Genomics, molecular targets and the discovery of antifungal drugs

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Summary

Comparative analyses of fungal genomes and molecular research on genes associated with fungal viability and virulence has led to the identification of many putative targets for novel antifungal agents. So far the rational approach to antifungal discovery, in which compounds are optimized against an individual target then progressed to efficacy against intact fungi and ultimately to infected humans has delivered no new agents. However, the approach continues to hold promise for the future. This review critically assesses the molecular target-based approach to antifungal discovery, outlines problems and pitfalls inherent in the genomics and target discovery strategies and describes the status of heavily investigated examples of target-based research.

Key words Genomics, Antifungal drugs, Molecular targets

Genómica, dianas moleculares y el descubrimiento de fármacos antifúngicos

Resumen

Los análisis comparativos de los genomas fúngicos y la investigación molecular en genes asociados con la viabilidad fúngica y la virulencia han conducido a la identificación de muchas dianas potenciales para nuevos antifúngicos. Por el momento, la vía racional para el descubrimiento de antifúngicos, en la que los compuestos son optimizados contra una diana individual, después mejorada su eficacia contra hongos intactos y, finalmente, frente a pacientes infectados, no ha proporcionado nuevos agentes. Sin embargo, esta vía continúa manteniendo promesas para el futuro. Este artículo revisa críticamente la vía basada en la diana molecular para el descubrimiento de antifúngicos, señala los problemas y trampas inherentes a las estrategias genómicas y de descubrimiento de dianas, y describe la situación de ejemplos profundamente estudiados en investigación basada en dianas moleculares.

Palabras clave Genómica, Fármacos antifúngicos, Dianas moleculares

Determination of the DNA sequence of the entire genome of a living organism provides a superb resource for research into the molecular genetics and physiology of that organism. Comparative genomics reveals important differences between organisms that may account for their known behaviours, for example, as inhabitants of unusual ecological niches or for pathogenic potential. Many claims have been made for the medical importance of genomic data in revolutionizing approaches to diagnosis and treatment of disease. This review will examine the role of genomics and molecular targets in discovery of novel antifungal drugs. Very many individual publications describe gene products as putative antifungal targets, and the literature on drug discovery via genomics and computer-aided design is vast. The references cited in this review are therefore illustrative examples of points made, rather than comprehensive in scope.

The antifungal drugs most recently introduced into clinical use or presently undergoing phase 3 clinical trials are the echinocandins anidulafungin, caspofungin and micafungin, and the triazoles voriconazole and posaconazole, with albaconazole and ravuconazole available for clinical development. None of these agents was discovered from research based on genomic approaches to drug discovery. All began pre-clinical development in the early 1990s, at which point no fungal genomic information was available. Early, incomplete versions of the genomes of important fungal pathogens such as *Candida albicans* were available commercially (and expensively) in the mid 1990s, and there has been no shortage of papers describing novel antifungal targets. However, at the time of writing, no new antifungal agents discovered from molecular targets have yet entered clinical development. Publicly available genome projects for several fungi, including *C. albicans* and *Aspergillus fumigatus* have been completed only recently, while many other fungal genome projects are currently under way, so the full impact of fungal genomics on antifungal discovery will require more time to show through.

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It is important to note the long development times – typically 10 years – for novel antifungal agents to gain approval for clinical use. The many hurdles between discovery of an antifungally active molecule and the marketing of an antifungal pharmaceutical include toxicological and pharmacokinetic testing, scaled-up production, and formulation as a stable, easily administered product. Finding a novel compound with potentially useful antifungal activity is obviously an essential first step, but the difference between a newly discovered inhibitor and a pharmaceutically useful drug is much greater than many scientists concerned with discovery research recognize.

Principles of genomics-based antifungal drug discovery

There is no conceptual difference between antifungal agents and other antimicrobials such as antibacterial and antiviral agents where the use of genomics is concerned (Figure 1). Once the complete DNA sequence of a pathogen has been determined, a combination of computerized approaches and manual scrutiny (bioinformatics) allows designation of the open reading frames (ORFs) that encode functional products. Even for such thoroughly analysed and annotated genomes as that of *Saccharomyces cerevisiae* [64], the accurate delineation of all ORFs remains an on-going process. To define potential antimicrobial targets, the sequences of microbial genes are compared with those of the human genome, and microbial genes with a low degree of amino acid sequence homology to human genes are listed as potentially microbe-specific and therefore of interest.

This strategy is intended to reduce the large number of ORFs found by whole genome sequencing to a shortlist of genes specific to the microorganism. The search can be extended to include, for example, only similar genes across a range of target species; this is appropriate to find targets for potentially broad-spectrum antimicrobials. The assumption underlying the genomic approach is that an inhibitor of the products of microbial genes will not have an equivalent target in a human host and will therefore work as a specific antimicrobial agent. The many approaches to determination of function for unknown genes include specific mutagenesis to determine phenotypic consequences or changes in gene expression profiles [17], transposon mutagenesis to reveal gene functions [26], two-hybrid screens to find sequences that interact with a gene of interest, post-transcriptional gene silencing with antisense RNA [16,36,57], and overexpression of the gene in a foreign host. Sometimes genes are assumed to encode putative targets mainly on theoretical grounds [43].

Once a list of genes of interest has been compiled, the list is further reduced by consideration of issues such as target function, patentability and the ease with which a high-throughput screen can be devised for inhibitors of the gene product. All these considerations and approaches to scoring data to assess the potential value of particular gene products as antifungal targets have been reviewed by Spaltmann and colleagues [95]. Roemer et al. have described approaches for large-scale identification of essential genes in *C. albicans* [81] while other comparative genomics approaches for identification of antifungal targets are detailed in [40], [106] and [65].

All that remains is for investigators to design novel chemical compounds or classes that can be predicted to inhibit the newly selected target [2], or screen existing compound libraries for target inhibitors. "Hits" (the first

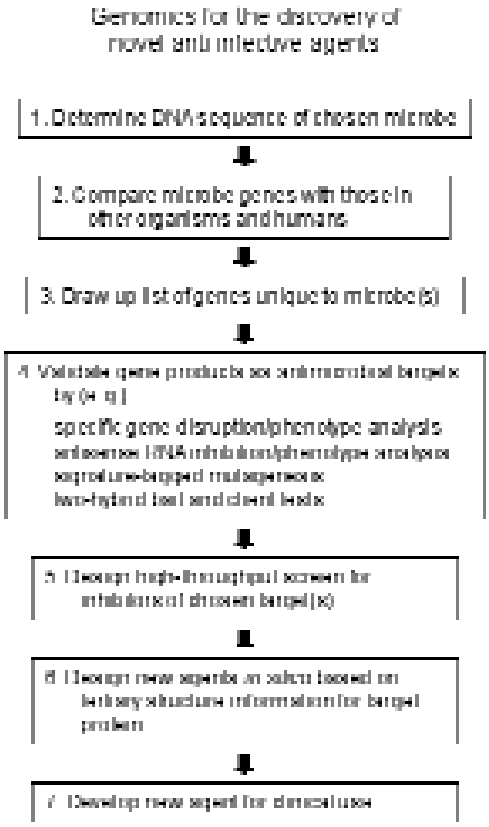

Figure 1. The strategy for use of genomic information to determine novel antifungal targets, which then form the basis for drug design and development.

inhibitors, not necessarily the optimum molecules) can be optimized to "leads" (more potent inhibitors) by computer analysis of drug-target interactions and of predicted pharmacokinetics (absorption, distribution, metabolism and excretion – ADME) [96]. The idealized lead is then ready to be developed for clinical use.

The genomic approach to antifungal target discovery is not without pitfalls. The steps in Figure 1 look very rational and very simple. They are to be found set out, with only minor differences, in countless articles that explain the ways in which genomic information is translated into drug discovery (examples of recent general reviews are references 2 and 96). One might therefore reasonably ask why we are not already overwhelmed with novel antimicrobial drugs, including antifungal agents, well on their way to full development for clinical use. Why, indeed, are there articles suggesting that the genomics/molecular target approach to antimicrobial discovery does not even necessarily accelerate drug discovery [20,41,42,71]. "Maybe", as Soll and Strosberg [92] suggested, "it is time for molecular biologists to realize that identifying new potential targets for drug development or pathways for manipulation is only half the game". Considering the many stages at which compounds fail on the path from pharmacological activity to clinical product, the identification of new targets may in fact represent a lot less than "half of the game".

Careful examination of the genomics-based strategy summarized in Figure 1 reveals areas in which the discovery of potential targets from genomic information may fail. The concept of selecting genes specific to a chosen

microbe or microbes requires a homology cut-off point, beyond which a gene will not be considered eligible for further consideration. The decision as to what level of homology – more precisely of derived amino acid sequence identity – defines an antifungal target may result in exclusion of some potential targets. For example, the derived amino acid sequence of the *ERG11* (= *CYP51*) gene in *C. albicans*, which encodes the target protein for azole antifungals, is 38% identical to that of its human equivalent [99]. The target of sordarin antifungals, fungal elongation factor 2, is a protein with 85% sequence homology to its human equivalent [50]. So a genomics-based search for fungus-specific genes with a cut-off chosen below 85% identity for human derived amino acid sequence would preclude discovery of the target for sordarins; and a cut-off below 38% would preclude discovery of the target for azole antifungals.

Additionally there are many uncertainties in gene annotation and functional assignments of genes [96]. The typical approach to annotation of new genomes is to assume that gene products with high amino acid sequence similarity to a well-studied peptide in a closely related species (in many cases the terms "well studied" or "closely related species" may not apply). For example, in the case of *C. albicans* it is commonly assumed that knowledge obtained with *S. cerevisiae* can be readily extrapolated to the pathogenic yeast. Yet, for example, the genes *SNF1* [80] and *CHS1* [66] prove to be essential for *C. albicans* and non-essential for *S. cerevisiae*, while *ERG24* is non-essential for *C. albicans* but essential for *S. cerevisiae* [46]. Similarly, *CDC43*, which encodes a subunit of geranylgeranyltransferase I (GGTase I) in both species is essential in *S. cerevisiae* but not in *C. albicans* [90]. In this particular case the assumption of homologous GGTase I essentiality between the two yeasts led to surprise when inhibitors of the *C. albicans* enzyme failed to inhibit fungal growth [52]. A similar problem in extrapolation was encountered in experiments with compounds that were specific inhibitors of bacterial histidine kinases. The compounds inhibited growth of both *S. cerevisiae* and *C. albicans* in vitro, but this activity persisted even in a *S. cerevisiae* mutant with the sole histidine kinase gene (*SLN1*) deleted [19]. Clearly the inhibitory action of the compounds in fungi was via a different mechanism from their effects in bacteria. This study also serves as a *caveat* for those who assume that toxicity of pharmaceuticals is inevitably the result of the same target being present in *Homo sapiens* and an infectious pathogen: small molecules may affect different targets in different species.

The mRNA capping enzyme, mRNA 5'-guanylyltransferase, in *C. albicans* has received a lot of attention as a potential target for antifungal agents. The *S. cerevisiae* gene encoding the homologous enzyme, *CEG1*, was shown to be essential for viability of this yeast and it was therefore assumed that the *C. albicans* equivalent gene (*CGT1*) would similarly be essential [109]. The *C. albicans* mRNA capping apparatus, based on the products of three genes – *CGT1*, *CET1,* which encodes an RNA triphosphatase, and *CCM1*, which encodes the mRNA methyltransferase – therefore underwent extensive characterization [76,82,84]. Some six years after the first published interest in mRNA capping as an antifungal target it was discovered that deletion of *CET1* or *CCM1* in *C. albicans* was not a lethal event, unlike the equivalent deletions in *S. cerevisiae*, although *CGT1* still does appear to be essential in both organisms [22]. Clearly, mRNA capping processes in *C. albicans* differ sufficiently from those in *S. cerevisiae* as now to raise doubt of their value as targets in pathogenic yeasts.

Genes of unknown function present a curious difficulty for the rational approach to target discovery. In principle, genes without equivalents of known function in other organisms should represent a particularly interesting area of exploration for new targets, but they require much more investigative effort to determine their physiological role than do genes encoding products of (presumed) known function. For this reason there have so far been no reports of the exploitation of unknown genes in the direction of antifungal discovery.

What makes a good antifungal target?

The genes of greatest interest as antifungal targets are those that can be shown to be essential for the fungus (or a range of fungi). Existing antifungal agents either kill a range of fungal pathogens or considerably retard their growth, yet they are not always clinically successful. Against that background the logic seems weak when authors claim that molecules with effects against virulence factors should be clinically effective antifungal agents. The number of published molecular virulence factors in opportunistic pathogens such as *C. albicans* exceeds 70 gene products [8,67] and homologous factors do not necessarily operate in other *Candida* species. Since almost all the gene knockouts defining *C. albicans* virulence factors to date have been done in the background of a single *C. albicans* strain, it is not even certain that the many molecules defined as virulence factors are common to all isolates of the species. While many authors argue a case for virulence factors as antifungal targets [e.g. 1,7,69,79,85]), antifungal agents are usually required *after* such factors have done their work and tissues are already infected. Virulence factors are therefore conceptually more likely to be targets for prophylactic rather than therapeutic agents, and may be highly specific to a single species or strains within a species. Notwithstanding such theoretical limitations, a number of virulence genes have been presented as candidate antifungal targets, including *MAN1* in *Cryptococcus neoformans* [107], *lysF* [56] and *pabaA* [9] in *A. fumigatus* and *COS1* [85], *VPS34* [10], *HWP1* [100] and *TPS2* [102] (among many others) in *C. albicans*.

Enzymes involved in mannosylation of cell wall proteins are particularly popular choices as potential antifungal targets in *C. albicans* [24,38,94] and *C. neoformans* [21]. However, few of these targets seem to be essential for fungal growth and therefore belong to the "virulence factor target" category. Patents have been assigned for thiazolidine compounds as inhibitors of *C. albicans* mannosyl transferases [5] but the company that owns the patent has now abandoned antifungal research.

Antifungal targets essential for fungal growth

Since many invasive fungal infections are opportunistic in nature and difficult to diagnose accurately, treatment of fungal disease is often empirical, rather than specifically targeted [54,61]. Commercially, the market for antifungal agents is much smaller than that for antibacterials, so a niche product that is efficacious against, say, *Aspergillus* species only is unlikely even to recoup its development costs. An ideal antifungal agent is therefore one that acts against as broad a range of fungi as possible and has a fungicidal effect. For the reasons discussed above, virulence factors rarely, if ever, offer useful thera-

Table. Examples of published putative targets for antifungal agents (excluding targets designated only as virulence factors).

Fungus	Gene	Product/function	Inhibitors known?	References
A. fumigatus, Aspergillus nidulans, C. neoformans, Histoplasma capsulatum	*PRP8*	Prp8 intein	No	[12,58]
Candida, Aspergillus spp.		Inositol phosphorylceramide synthase	Yes (natural products)	[34,110]
A. fumigatus, C. neoformans, others	*PMA1*	Plasma membrane H^+-ATPase	Yes (ebselen, a seleno-organic compound and conjugated stryryl ketone NC1175)	[11,60,86,93]
C. albicans	*CDC68*	Transcript elongation factor	No	[13]
C. albicans	*VRG4*	Golgi apparatus GDP-mannose transporter	No	[70]
C. albicans	*CHS1*	Chitin synthase 1	Yes (HWY-289)	[66,75]
C. albicans		2,3-oxidosqualene-lanosterol cyclase	Yes (squalene analogues)	[48,49]
C. albicans	*NMT*	*N*-myristoyl transferase	Yes (RO-09-4609, RO-09-4879)	see text
C. albicans	*CGT1*	mRNA 5'-guanylyltransferase	No	see text
C. albicans	*TOP1*	DNA topoisomerase I	Yes (eupolauridine)	see text
C. neoformans	*RAM1*	protein-farnesyltransferase β-subunit homologue	Yes (FPT inhibitor 3; Calbiochem)	[101]
C. albicans, C. neoformans	*CNA1/CNB1*	calcineurin A or B	Yes (cyclosporine and FK506)	see text
Unspecified		protein elongation factor 2	Yes (sordarins)	US patent no. 6,096,511
Unspecified		1,3-β-glucan synthase	No	US patent no. 5,912,153
Unspecified	*yTFIIB*	yeast transcription factor IIB	No	US patent no. 5,908,748
Yeasts	*BOT1*	mitochondrial ribosome subunit	No	US patent application no. 20040106173

peutic targets: their interest is more for prophylaxis or, possibly, for vaccine development.

The table lists examples of targets that have been proposed as essential for growth (therefore offering opportunities for fungicidal effects of inhibitors in vivo). Some examples have been drawn from patent details, but it is perhaps surprising that very few antifungal targets seem to have been patented (the search was based on the keywords "antifungal" and "target" in abstracts of US patents and patent applications since 1976). US patent application no. 20040082532 from 2004 seeks to patent no fewer than 77 fungal genes as putative antifungal targets, but this application appears to be unique in having such a broad scope. For most of the targets listed in the table no inhibitors have been found that could be developed as clinically effective agents, even though occasional prototype inhibitors have been described. For a small number of targets considerable efforts have been expended to discover inhibitors, and these targets merit more detailed discussion.

Protein N-*myristoyl transferases. N*-myristoylation is a process in which the 14-carbon fatty acid myristate is added to a terminal glycine residue of a protein during its formation at the stage of translation. Proteins carrying the terminal lipid moiety are involved in a large number of essential cellular processes. The biochemistry of the *N*–myristoylation process in eukaryotic cells was worked out in the 1980s, and reviews of *N*-myristoylation [25] and of research into the enzyme catalysing the process as an antifungal target [33] were published in recent years. Although the target was chosen earlier than would be possible by genomic approaches, its history is an excellent example of the way in which molecular targets can be used for discovery of novel antifungal agents.

The enzyme concerned, myristoyl-CoA:protein *N*–myristoyltransferase (Nmt1p), was shown experimentally to be essential both for *C. neoformans* [59] and *C. albicans* [104]. The *C. albicans* protein has been crystallized and its active site structure used to investigate interactions with a variety of inhibitors [39,91,105]. Various simple assays for inhibition of Nmt1p activity have been described [32,77], allowing for high-throughput screening of compound libraries. Novel derivatives of benzofuran inhibitors (originally discovered serendipitously by screening, not by design) have been synthesized to enhance inhibitory activity against Nmt1p, some of which showed activity against intact fungal cells in vitro, and even activity in mouse models of *C. albicans* infection [23,51,62,63]. It is to be hoped that this promising research, now spread over more than 10 years since the first proofs of essentiality of the target, will ultimately lead to the development of clinically useful antifungal agents. In the context of the present review it is perhaps worth mentioning that a blastp comparison of the 451 amino acid sequence of the *C. albicans* Nmt1p shows it to be 61% similar and 45% identical with its human homologue: this relatively high identity means the target may not have emerged as an ideal candidate from a genomic screen.

DNA topoisomerases. DNA topoisomerases are involved in the unpackaging of DNA supercoils. *C. albicans* DNA topoisomerase I was chosen and investigated as a potential antifungal target on biochemical grounds before any form of the *C. albicans* genome was available [28,87]. According to the annotated genome sequence now available (http://genolist.pasteur.fr/CandidaDB/) *C. albicans* possesses three DNA topoisomerase-encoding genes (*TOP1, TOP2, TOP3*) with amino acid sequences that are, respectively, 64%, 64% and 58% similar and 49%, 46% and 41% identical to the human topoisomerases I, II and III. Once again, a target selected before genomic approaches were possible has a level of homology to its human equivalent that may be on the borderline of interest for selection in a genomics screen.

Gene disruption experiments showed the *TOP1* gene was essential for *C. neoformans* [18] though more equivocal results were obtained with *C. albicans* [47] and the gene is not essential for *S. cerevisiae* [37]. Inhibitors of mammalian and microbial topoisomerases seldom showed any useful whole-cell antifungal activity in vitro [4,27,78]. There still appear to be mysteries requiring resolution around DNA topoisomerases as antifungal targets. Eupoleuridine, an alkaloid with growth-inhibitory activity for *C. albicans* [44], inhibits the activity of *S. cerevisiae* DNA topoisomerase I, but mutants lacking the *TOP1* gene were *more* sensitive to the agent than wild type. No inhibitors of fungal DNA topoisomerases have so far emerged as candidates for clinical development.

H^+-ATPase. Proton-pumping ATPases have been studied for many years in a variety of fungi and fungal H^+-ATPase began to be decribed as potential antifungal targets in the 1990s [86]. The H^+-ATPase encoded by *PMA1* in *C. neoformans* has recently emerged as a particularly interesting target with the discovery of fungicidal effects from an ATPase antagonist, ebselen [93] and a conjugated styryl ketone, NC1175 [60]. Comparative genomic analyses suggest the target may provide a basis for broad-spectrum antifungal activity [93].

Calcineurin. Calcineurin is a heterodimeric protein involved in a variety of calcium-dependent regulatory processes in eukaryotic cells. Its catalytic action as a protein phosphatase is mediated by the calcineurin A subunit; calcineurin B is the regulatory subunit of the heterodimer. Calcineurin action in fungi and in mammalian cells is inhibited by the immunosuppressive drugs cyclosporine and FK506: an observation that might suggest difficulties in finding a fungus-specific inhibitor. However, there is considerable interest in calcineurin as an antifungal target because it has been shown to be essential for virulence and, in some circumstances, for survival in *C. albicans* and *C. neoformans* [6,7,14,15,29,30,68,73,83,103]. The *CNA1* gene of *C. neoformans* is targetable by antisense repression [36] so the tools are in place for thorough exploration of the calcineurin-encoding genes, their products and their function. At present the main interest is in the effect of calcineurin inhibitors to synergize with existing azole antifungals to generate a fungicidal effect [73,74,83,97,98] but it is conceivable that inhibitors specific to fungal calcineurin may emerge as antifungal agents in their own right in the course of time.

Rational design of novel antifungal agents

It is by now obvious that molecular approaches to target identification and the input of genomic information have successfully validated very many targets for new inhibitory drugs. However, even for targets more than 10 years old there is, as yet, no sign of novel antifungal agents moving into clinical development. The expression "drug design" may suggest a more confident process than its reality. Computerized models of protein structures and the technology for determination of protein structures have both made enormous advances, particularly in relatively recent years. However, specialized journals publish research on methods for modelling of the complex interactions between protein molecules and small-molecule inhibitors; so many problems of modelling for molecular design evidently remain to be solved. At present it is almost impossible for a computerized molecular docking approach to allow design of ideal inhibitory molecules *de novo*. It is usually necessary for many iterations of tests in vitro and synthetic modifications to be done before successful inhibitors emerge which are then claimed as having been "designed". Most commonly, a high-throughput screen of existing chemical libraries identifies a small number of candidate inhibitor classes whose structure can then be adapted according to results obtained *in silico*. The word "design" implies that hit-and-miss approaches have been eliminated; but comments, for instance, that "rational design" was used as the basis for synthesis of more than 1,000 possible inhibitors of a bacterial target in order to find a molecule with good target inhibitory activity [31] may leave the suspicion that the serendipitous component of drug discovery is not yet a thing of the past.

There are many examples of the brilliant molecular modelling work that can be done to investigate interactions of drugs with their targets *in silico*. The azole antifungal target, cytochrome P450-14DM has received a lot of attention since the first protein of its type was crystallized [35,88,89,108], so it may be too early to dismiss the azole antifungal class as exhausted for its potential to yield novel compounds. However, as discussed above, most prototype inhibitors for newly determined antifungal targets have been discovered by screening existing libraries of synthetic compounds and natural products for activities; computerized design approaches are most effective when such prototype inhibitors already exist. The paper by Kumar, for example, illustrates the molecular design process applied to discovery of inhibitors for the *C. albicans* Sap2p proteinase [55].

Pharmacogenetics and pharmacogenomics

Pharmacogenetics, where human genetic polymorphisms are used to predict differences in the responses of patients to drugs, has already entered the antifungal arena with voriconazole. The agent is metabolised principally by the cytochrome P450 enzyme CYP2C19, and humans differ in their genotypes for this enzyme, with consequences for voriconazole pharmacokinetics [45]. Pharmacogenomics take the pharmacogenetic principle to the even higher level of using genomic information from human patients to predict the likelihood of success of drug treatment in each individual. This goal seems far off for antifungal therapy at present, but the technology for gene sequencing becomes ever faster and cheaper, so it is impossible to predict how soon the pharmacogenomic approach might become clinical reality.

Discussion

This article has critically reviewed the status of genomics and molecular target development as a means of accelerating discovery and development of novel antifungal agents. Like Horrobin [42], the present author has no doubt that, in time, new drugs will be used clinically that have been discovered by the strategy of genomics-based target identification and molecular design; the concern is the actual length of time it may take before the elegant scientific strategies deliver new agents. For antimicrobial discovery, the use of target-based approaches does not conceptually accelerate the process. Figure 2 illustrates the basic problem: basing drug discovery on a molecular target moves the focus of research one step backwards from random screening of compounds against intact cells. This is not a disadvantage if all the assumptions that underlie the choice of target are reasonable; however, sometimes it appears that the potential problems of the molecular approach have not always been thought through.

The assumption that small molecule inhibitors have unique actions is to be questioned, even in the antifungal context. For example, the compound HWY-289, a protoberberine derivative, inhibits multiple targets – two chitin synthases and acyl-CoA:sterol transferase – in *C. albicans* [53,75] yet still has the properties at whole-cell level of a potentially specific antifungal agent. The examples of histidine kinase inhibitors and DNA topoisomerase inhibitors, which have different or extra targets in fungi, have already been mentioned above.

Enthusiasm for virulence factors as antifungal targets may make scientific sense, but in realistic, commercial terms an antifungal agent needs to have as broad as possible a spectrum of susceptible fungal species to achieve clinical success, and this seems unlikely to happen for virulence factors that are species- or strain-specific. The fast-growing availability of complete genomic information for a large number of species and strains of pathogenic fungi will greatly facilitate comparative genomics and identify targets for broad-spectrum antifungal attack. However, it is relatively inexpensive to screen compounds against a panel of different fungi which contain all possible targets and which are likely to be expressing those essential for viability when grown in vitro. This comment goes to the heart of what is required for pragmatic drug discovery.

Molecular target-based strategies for antifungal discovery have the great advantage that they offer chemists the opportunity to invent new compounds with the potential to interact with the target. Without targets there is no inspiration for novel inhibitor design. However, the prototypes for successful inhibitors cannot easily be designed *de novo*: mimetics of enzyme substrates, for example, are sometimes, but not often, capable of development as pharmaceuticals. But, more usually, within pharmacologically active research groups, existing libraries of compounds are screened over and over for activity against newly validated targets. Often the compounds have already been tested for growth-inhibitory antimicrobial activities, so the target screening either detects older, known antimicrobial molecules which have previously been abandoned (this happened, for example, with the sordarins [72]) or the search is aimed at finding target-active molecules *without* antimicrobial activity against intact cells. This is not the ideal starting point to find clinically useful molecules since it sets out to eliminate molecules with proven growth-inhibitory activity. The rejection of compounds with demonstrable antimicrobial activity reaches its zenith in screens based on mutant microorganisms that have been modified to reveal differential activity against a chosen target: compounds that equally inhibit the growth of wild type and mutant organisms are regarded as of no interest, even though these may be ideal candidates for development. Projects based on screening of hyper-sensitive or hypo-sensitive mutants require special confidence in the importance of the target involved.

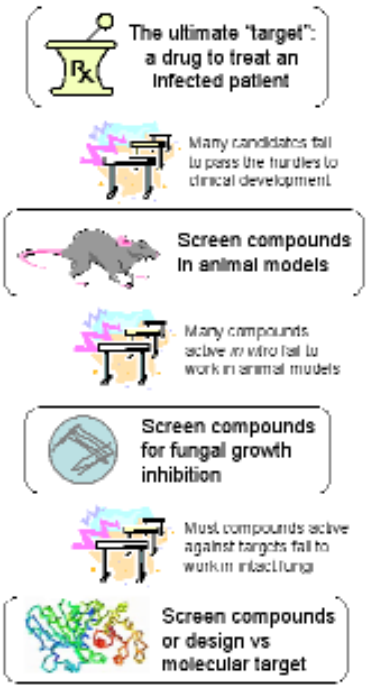

Figure 2. Starting points for discovery of novel antifungal agents. Screening compounds in animal models of fungal disease starts the process very close to the goal of clinically useful drugs, but is limited in scope by many considerations (apart from ethical ones). Starting with an antifungal target offers the possibility of drug design and screening large numbers of compounds, but it means the strategy is based on a single macromolecule from one fungal pathogen and is therefore strategically one stage behind "traditional" screening of compounds against intact fungi in vitro.

A major motivation for the shift towards rational, target-based antifungal discovery was the perception that traditional hit-and-miss, whole-microbe screening approaches had become unproductive. However, the rate of discovery of novel antifungal agents has not perceptibly increased since the mid 1990s, when the first partial fungal genome sequences became available. Meanwhile, a recent report on the discovery of a totally novel and apparently attractive agent against *Mycobacterium tuberculosis* [3] illustrates that it is still possible to discover a new agent by whole-microbe screening while using molecular technologies to find the agent's target. As illustrated in figure 2, the refined, rational, scientific approaches do not yet necessarily accelerate the pace of antifungal discovery since compounds with high activity against molecular targets then need to be tailored to work against intact cells. Their molecular activities commonly fail to extrapolate to intact fungi, often because they are unable to gain access to an intracellular target. And, of course, not all compounds that work against intact fungi show activity in animal models of fungal infection. Clearly a combination of technologies, old and new, provides the optimum platform for antifungal discovery.

However, the excellent progress that has been made, for example, with construction of computerized databases that predict the ADME properties of drugs [96] encourage confidence that, in the future, many of the present difficulties experienced in taking compounds from target inhibitors through to clinically effective drugs will be overcome and the potential for genomics-based molecular approaches to antifungal discovery will eventually be realized.

References

1. Alonso-Monge R, Navarro-Garcia F, Roman E, Eisman B, Nombela C, Pla J. Strategies for the identification of virulence determinants in human pathogenic fungi. Curr Genet 2003; 42: 301-312.
2. Anderson AC. The process of structure-based drug design. Chem Biol 2003; 10: 787-797.
3. Andries K, Verhasselt P, Guillemont J, Gohlmann HW, Neefs JM, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E, Williams P, de Chaffoy D, Huitric E, Hoffner S, Cambau E, Truffot-Pernot C, Lounis N, Jarlier V. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. Science 2005; 307: 223-227.
4. Anizon F, Belin L, Moreau P, Sancelme M, Voldoire A, Prudhomme M, Ollier M, Severe D, Riou JF, Bailly C, Fabbro D, Meyer T. Syntheses and biological activities (topoisomerase inhibition and antitumor and antimicrobial properties) of rebeccamycin analogues bearing modified sugar moieties and substituted on the imide nitrogen with a methyl group. J Med Chem 1997; 40: 3456-3465.
5. Anonymous. Thiazolidine and benzylidene thiazolidinedione inhibitors of mannosyltransferease as antifungal agents. Expert Opin Ther Pat 2002; 12: 1285-1287.
6. Bader T, Bodendorfer B, Schroppel K, Morschhauser J. Calcineurin is essential for virulence in *Candida albicans*. Infect Immun 2003; 71: 5344-5354.
7. Blankenship JR, Wormley FL, Boyce MK, Schell WA, Filler SG, Perfect JR, Heitman J. Calcineurin is essential for *Candida albicans* survival in serum and virulence. Eukaryot Cell 2003; 2: 422-430.
8. Brand A, MacCallum DM, Brown AJP, Gow NAR, Odds FC. Ectopic expression of *URA3* can influence the virulence phenotypes and proteome of *Candida albicans* but can be overcome by targeted reintegration of *URA3* at the *RPS10* Locus. Mol Microbiol 2004; 3: 900-909.
9. Brown JS, Aufauvre-Brown A, Brown J, Jennings JM, Arst H, Holden DW. Signature-tagged and directed mutagenesis identify PABA synthetase as essential for *Aspergillus fumigatus* pathogenicity. Mol Microbiol 2000; 36: 1371-1380.
10. Bruckmann A, Kunkel W, Hartl A, Wetzker R, Eck R. A phosphatidylinositol 3-kinase of *Candida albicans* influences adhesion, filamentous growth and virulence. Microbiology 2000; 146: 2755-2764.
11. Burghoorn HP, Soteropoulos P, Paderu P, Kashiwazaki R, Perlin DS. Molecular evaluation of the plasma membrane proton pump from *Aspergillus fumigatus*. Antimicrob Agents Chemother 2002; 46: 615-624.
12. Butler MI, Goodwin TJD, Poulter RTM. A nuclear-encoded intein in the fungal pathogen *Cryptococcus neoformans*. Yeast 2001; 18: 1365-1370.
13. Buurman ET, Jiang WD, McCoy M, Averett DR, Thompson CM, Wobbe CR. Validation of Cdc68p as a novel antifungal target. Arch Microbiol 2002; 178: 428-436.
14. Cruz MC, DelPoeta M, Wang P, Wenger R, Zenke G, Quesniaux VFJ, Movva NR, Perfect JR, Cardenas ME, Heitman J. Immunosuppressive and nonimmunosuppressive cyclosporine analogs are toxic to the opportunistic fungal pathogen *Cryptococcus neoformans* via cyclophilin-dependent inhibition of calcineurin. Antimicrob Agents Chemother 1999; 44: 143-149.
15. Cruz MC, Goldstein AL, Blankenship JR, Del Poeta M, Davis D, Cardenas ME, Perfect JR, McCusker JH, Heitman J. Calcineurin is essential for survival during membrane stress in *Candida albicans*. EMBO J 2002; 21: 546-559.
16. De Backer MD, Nelissen B, Logghe M, Viaene J, Loonen I, Vandoninck S, de Hoogt R, Dewaele S, Simons FA, Verhasselt P, Vanhoof G, Contreras R, Luyten WML. An antisense-based functional genomics approach for identification of genes critical for growth of *Candida albicans*. Nat Biotechnol 2001; 19: 235-241.
17. De Backer MD, Van Dijck P. Progress in functional genomics approaches to antifungal drug target discovery. Trends Microbiol 2003; 11: 470-478.
18. Del Poeta M, Toffaletti DL, Rude TH, Dykstra CC, Heitman J, Perfect JR. Topoisomerase I is essential in *Cryptococcus neoformans*: role in pathobiology and as an antifungal target. Genetics 1999; 152: 167-178.
19. Deschenes RJ, Lin H, Ault AD, Fassler JS. Antifungal properties and target evaluation of three putative bacterial histidine kinase inhibitors. Antimicrob Agents Chemother 1999; 43: 1700-1703.
20. Dixon B. Genomics and innovation in antibiotics. ASM News 2002; 68: 106-107.
21. Doering TL. A unique a-1,3 mannosyltransferase of the pathogenic fungus *Cryptococcus neoformans*. J Bacteriol 1999; 181: 5482-5488.
22. Dunyak DS, Everdeen DS, Albanese JG, Quinn CL. Deletion of individual mRNA capping genes is unexpectedly not lethal to *Candida albicans* and results in modified mRNA cap structures. Eukaryot Cell 2002; 1: 1010-1020.
23. Ebiike H, Masubuchi M, Liu PL, Kawasaki K, Morikami K, Sogabe S, Hayase M, Fujii T, Sakata K, Shindoh H, Shiratori Y, Aoki Y, Ohtsuka T, Shimma N. Design and synthesis of novel benzofurans as a new class of antifungal agents targeting fungal *N*-myristoyltransferase. Part 2. Bioorganic & Medicinal Chemistry Letters 2002; 12: 607-610.
24. Ernst JF, Prill SKH. *O*-glycosylation. Med Mycol 2001; 39: 67-74.
25. Farazi TA, Waksman G, Gordon JI. The biology and enzymology of protein *N*-myristoylation. J Biol Chem 2001; 276: 39501-39504.
26. Firon A, Villalba F, Beffa R, d'Enfert C. Identification of essential genes in the human fungal pathogen *Aspergillus fumigatus* by transposon mutagenesis. Eukaryot Cell 2003; 2: 247-255.
27. 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.
28. Fostel JM, Montgomery DA, Shen LL. Characterization of DNA topoisomerase-I from *Candida albicans* as a target for drug discovery. Antimicrob Agents Chemother 1992; 36: 2131-2138.
29. Fox DS, Cruz MC, Sia RAL, Ke HM, Cox GM, Cardenas ME, Heitman J. Calcineurin regulatory subunit is essential for virulence and mediates interactions with FKBP12-FK506 in *Cryptococcus neoformans*. Mol Microbiol 2001; 39: 835-849.
30. Fox DS, Heitman J. Good fungi gone bad: the corruption of calcineurin. Bioessays 2002; 24: 894-903.
31. Fox JL. PDF inhibitor: rare success with rational drug design. ASM News 2001; 67: 438.
32. French SA, Christakis H, O'Neill RR, Miller SP. An assay for myristoyl CoA:protein *N*-myristoyl transferase activity based on ion-exchange exclusion of [^{3}H]myristoyl peptide. Anal Biochem 1994; 220: 115-121.
33. Georgopapadakou NH. Antifungals targeted to protein modification: focus on protein *N*-myristoyltransferase. Expert Opinion on Investigational Drugs 2002; 11: 1117-1125.
34. Georgopapadakou NH. Antifungals targeted to sphingolipid synthesis: focus on inositol phosphorylceramide synthase. Expert Opinion on Investigational Drugs 2000; 9: 1787-1796.
35. Gollapudy R, Ajmani S, Kulkarni SA. Modeling and interactions of *Aspergillus fumigatus* lanosterol 14-alpha demethylase 'A' with azole antifungals. Bioorganic & Medicinal Chemistry 2004; 12: 2937-2950.
36. Gorlach JM, McDade HC, Perfect JR, Cox GM. Antisense repression in *Cryptococcus neoformans* as a laboratory tool and potential antifungal strategy. Microbiology 2002; 148: 213-219.
37. Goto T, Wang JC. Cloning of yeast *TOP1*, the gene encoding DNA topoisomerase I, and construction of mutants defective in both DNA topoisomerase I and DNA topoisomerase II. PNAS 1985; 82: 7178-7182.
38. Gow NAR, Bates S, Brown AJP, Buurman ET, Thomson LM, Westwater C. *Candida* cell wall mannosylation: importance in host-fungus interaction and potential as a target for the development of antifungal drugs. Biochem Soc Trans 1999; 27: 512-516.
39. Hasegawa K, Morikami K, Shiratori Y, Ohtsuka T, Aoki Y, Shimma N. 3D-QSAR study of antifungal *N*-myristoyltransferase inhibitors by comparative molecular sur-

face analysis. Chemometrics Intell Lab Syst 2003; 69: 51-59.

40. Haselbeck R, Wall D, Jiang B, Ketela T, Zyskind J, Bussey H, Foulkes JG, Roemer T. Comprehensive essential gene identification as a platform for novel anti-infective drug discovery. Curr Pharm Des 2002; 8: 1155-1172.
41. Horrobin DF. Innovation in the pharmaceutical industry. J R Soc Med 2000; 93: 341-345.
42. Horrobin DF. Realism in drug discovery – could Cassandra be right? Nat Biotechnol 2001; 19: 1099-1100.
43. Horsch M, Mayer C, Sennhauser U, Rast DM. ß-*N*-acetylhexosaminidase – a target for the design of antifungal agents. Pharmacol Ther 1997; 76: 187-218.
44. Hufford CD, Liu S, Clark AM, Oguntimein BO. Anticandidal activity of eupolauridine and onychine, alkaloids from *Cleistopholis patens*. J Nat Prod 1987; 40: 961-964.
45. Hyland R, Jones BC, Smith DA. Identification of the cytochrome P450 enzymes involved in the *N*-oxidation of voriconazole. Drug Metabolism and Disposition 2003; 31: 540-547.
46. Jia N, Arthington-Skaggs B, Lee W, Pierson CA, Lees ND, Eckstein J, Barbuch R, Bard M. *Candida albicans* sterol C-14 reductase, encoded by the ERG24 gene, as a potential antifungal target site. Antimicrob Agents Chemother 2002; 46: 947-957.
47. Jiang WD, Gerhold D, Kmiec EB, Hauser M, Becker JM, Koltin Y. The topoisomerase I gene from *Candida albicans*. Microbiology 1997; 143: 377-386.
48. Jolidon S, Polak AM, Guerry P, Hartman PG. Inhibitors of 2,3-oxidosqualene lanosterol cyclase as potential antifungal agents. Biochem Soc Trans 1990; 18: 47-48, 1990.
49. Jolidon S, Polak-Wyss A, Hartman PG, Guerry P. 2,3-Oxidosqualene-lanosterol cyclase: an attractive target for antifungal drug design. Recent Advances in the Chemistry of Anti-Infective Agents 1993; RSC: 223-233.
50. Justice MC, Hsu MJ, Tse B, Ku T, Balkovec J, Schmatz D, Nielsen J. elongation factor 2 as a novel target for selective inhibition of fungal protein synthesis. J Biol Chem 1998; 273: 3148-3151.
51. Kawasaki K, Masubuchi M, Morikami K, Sogabe S, Aoyama T, Ebiike H, Niizuma S, Hayase M, Fujii T, Sakata K, Shindoh H, Shiratori Y, Aoki Y, Ohtsuka T, Shimma N. Design and synthesis of novel benzofurans as a new class of antifungal agents targeting fungal *N*-myristoyltransferase. Part 3. Bioorganic & Medicinal Chemistry Letters 2003; 13: 87-91.
52. Kelly R, Card D, Register E, Mazur P, Kelly T, Tanaka KI, Onishi J, Williamson JM, Fan HX, Satoh T, Kurtz M. Geranylgeranyltransferase I of *Candida albicans*: null mutants or enzyme inhibitors produce unexpected phenotypes. J Bacteriol 2000; 182: 704-713.
53. Kim KY, Shin YK, Park JC, Kim JH, Yang HY, Han DM, Paik YK. Molecular cloning and biochemical characterization of *Candida albicans* acyl-CoA: sterol acyltransferase, a potential target of antifungal agents. Biochem Biophys Res Commun 2004; 319: 911-919.
54. Klastersky J. Empirical antifungal therapy. Int J Antimicrob Agents 2004; 23: 105-112.
55. Kumar SKP, Kulkarni VM. Insights into the selective inhibition of *Candida albicans* secreted aspartyl protease: A docking analysis study. Bioorg Med Chem 2002; 10: 1153-1170.
56. Liebmann B, Muhleisen TW, Muller M, Hecht M, Weidner G, Braun A, Brock M, Brakhage AA. Deletion of the *Aspergillus fumigatus* lysine biosynthesis gene lysF encoding homoaconitase leads to attenuated virulence in a low-dose mouse infection model of invasive aspergillosis. Arch Microbiol 2004; 181: 378-383.
57. Liu H, Cottrell TR, Pierini LM, Goldman WE, Doering TL. RNA interference in the pathogenic fungus *Cryptococcus neoformans*. Genetics 2002; 160: 463-470.
58. Liu XQ, Yang J. Prp8 intein in fungal pathogens: target for potential antifungal drugs. FEBS Lett 2004; 572: 46-50.
59. Lodge JK, Jacksonmachelski 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 Natl Acad Sci U S A 1994; 91: 12008-12012.
60. Manavathu EK, Dimmock JR, Vashishtha SC, Chandrasekar PH. Inhibition of H+-ATPase-mediated proton pumping in *Cryptococcus neoformans* by a novel conjugated styryl ketone. J Antimicrob Chemother 2001; 47: 491-494.
61. Marr KA. Empirical antifungal therapy – new options, new tradeoffs. N Engl J Med 2002; 346: 278-280.
62. Masubuchi M, Ebiike H, Kawasaki E, Sogabe S, Morikami K, Shiratori Y, Tsujii S, Fujii T, Sakata K, Hayase M, Shindoh H, Aoki Y, Ohtsuka T, Shimma N. Synthesis and biological activities of benzofuran antifungal agents targeting fungal *N*-myristoyltransferase. Bioorganic & Medicinal Chemistry 2003; 11: 4463-4478.
63. Masubuchi M, Kawasaki K, Ebiike H, Ikeda Y, Tsujii S, Sogabe S, Fujii T, Sakata K, Shiratori Y, Aoki Y, Ohtsuka T, Shimma N. Design and synthesis of novel benzofurans as a new class of antifungal agents targeting fungal *N*-myristoyltransferase. Part 1. Bioorganic & Medicinal Chemistry Letters 2001; 11: 1833-1837.
64. Mewes HW, Albermann K, Bähr M, Frishman D, Gleissner A, Hani J, Heumann K, Kleine K, Maierl A, Oliver SG, Pfeiffer F, Zollner A. Overview of the yeast genome. Nature 1997; 387 suppl: 7-8.
65. Monteoliva L, Matas ML, Gil C, Nombela C, Pla J. Large-scale identification of putative exported proteins in *Candida albicans* by genetic selection. Eukaryot Cell 2002; 1: 514-525.
66. Munro CA, Winter K, Buchan A, Henry K, Becker JM, Brown AJP, Bulawa CE, Gow NAR. Chs1 of *Candida albicans* is an essential chitin synthase required for synthesis of the septum and for cell integrity. Mol Microbiol 2001; 39: 1414-1426.
67. Navarro-Garcia F, Sanchez M, Nombela C, Pla J. Virulence genes in the pathogenic yeast *Candida albicans*. FEMS Microbiol Rev 2001; 25: 245-268.
68. Nelson RT, Hua J, Pryor B, Lodge JK. Identification of virulence mutants of the fungal pathogen *Cryptococcus neoformans* using signature-tagged mutagenesis. Genetics 2001; 157: 935-947.
69. Niimi M, Cannon RD, Monk BC. *Candida albicans* pathogenicity: a proteomic perspective. Electrophoresis 1999; 20: 2299-2308.
70. Nishikawa A, Poster JB, Jigami Y, Dean N. Molecular and phenotypic analysis of *CaVRG4*, encoding an essential Golgi apparatus GDP-mannose transporter. J Bacteriol 2002; 184: 29-42.
71. Odds FC. Are molecular methods the optimum route to antimicrobial drugs? In Andrew PW, Oyston P, Smith GL, Stewart-Tull DE (eds.) Fighting Infection in the 21st Century. Oxford, Blackwell Science Ltd, 2000: 237-258.
72. Odds FC. Sordarin antifungal agents. Expert OpinTher Patents 2001; 11: 283-294.
73. Odom A, Muir S, Lim E, Toffaletti DL, Perfect J, Heitman J. Calcineurin is required for virulence of *Cryptococcus neoformans*. EMBO J 1997; 16: 2576-2589.
74. Onyewu C, Wormley FL, Perfect JR, Heitman J. The calcineurin target, crz1, functions in azole tolerance but is not required for virulence of *Candida albicans*. Infect Immun 2004; 72: 7330-7333.
75. Park KS, Kang KC, Kim KY, Jeong PY, Kim JH, Adams DJ, Paik YK. HWY-289, a novel semi-synthetic protoberberine derivative with multiple target sites in *Candida albicans*. J Antimicrob Chemother 2001; 47: 513-519.
76. Pei Y, Lehman K, Tian LG, Shuman S. Characterization of *Candida albicans* RNA triphosphatase and mutational analysis of its active site. Nucleic Acids Research 2000; 28: 1885-1892.
77. Pennise CR, Georgopapadakou NH, Collins RD, Graciani NR, Pompliano DL. A continuous fluorometric assay of myristoyl-coenzyme A : protein *N*-myristoyltransferase. Anal Biochem 2002; 300: 275-277.
78. Pereira ER, Belin L, Sancelme M, Prudhomme M, Ollier M, Rapp M, Severe D, Riou JF, Fabbro D, Meyer T. Structure-activity relationships in a series of substituted indolocarbazoles – topoisomerase I and protein kinase C inhibition and antitumoral and antimicrobial properties. J Med Chem 1996; 39: 4471-4477.
79. Perfect JR. Fungal virulence genes as targets for antifungal chemotherapy. Antimicrob Agents Chemother 1996; 40: 1577-1583.
80. Petter R, Chang YC, Kwon-Chung KJ. A gene homologous to *Saccharomyces cerevisiae snf1* appears to be essential for the viability of *Candida albicans*. Infect Immun 1997; 65: 4909-4917.
81. Roemer T, Jiang B, Davison J, Ketela T, Veillette K, Breton A, Tandia F, Linteau A, Sillaots S, Marta C, Martel N, Veronneau S, Lemieux S, Kauffman S, Becker J, Storms R, Boone C, Bussey H. Large-scale essential gene identification in *Candida albicans* and applications to antifungal drug discovery. Mol Microbiol 2003; 50: 167-181.
82. Saha N, Schwer B, Shuman S. Characterization of human, *Schizosaccharomyces pombe*, and *Candida albicans* mRNA cap methyltransferases and complete replacement of the yeast capping apparatus by mammalian enzymes. J Biol Chem 1999; 274: 16553-165624.
83. Sanglard D, Ischer F, Marchetti O, Entenza J, Bille J. Calcineurin A of *Candida albicans*: involvement in antifungal tolerance, cell morphogenesis and virulence. Mol Microbiol 2003; 48: 959-976.
84. Schwer B, Lehman K, Saha N, Shuman S. Characterization of the mRNA capping apparatus of *Candida albicans*. J Biol Chem 2001; 276: 1857-1864.
85. Selitrennikoff CP, Alex L, Miller TK, Clemons KV, Simon MI, Stevens DA. *COS-I*, a putative two-component histidine kinase of *Candida albicans*, is an in vivo virulence factor. Med Mycol 2001; 39: 69-74.
86. Setoyoung D, Monk B, Mason AB, Perlin DS. Exploring an antifungal target in the plasma membrane h(+)ATPase of fungi. Biochim Biophys Acta – Biomembranes 1997; 1326: 249-256.

87. 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.
88. Sheng CQ, Zhang WN, Ji HT, Song YL, Zhang M, Zhou YJ, Lu JG, Zhu J. Design, synthesis and antifungal activity of novel triazole derivatives. Chin Chem Lett 2004; 15: 404-407.
89. Sheng CQ, Zhang WN, Zhang M, Song YL, Ji HT, Zhu J, Yao JZ, Yu JX, Yang S, Zhou YJ, Lu JG. Homology modeling of lanosterol 14 a-demethylase of *Candida albicans* and *Aspergillus fumigatus* and insights into the enzyme-substrate interactions. Journal of Biomolecular Structure & Dynamics 2004; 22: 91-99.
90. Smalera I, Williamson JM, Baginsky W, Leiting B, Mazur P. Expression and characterization of protein geranylgeranyltransferase type I from the pathogenic yeast *Candida albicans* and identification of yeast selective enzyme inhibitors. Biochim Biophys Acta-Protein Struct Molec Enzym 2000; 1480: 132-144.
91. Sogabe S, Masubuchi M, Sakata K, Fukami TA, Morikami K, Shiratori Y, Ebiike H, Kawasaki K, Aoki Y, Shimma N, D'Arcy A, Winkler FK, Banner DW, Ohtsuka T. Crystal structures of *Candida albicans N*-myristoyltransferase with two distinct inhibitors. Chem Biol 2002; 9: 1119-1128.
92. Soll DR, Strosberg D. Genome-wide technologies – the first half of the story. Curr Opin Microbiol 2002; 5: 311-312.
93. Soteropoulos P, Vaz T, Santangelo R, Paderu P, Huang DY, Tamas MJ, Perlin DS. Molecular characterization of the plasma membrane H+-ATPase, an antifungal target in *Cryptococcus neoformans*. Antimicrob Agents Chemother 2000; 44: 2349-2355.
94. Southard SB, Specht CA, Mishra C, ChenWeiner J, Robbins PW. Molecular analysis of the *Candida albicans* homolog of *Saccharomyces cerevisiae MNN9*, required for glycosylation of cell wall mannoproteins. J Bacteriol 1999; 181: 7439-7448.
95. Spaltmann F, Blunck M, Ziegelbauer K. Computer-aided target selection – prioritizing targets for antifungal drug discovery. Drug Discovery Today 1999; 4: 17-26.
96. Stahura FL, Bajorath J. Bio- and chemoinformatics beyond data management: crucial challenges and future opportunities. Drug Discovery Today 2001; 7: S41-S47.
97. Steinbach WJ, Schell WA, Blankenship JR, Onyewu C, Heitman J, Perfect JR. In vitro interactions between antifungals and immunosuppressants against *Aspergillus fumigatus*. Antimicrob Agents Chemother 2004; 48: 1664-1669.
98. Steinbach WJ, Singh N, Miller JL, Benjamin DK, Schell WA, Heitman J, Perfect JR. In vitro interactions between antifungals and immunosuppressants against *Aspergillus fumigatus* isolates from transplant and nontransplant patients. Antimicrob Agents Chemother 2004; 48: 4922-4925.
99. Stromstedt M, Rozman D, Waterman MR. The ubiquitously expressed human CYP51 encodes lanosterol 14 a-demethylase, a cytochrome P450 whose expression is regulated by oxysterols. Arch Biochem Biophys 1996; 329: 73-81.
100. Sundstrom P, Balish E, Allen CM. Essential role of the *Candida albicans* transglutaminase substrate, hyphal wall protein 1, in lethal oroesophageal candidiasis in immunodeficient mice. J Infect Dis 2002; 185: 521-530.
101. Vallim MA, Fernandes L, Alspaugh JA. The RAM1 gene encoding a protein-farnesyltransferase b- subunit homologue is essential in *Cryptococcus neoformans*. Microbiology 2004; 150: 1925-1935.
102. Van Dijck P, De Rop L, Szlufcik K, Van Ael E, Thevelein JM. Disruption of the *Candida albicans TPS2* gene encoding trehalose-6-phosphate phosphatase decreases infectivity without affecting hypha formation. Infect Immun 2002; 70: 1772-1782.
103. Wang P, Cardenas ME, Cox CM, Perfect JR, Heitman J. Two cyclophilin A homologs with shared and distinct functions important for growth and virulence of *Cryptococcus neoformans*. EMBO Reports 2001; 2: 511-518.
104. Weinburg RA, Mcwherter CA, Freeman KS, Wood DC, Gordon JI, Lee SC. Genetic studies reveal that myristoylCoA:protein *N*-myristoyltransferase is an essential enzyme in *Candida albicans*. Mol Microbiol 1995; 16: 241-250.
105. Weston SA, Camble R, Colls J, Rosenbrock G, Taylor I, Egerton M, Tucker AD, Tunnicliffe A, Mistry A, Mancia F, Delafortelle E, Irwin J, Bricogne G, Pauptit RA. Crystal structure of the anti-fungal target *N*-myristoyl transferase. Nat Struct Biol 1998; 5: 213-221.
106. Willins DA, Kessler M, Walker SS, Reyes GR, Cottarel G. Genomics strategies for antifungal drug discovery – from gene discovery to compound screening. Curr Pharm Des 2002; 8: 1137-1154.
107. Wills EA, Roberts IS, Del Poeta M, Rivera J, Casadevall A, Cox GM, Perfect JR. Identification and characterization of the *Cryptococcus neoformans* phosphomannose isomerase-encoding gene, *MAN1*, and its impact on pathogenicity. Mol Microbiol 2001; 40: 610-620.
108. Xiao L, Madison V, Chau AS, Loebenberg D, Palermo RE, McNicholas PM. Three-dimensional models of wild-type and mutated forms of cytochrome P450 14 a-sterol demethylases from *Aspergillus fumigatus* and *Candida albicans* provide insights into posaconazole binding. Antimicrob Agents Chemother 2004; 48: 568-574.
109. Yamada-Okabe T, Shimmi O, Doi R, Mizumoto K, Arisawa M, Yamada-Okabe H. Isolation of the mRNA-capping enzyme and ferric-reductase-related genes from *Candida albicans*. Microbiology 1996; 142: 2515-2523.
110. Zhong WY, Jeffries MW, Georgopapadakou NH. Inhibition of inositol phosphorylceramide synthase by aureobasidin A in *Candida* and *Aspergillus* species. Antimicrob Agents Chemother 2000; 44: 651-653.