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Emerging targets for the development of novel antifungal therapeutics

Andreas H. Groll, Anthony J. De Lucca and Thomas J. Walsh

Invasive mycoses have become important causes of morbidity and mortality in immunocompromised patients. New approaches for antifungal therapy are required to meet the challenges imposed by these life-threatening infections. Such approaches are being developed through identification of novel biochemical and molecular targets of pathogenic fungi.

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Together with the growing number of individuals with impaired host defenses, invasive fungal infections have emerged as major causes of morbidity and mortality[1–3]. For many years, the treatment of these infections was essentially limited to amphotericin B (AmB). Therapeutic options did not emerge until the late 1980s when fluconazole and itraconazole were introduced. The past decade, however, has seen a major expansion in antifungal drug research; these efforts have resulted in the development of lipid-based formulations of AmB and the discovery of several new antifungal compounds that are currently at various stages of clinical investigation (Tables 1,2).

The key to the development of new drugs is the identification and characterization of new targets. Current strategies include the classical screening of natural products or synthetic substances, the design of compounds directed against essential biochemical or molecular targets, and the novel approach of whole genome sequencing and bioinformatics. Owing to their eukaryotic nature, however, fungal cells have only a restricted set of specific targets that do not overlap with their mammalian counterparts. Here, we look beyond current investigational compounds and aim to provide a structured update on recent trends in the search for new targets for antifungal therapeutics (Fig. 1).

Fungal cell wall

The dynamics of the fungal cell wall are closely coordinated with growth and division, and its predominant function is to control the internal turgor pressure of the cell; interference with its assembly or integrity eventually leads to cell lysis and cell death. With considerable variation among different species, the gross macromolecular components of the cell wall of most fungi include chitin, β- or α-linked glucans and a variety of mannoproteins. Glucans and chitin are predominantly found

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Table 1. Systemic antifungal agents in clinical use

Class and compound	Mechanism of action
Polyene antibiotics	
Amphotericin B Lipid formulations of amphotericin B	Interaction with ergosterol, formation of aqueous channels, increased membrane permeability to univalent cations and, ultimately, cell death
Nucleoside analog	
5-Fluorocytosine	Intracellular deamination to 5-fluorouracil; interference with both DNA and RNA synthesis/function
Antifungal azoles	
Ketoconazole Fluconazole Itraconazole	Interaction with cytochrome P-450; inhibition of C-14 demethylation of lanosterol, causing ergosterol depletion and accumulation of aberrant and toxic sterols in the cell membrane
Allylamines	
Terbinafine[a]	Inhibition of squalene epoxidase, resulting in ergosterol depletion and accumulation of aberrant and toxic sterols in the membrane
Cell mitosis inhibitor	
Griseofulvin[a]	Inhibition of fungal cell mitosis at metaphase by interaction with polymerized microtubules

[a]For systemic treatment of fungal infections of the skin and its appendages only.

in the inner layer, while mannoproteins are predominantly found in the outer layer of the wall. While chitin and the rope-like glucan fibrils are essential and responsible for the strength and shape of the cell wall, interstitial mannoproteins, which are neither unique nor considered to be essential to fungi, account for its porosity, antigenicity and, as in *Candida albicans*, its capability for adhesion[4,5].

Current investigational systemic antifungal agents directed against or involving the major constituents of the fungal cell wall include the nucleoside–peptide antibiotic nikkomycin Z (a competitive inhibitor of chitin synthase), the echinocandin family of antibiotics (acetylated cyclic hexapeptides that act as inhibitors of glucan assembly and cell polarization) and, with limitations, the pradimicins (D-alanine-substituted benzonaphthacene quinones with mono- and disaccharide side chains, which exert their effects on the cell membrane by complexing with mannoproteins). These new classes of antifungal agents have been reviewed recently[6].

Table 2. Current investigational systemic antifungal agents

Class and compound	Mechanism of action
Antifungal triazoles	
UK 109496 (voriconazole) SCH 56592	Interaction with cytochrome P-450; inhibition of C-14 demethylation of lanosterol, causing ergosterol depletion and accumulation of aberrant and toxic sterols in the cell membrane
Echinocandins	
LY 303366 L 743872 (MK-0991)	Inhibition of the fungal β-(1,3)glucan synthase complex, leading to depletion of cell wall glucan and osmotic instability
Pradimicins	
BMS 181184[a] Benanomycin A	Ca^{2+}-dependent complexing with cell wall mannoproteins, leading to perturbation of the cell membrane and, ultimately, cell death
Polyene antibiotics	
Liposomal nystatin	Interaction with ergosterol, formation of aqueous channels, increased membrane permeability to univalent cations and, ultimately, cell death
Nikkomycins	
Nikkomycin Z	Inhibition of fungal chitin synthase, leading to inhibition of growth and osmotic fragility
Sordarins	
GM 237354	Interaction with fungal elongation factor 2

[a]Development stopped owing to laboratory signs of hepatic toxicity in early human studies.

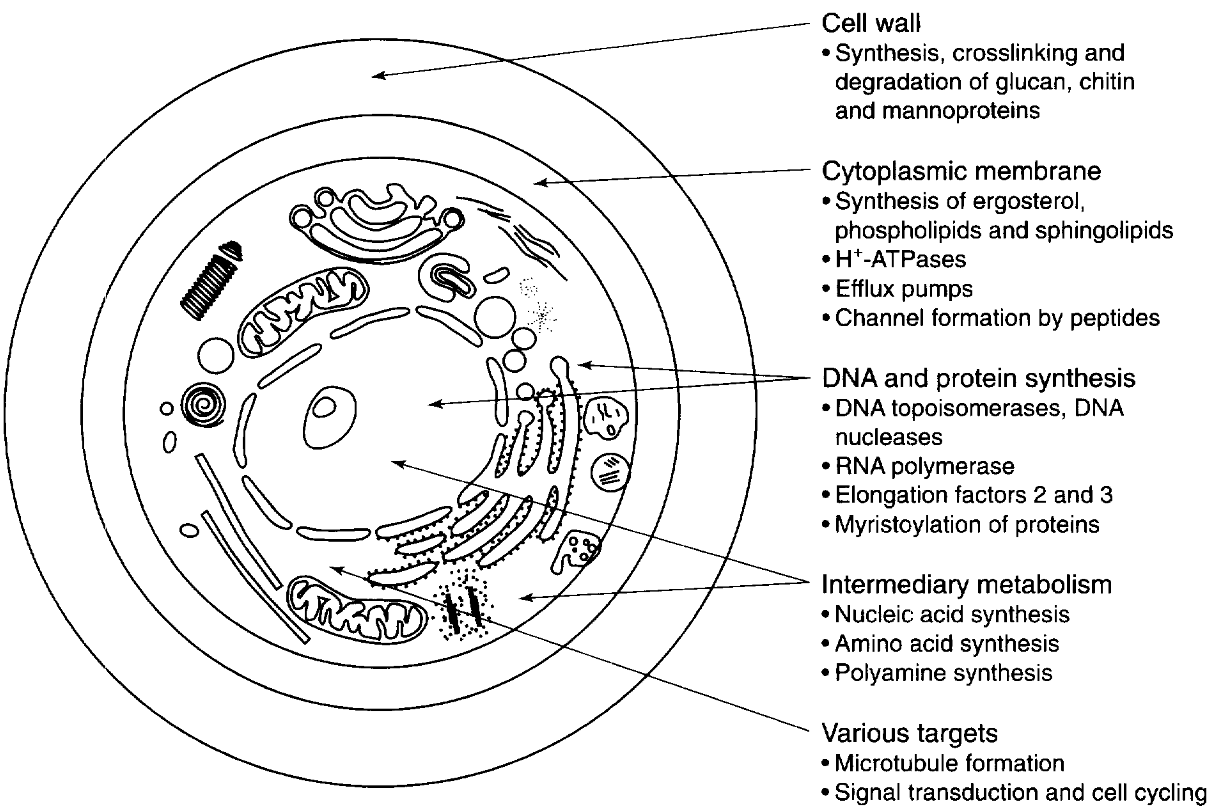

Fig. 1. Schematic overview of emerging targets and compounds for antifungal drug development. Adapted with permission from Rapid Science and Ref. 70.

Many details of the synthetic machinery of the fungal cell wall and its coordination with the cell cycle remain to be discovered. Although no novel targets in the biosynthetic pathways of mannoproteins and chitin have emerged, much progress has been made in elucidating the crosslinks between cell wall polysaccharides and mannoproteins. Recent studies suggest that β-(1,6)glucan has a pivotal role in the organization of the yeast cell wall, linking mannoproteins, glucan and chitin together[7–10]. In this model, mannoproteins of the outer cell wall appear to be attached to β-(1,6)glucan through a remnant of their original membrane-based glycosylphosphatidylinositol (GPI) anchor. β-(1,6)glucan in turn possesses some β-(1,3)glucan branches, whose nonreducing ends are linked by β-(1,4) or β-(1,2) linkages to the terminal reducing residue of chitin chains. The connection of β-(1,6)glucan to the nonreducing terminal glucose of β-(1,3)glucan is accomplished through a yet unknown linkage[10]. It is conceivable that these linkages[9,10], the synthesis of the GPI anchor[11,12] and the unelucidated transfer of mannoproteins from the membrane-bound GPI anchor to β-(1,6)glucan[4] may evolve into novel and specific targets for antifungal drug design.

Other potential cell wall-based targets include chitinases and glucanases, which are important for cell wall plasticity during growth and proliferation. Disruption of the genes encoding chitinase in *Saccharomyces cerevisiae* results in clumping of the cells and failure to separate after cell division[13]. Similar effects can be provoked by demethyl-allosamidine, a potent inhibitor of chitinases in yeasts[5], and the cyclic peptide antibiotic Cl4 (Ref. 14). The exact role of glucanases in cell wall morphogenesis, however, is less clear[15].

Fungal plasma membrane

The fungal plasma membrane functions as a permeability barrier and as a conduit for the transport of small molecules and the transmission of signals. It is mainly composed of phospholipids, sphingolipids and sterols and serves as a matrix for proteins with various functions[4,16].

Ergosterol synthesis

As fungal sterols are structurally distinct from their mammalian counterparts and as their biosynthetic pathways have been elucidated, their synthesis has been a major target for antifungal drug development[16]. Apart from the antifungal azoles, approved antifungal drugs that interfere with the synthesis of ergosterol include the allylamines, the thiocarbamates and the morpholine amorolfine, which, with the exception of terbinafine, are only used as topical agents (Fig. 2). Apart from these drugs, 2,3-oxydosqualene-lanosterol cyclase and δ-24-sterol methyltransferase are the most promising targets in the postsqualene segment of the biosynthesis of ergosterol[5,16–18]. Potential targets in the presqualene steps of ergosterol biosynthesis include inhibitors of hydroxymethylglutaryl coenzyme A (HMG-CoA) and mevalonic acid synthesis[16]. Indeed, fluvastatin, a

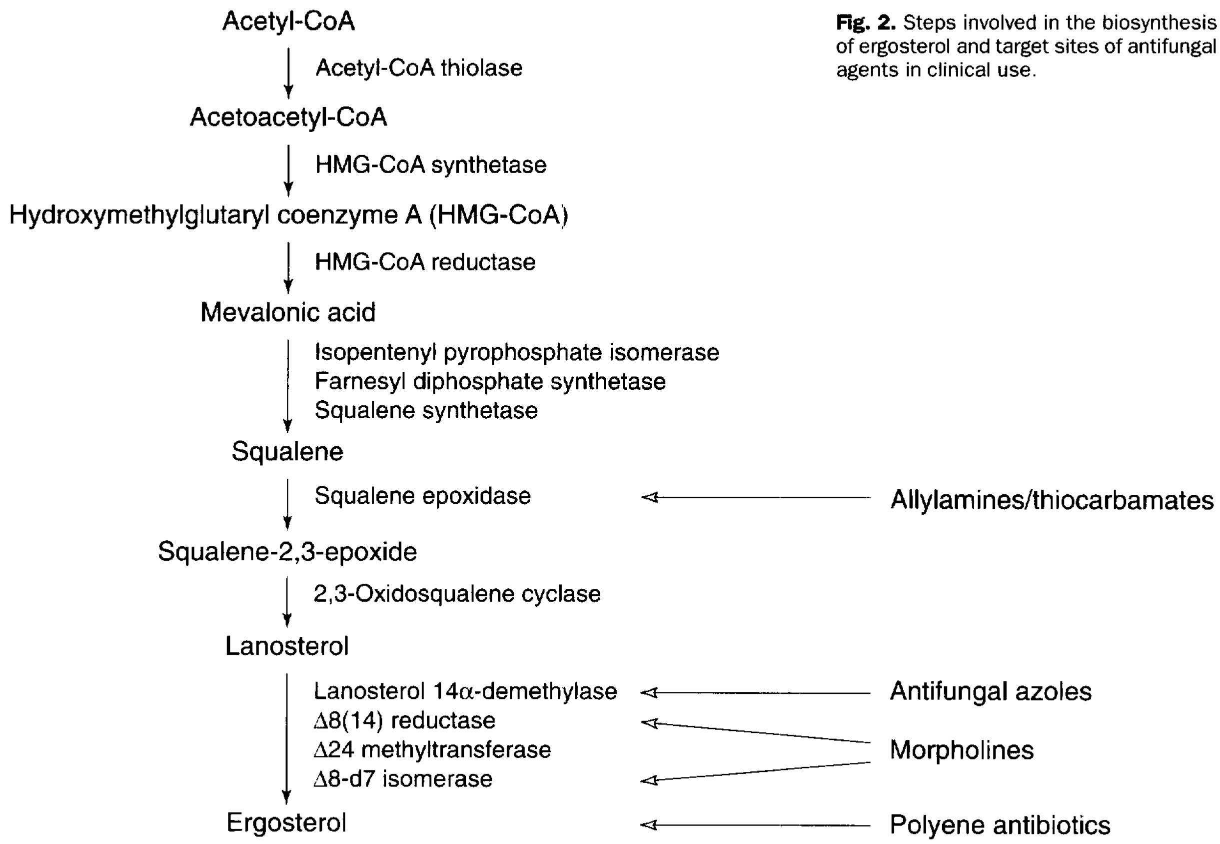

Fig. 2. Steps involved in the biosynthesis of ergosterol and target sites of antifungal agents in clinical use.

synthetic HMG-CoA reductase inhibitor and a cholesterol-lowering agent, has demonstrated synergistic fungicidal activity *in vitro* with fluconazole and itraconazole[19]. The zaragozic acids or squalestatins, a novel family of fungal metabolites containing a 4,6,7-trihydroxy-2,8-dioxobicyclo[3,2,1]octane-3,4,5-tricarboxylic acid core, are potent inhibitors of squalene synthase. In addition to their potential as cholesterol-lowering agents, they also inhibit fungal ergosterol synthesis and have broad-spectrum, fungicidal activity *in vitro*[20,21].

Phospholipid synthesis
Although assays for high-throughput screening are available[5], no specific target or compound to inhibit fungal phospholipid synthesis has yet been reported.

Sphingolipid synthesis
Sphingolipids are essential components of the cell membrane of both fungal and mammalian cells; they have important functions in signal transduction and are involved in cell differentiation, proliferation and apoptosis[22]. The biosynthesis of sphingolipids (Fig. 3) starts with the condensation of a fatty acyl CoA, usually palmitoyl CoA, with serine, which is catalyzed by serine palmitoyltransferase. Structurally distinct natural product inhibitors of this reaction have been isolated and include myriocin, lipoxamycin, the sphingofungins and viridiofungins. Members of the last two classes of compounds are potent, but nonselective, inhibitors of the target enzyme and show relatively broad-spectrum antifungal activity[22–24]. Another fermentation-derived product, australifungin, inhibits sphinganine *N*-acyltransferase, the enzyme catalyzing the conversion of sphinganine to ceramide, and has similar potent and broad-spectrum antifungal activity *in vitro*[25].

The aureobasidins are highly lipophilic, cyclic depsipeptide antibiotics derived from fermentation broths of *Aureobasidium pullulans*. They are composed of eight α-amino acids and one hydroxy acid such as 2-hydroxy-3-methylpentanoic acid[26]. Aureobasidin A (R-106; LY 295337), the lead compound, has potent activity *in vitro* against many clinically relevant fungi, including *Candida* spp., *Cryptococcus neoformans*, *Blastomyces dermatidis*, *Histoplasma capsulatum* and some *Aspergillus* species[27,28]. *In vivo*, in a murine model of systemic candidiasis, the compound was well tolerated and more effective than AmB and fluconazole[27]. Molecular studies of aureobasidin A-resistant mutants of *S. cerevisiae* have led to the discovery of the aureobasidin resistance (*AUR1*$^-$) gene[29,30], and it has been suggested that the gene product of the wild-type *AUR1*$^+$ is the molecular target for aureobasidin A (Ref. 30). Recent experiments in a mutant *S. cerevisiae* clone defective in inositolphosphorylceramide synthase (IPC synthase), an enzyme involved in fungal, but not mammalian sphingolipid biosynthesis, have demonstrated that *AUR1*$^+$ can fully restore the activity of IPC synthase and sphingolipid synthesis, and that aureobasidin A does indeed function as a potent inhibitor of IPC synthase[31]. The inhibition of this essential biosynthetic step leads to the accumulation of ceramide in growing cells and cell death. Cytological studies in *S. cerevisiae* after aureobasidin A exposure and after disruption of the *AUR1*$^+$ gene suggest that the ultimate effect of the compound is the destruction of the cell membrane and disturbance of intracellular microtubule organization[29,30].

Proton ATPase and efflux pumps
Provided that they are sufficiently distinct from related host enzymes, fungal plasma membrane (P-type) and vacuolar (V-type) proton-translocating ATPases, which are mainly involved in intracellular pH regulation, may evolve into potential targets for rational drug design[5].

Efflux pumps have been reported in various laboratory yeasts, as well as in *Candida* species[32], and their overexpression or deregulation can be responsible for clinical drug resistance to the antifungal azoles[33]. Importantly, deletion of multidrug resistance genes in *C. albicans* resulted not only in hypersusceptibility to antifungal agents[34] but also in marked attenuation of virulence[35]. Very recently, it has been reported that aureobasidin A, in addition to possessing promising antifungal activity, strongly inhibits the expression of CDR2 [K. Takesako *et al.* (1997) 37th International Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Toronto, Canada, Abstr. F105]. If confirmed, this property would make the compound particularly attractive for investigation in combination with antifungal triazoles.

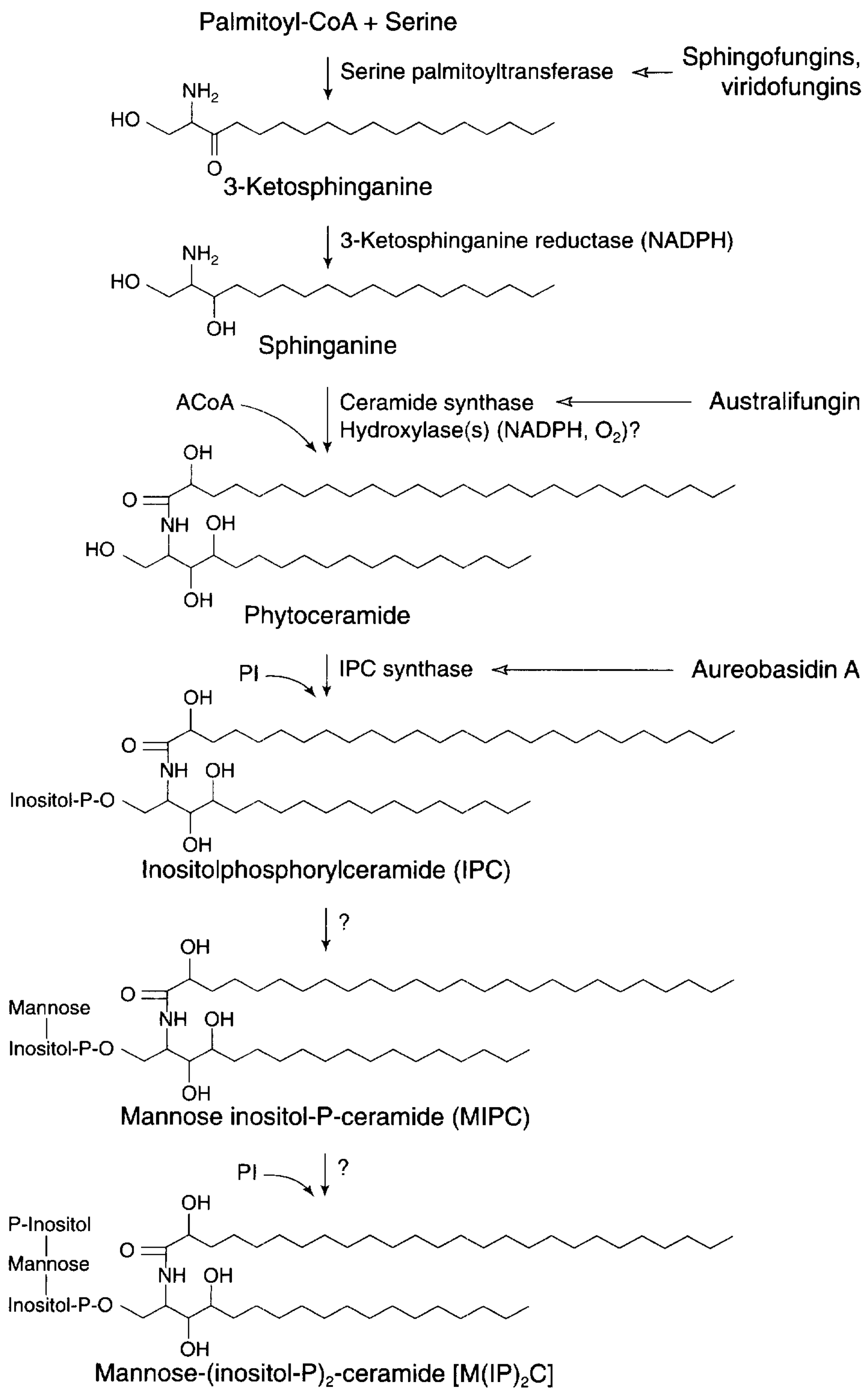

Fig. 3. Biosynthetic pathways of the sphingolipids in *Saccharomyces cerevisiae*[31], and target sites of inhibitors of fungal sphingolipid synthesis (open arrowheads). Steps that have not yet been elucidated are indicated by question marks. Abbreviations: ACoA, acyl-CoA; PI, phosphoinositol.

Naturally occurring antimicrobial peptides
The investigation of antimicrobial peptides from a wide range of biological sources, and their synthetic derivatives, is a novel inroad to new antifungal agents. These peptides bind to the lipid bilayer of biological membranes, form pores and ultimately kill the cell; others may traverse the membrane and interact with intracellular molecules. Although some of these peptides are toxic, others possess only weak activity against mammalian cells[36].

Naturally occurring, cationic peptides with potentially exploitable antifungal activity *in vitro* include the defensins, the protegrins, gallinacin I, cecropin A, thanatin and the dermaseptins. *In vivo*, a liposomal formulation of indolicin was effective against experimental systemic aspergillosis[37]. Similarly, synthetic derivatives of the bactericidal/permeability-increasing protein (BPI) demonstrate dose-dependent effects on survival and fungal burden in mouse models of systemic candidiasis [L. Appenzeller *et al.* (1996) 36th ICAAC, New Orleans, LA, USA, Abstr. F187] and disseminated aspergillosis [W.S. Ammons *et al.* (1997) 37th ICAAC, Abstr. B16]; in the latter study, combination with AmB significantly increased survival when compared with either agent alone. Finally, genes encoding cationic peptides with antifungal activity have been successfully transferred to the salivary glands of laboratory animals, thus allowing the investigation of their effect on mucosal candidiasis in permanently immunosuppressed subjects[38]. The first clinical trials involving antimicrobial therapy with cationic peptides are now under way[36]; however, the potential usefulness of these peptides in treating human diseases of infectious origin remains to be determined.

The cyclic lipodepsinonapeptides (CLPs) produced by *Pseudomonas syringae* are a distinct class of water-soluble, natural products of individual strains of the plant bacterium *P. syringae* pv. *syringae* with broad-spectrum fungicidal activity *in vitro*; they include the syringomycins, syringotoxins, syringostatins and pseudomycins[39,40]. The CLPs are composed of a nonapeptide moiety with the carboxy-terminal sequence dehydroamino butanoic acid–Asp(3-OH)–Thr(4-Cl) and an amino-terminal serine *N*-acetylated by a long-chain, unbranched 3-hydroxy fatty acid and O-acetylated by the carboxy-terminal carboxyl to form a macrolactone ring; the five amino acids between the carboxy-terminal tripeptide and the amino-terminal serine form the variable region of the peptide part[40]. The CLPs alter several cell membrane functions but are essentially channel-forming cytotoxins; molecular genetic studies indicate that ergosterol and cholesterol are involved in their binding to the plasma membrane[41]. Not surprisingly, the currently known *P. syringae*-derived CLPs all cause considerable hemolysis *in vitro*. Nevertheless, these and other cyclic lipopeptide antibiotics, such as the bacillopeptins and bacillomycins, the fusaricidins and the cyclic lipoglycopeptide cepacidine A, are used as lead compounds for the design of specific congeners with tolerable toxicity and improved antifungal activity.

DNA and protein synthesis

DNA synthesis

DNA topoisomerases I and II are ubiquitous enzymes found in both prokaryotic and eukaryotic organisms. They act by introducing transient enzyme-linked DNA breaks that allow the passing of DNA strands; inhibitors of these enzymes lead to the stabilization of the enzyme–DNA complex, resulting in inhibition of DNA synthesis, inhibition of cell division, disintegration of DNA and cell death[42].

Topoisomerase inhibitors, such as the anthracyclines, epipodophyllotoxins and quinolones, are important agents in cancer chemotherapy or in the treatment of bacterial infections. The fact that pathogenic fungi contain high levels of both type I and II topoisomerases points to the attractiveness of topoisomerases as targets for new antifungal compounds[5]. Indeed, recent studies demonstrate that fungal topoisomerase I can be inhibited selectively[43].

Diaryl furans, dicationic aromatic compounds with activity against yeast isolates and *Pneumocystis carinii*, appear to act by binding to DNA and inhibiting endo- and/or exonuclease activity [C.C. Dykstra *et al.* (1997) 37th ICAAC, Abstr. F240; M. Del Poeta *et al.* (1997) 37th ICAAC, Abstr. F101]. Toxicity, bioavailability and antifungal spectrum will determine whether these compounds will evolve into serious candidates for further investigation.

Protein synthesis

The rifamycin class of antibiotics exerts its effects by inhibition of DNA-dependent RNA polymerase, which affects chain initiation but not chain elongation. The clinical significance of the experimentally observed synergy of rifampin with AmB is uncertain. Rifabutin, a newer semisynthetic derivative of rifamycin S, has similar synergy with AmB *in vitro* [C.J. Clancy *et al.* (1997) 37th ICAAC, Abstr. E80]. In contrast to rifampin, however, this compound shows greater tissue penetration and might therefore be a candidate for *in vivo* investigation in combination with AmB.

In both fungal and mammalian cells, two soluble protein factors, elongation factor 1 (EF-1) and 2 (EF-2), are required for the ribosomal decoding of mRNA and the synthesis of the corresponding polypeptide chain[44]. Very recently, a novel class of antifungal agents has emerged, which appears to target the function of EF-2 in a highly selective fashion [J.M. Dominguez *et al.* (1997) 37th ICAAC, Abstr. F55]. The first compounds of this class, BE 31405 and SCH 57404, natural antibiotics containing a sordarin skeleton, produce potent growth inhibition of yeasts *in vitro*[45]. GM 237354, a novel synthetic sordarin derivative [J.M. Bueno *et al.* (1997) 37th ICAAC, Abstr. F54], shows promising activity *in vitro* and *in vivo* against yeasts, certain dimorphic and dematiaceous fungi and *P. carinii*, and was well tolerated in laboratory animals.

Elongation factor 3 (EF-3), in contrast to the corresponding factor in mammalian cells, exists as a separate ribosomal polypeptide in fungi. It exhibits ATPase and GTPase activity and may play a role in the ribosomal optimization of the accuracy of protein synthesis. EF-3 is present in a wide range of fungal species and is apparently essential for viability, as disruption of its gene is lethal[5,46]. Extensive studies on the structure and function of EF-3 have been undertaken, and several candidate compounds are currently being analyzed to identify potential inhibitors.

A cotranslational target is myristoyl CoA–protein *N*-myristoyl transferase (NMT), a eukaryotic cytosolic enzyme that catalyzes the covalent attachment of a rare 14-C saturated fatty acid (myristate) to the amino-terminal glycine of certain cellular proteins. The myristoyl moiety appears to be involved in mechanisms by which these proteins associate with membranes[47]. Genetic studies have shown that NMT is essential for the viability of medically important fungal pathogens[48]. The substrate specificities of fungal and mammalian NMTs are different and, indeed, as a first step, selective peptidic and peptidomimetic inhibitors of the NMT of

C. albicans with fungistatic activity have recently been constructed[49–51].

Intermediary metabolism

Nucleic acids

Although highly effective against *P. carinii* infections, the combination of trimethoprim and sulfamethoxazole has no target in opportunistic fungi. More recently, phosphoribosyl-aminoimidazole carboxylase, an enzyme of the purine pathway, which is apparently essential for growth in cerebrospinal fluid, has been suggested as a potential target for treatment of *C. neoformans* meningoencephalitis[52].

Amino acids

Several natural amino acid analogs have been shown to possess promising antifungal activity. Whereas cispentacin (FR 109615) has several cellular targets, RI-331 selectively inhibits homoserine dehydrogenase and thereby impedes the biosynthesis of methionine, isoleucine and threonine[5]. Azoxybacillin and its synthetic ester derivatives have broad-spectrum antifungal activity, especially against the filamentous fungi, and inhibit the biosynthesis of sulfur-containing amino acids by interfering with the regulation of expression of intracellular sulfite reductase activity[5,53].

Polyamines

Difluoromethyl ornithine (eflornithine), difluoromethylarginine and monofluormethyldehydroornithine methylester (a methylester analog of ornithine) are irreversible suicide inhibitors of ornithine decarboxylase, the rate-limiting enzyme in polyamine synthesis, and possess some antifungal activity *in vitro*[5]. CGP 40.215A, an aminobenzylidene-guanylhydrazone derivative inhibiting *S*-adenosylmethionine decarboxylase, showed promising activity against *P. carinii in vivo* [S. Kunz *et al.* (1993) 33rd ICAAC, New Orleans, LA, USA, Abstr. 391].

Other cellular functions

Microtubules

Microtubules play an important role in cell morphology and cell growth. Both microtubule aggregation (inhibited by griseofulvin, the agricultural fungicide benomyl, and the anticancer drugs vincristine and vinblastine) and microtubule disintegration (inhibited by the anticancer drug taxol) may merit re-examination as possible targets for antifungal agents[54].

Signal transduction and the cell cycle

Other potential targets include the complex and not yet fully elucidated system of protein kinases and phosphatases involved in intracellular signal transduction and the cell cycle. For example, the yeast gene *PKC1* encodes a protein kinase C isoenzyme that is involved in the regulation of proliferation and growth[55–58]; loss of the *PKC1* gene function leads to cell-cycle-specific lysis resulting from deficits in cell wall construction[59]. *PKC1* functions to activate the SLT2 (MPK1) mitogen-activated protein (MAP) kinase cascade, a group of serine/threonine kinases that plays a critical role in signal transduction[56,58]. This cascade ultimately targets the SBF transcription factor, which is an important regulator of gene expression at the G1- to S-phase cell-cycle transition and, consequently, an important regulator of cell growth and proliferation[58]. Finally, independent of its vital function in the β-(1,3)glucan synthase multienzyme complex[60], the expression of *PKC1* and another protein kinase, PKN, are in turn regulated by Rho1p, a guanosine triphosphate-binding protein[61–63]. The suitability of these pathways as antifungal targets, however, remains to be determined.

Cyclosporin A (CSA) and tacrolimus (FK506) are immunosuppressive, natural antifungal products that act on T cells and in *S. cerevisiae* by complexing with intracellular proteins (immunophilins) to inhibit calcineurin, a Ca^{2+}/calmodulin-dependent serine/threonine protein phosphatase involved in intracellular signaling. *C. neoformans* is susceptible to FK506 *in vitro* at 37°C, but is resistant at 24°C, and it has been shown that calcineurin is required for growth *in vitro* at 37°C and pathogenicity *in vivo*[64]. In laboratory animals, CSA and FK506 have no or minimal effects in systemic aspergillosis[65] and exacerbate cryptococcal meningitis, probably because of the overwhelming dominance of the immunosuppressive effect[66]. However, a non-immunosuppressive, non-nephrotoxic FK506 analog (L 685818) has recently been shown to have fungicidal activity against *C. neoformans in vitro*[66].

Finally, the adenosine analog 3′deoxyadenosine (cordicepin) can selectively induce apoptotic cell death in certain cancer cell lines[67]. When adenosine deaminase is inactivated by concomitant administration of deoxycoformycin, the compound is as active as AmB in a systemic candidiasis model in mice [A.M. Sugar and R.P. McCaffrey (1996) 36th ICAAC, New Orleans, LA, USA, Abstr. F193].

Virulence factors

Although targeting fungal virulence mechanisms appears appealing at first sight, host-based factors are probably more important in both establishment and outcome of most opportunistic fungal infections. The species specificity of most virulence factors, the composite nature of the virulence phenotype, and strain

Questions for future research

- Will new classes of compounds, such as inhibitors of fungal sphingolipid biosynthesis, cationic antimicrobial peptides, novel lipopeptides and the sordarins, evolve into safe, potent and broad-spectrum systemic antifungal agents?
- Will combinations of new antifungal agents directed at different cellular targets provide effective, potent antifungal therapy?
- To what extent will resistance to these new compounds develop?
- What qualitative and quantitative impact will the strategy of whole genome sequencing and bioinformatics have on antifungal drug development?
- What will be the role of adjunctive immunotherapy with recombinant cytokines in the prevention and treatment of invasive fungal infections?
- Will it be possible to develop effective antifungal vaccines against *Cryptococcus neoformans* and dimorphic molds?

variability further limit their usefulness. However, in at least some fungi, virulence genes and their regulatory mechanisms may serve as potential targets. Strategies in *C. albicans* and other fungi have included inhibition of adhesion, iron uptake and germ tube formation, prevention of germination, and interference with fungal proteinases and phospholipases involved in tissue invasion. Other approaches are inhibition of melanin biosynthesis in *C. neoformans* and dematiaceous fungi, administration of anti-capsular monoclonal antibodies, and interference with the synthesis of the polysaccharide capsule of *C. neoformans*[68,69].

Conclusions

If current trends continue, invasive fungal infections will remain a frequent and important complication in patients with compromised host defenses. Several novel and promising antifungal agents are currently in the early stages of clinical investigation; the continuing need for safe, effective and affordable compounds, technological advances, and the current aggressive pursuit of novel biochemical and molecular targets will result in further candidate drugs. An expanded and refined drug arsenal and novel immunological approaches will hopefully provide significant prevention and improved clinical outcomes for invasive fungal infections.

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