resistance.** *Biochim Biophys Acta* 1994, **1187**:152-162.

147. •• Groll AH, De Lucca AJ, Walch TJ: **Emerging targets for the development of novel antifungal therapeutics.** *Trends Microbiol* 1998, **6**:117-124.

This is an excellent minireview on antifungal drugs currently in development and targets under investigation.

Patent

P1. Masubuchi M, Okuda T, Shimada H: **Antifungal agent, its preparation and microorganism there for.** European patent application, April/21/1993, EP 0537622A.