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Genomics and the development of new diagnostics and anti-*Candida* drugs

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Pathogenic *Candida* species remain a significant medical problem despite the availability of antifungal therapies. Two key issues must be addressed to improve the treatment of life-threatening systemic *Candida* infections. First, advanced diagnostic tools are required to facilitate the early identification of these infections, when therapeutic intervention is more likely to be effective. Second, improved antifungal therapies are needed. These therapies, which might include combinations of antifungals, need to be less toxic to the patient and more potent in killing a broader range of *Candida* species. Recent advances in unravelling the genomics of these species should facilitate efforts to achieve these goals. We discuss the contribution of genomics to the development of novel antifungals and new diagnostic tools.

Candida infections and genomics

Candida infections have a significant impact upon human health and the economics of medical service provision. Systemic, disseminated candidiases prolong hospital stays and cause significantly increased treatment costs for high-risk patients, such as haematologic and neutropenic patients, people undergoing abdominal surgery and preterm infants [1,2]. Although various antifungal drugs have been licensed, the clinicians treating such infections only have available a limited number of therapeutic treatment strategies. As a consequence, systemic *Candida* infections are still associated with a high attributable mortality rate [3]. New antifungal drug targets and novel classes of systemically applicable antifungals are highly desirable for use in synergistic or additive combination therapies that would be analogous to the successful treatment of life-threatening bacterial infections. Also, more rapid and accurate diagnostic tests are required to facilitate the early and reliable diagnoses that will enhance the chances of patients surviving systemic candidiasis. The clinician needs to know the fungal species and the resistance pattern of the strain that is infecting their patient so that an appropriate therapy can be applied. Is genomics addressing these clinical needs?

Functional genomics of *Candida albicans* has become well established in the past five years, following the release of *C. albicans* genome sequence data. This has led to a dramatic increase in the amount of biological information about this pathogen, for example through the publication of large transcript profiling and proteomics datasets (e.g. [4–9]). More recently, genome sequence data have become available for other pathogenic *Candida* species as well (Table 1), and more will become public in the near future. These new data provide a platform for the functional genomics of these species and for bioinformatic screens for those functions that might represent novel antifungal targets for selective treatment or diagnostic markers.

In this review we discuss the extent to which genomic approaches are contributing to the development of novel antifungal drugs and diagnostic approaches. We argue that although genomics is helping to provide global views of the impact of antifungal drugs upon the fungal cell, genomics has not yet led directly to the development of a clinically useful anti-*Candida* drug. Nevertheless, genomics is helping to identify novel targets for antifungal drugs and potentially useful diagnostic markers.

Epidemiology and significance of different *Candida* species

In the United States, *Candida spp*. have been identified among the four most common blood stream pathogens [10] and increasing frequencies of fatal *Candida* infections were reported extensively during the 1980s and 1990s (e.g. [11]). Although comprehensive longitudinal epidemiological data are missing, there are some national surveys that suggest a comparable clinical significance of *Candida spp*. for Europe [12,13]. Generally, more recent epidemiological studies confirm a stable or slightly decreasing trend in the frequency of invasive candidiasis [14], and a minor epidemiological shift towards more frequent mould infections [15].

Although *C. albicans* is still the most frequently isolated species from blood cultures and tissue samples, non-*albicans Candida spp*., such as *C. glabrata*, *C. parapsilosis, C. dubliniensis*, *C. tropicalis* and *C. krusei* have gained remarkable clinical significance [16,17]. This epidemiological change was observed throughout the 1990s and was explained by the widespread use of triazole-based antifungal prophylaxis and therapy [18]. The reported incidence of infections with non-*albicans Candida spp.* varies considerably between medical centres, countries and patient groups. Whereas *C. glabrata* is the second most isolated *Candida spp*. in the adult population, with reported isolation rates in blood cultures of up to 21% [19], *C. parapsilosis* seems to be the second most frequent cause of invasive candidiasis in paediatric patients [14,20]. This is significant because

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Table 1. *Candida* genome sequence databases

Species	Database	Host
C. albicans	Http://genome-www.stanford.edu/	Stanford Genome Database
	Http://www.candidagenome.org/	Candida Genome Database, NIH
	Http://genolist.pasteur.fr//CandidaDB/	CandidaDB, Pasteur Institute
C. dubliniensis	Http://www.sanger.ac.uk/sequencing/Candida/dubliniensis/	Wellcome Trust Sanger Centre
C. parapsilosis	Http://www.sanger.ac.uk/sequencing/Candida/parapsilosis/	Wellcome Trust Sanger Centre
C. tropicalis	Http://www.broad.mit.edu/annotation/genome/candida_tropicalis/Home.html	The Broad Institute
C. guillermondii	Http://www.broad.mit.edu/annotation/genome/candida_guilliermondii/Home.html	The Broad Institute
C. lusitaniae	Http://www.broad.mit.edu/annotation/genome/candida_lusitaniae/Home.html	The Broad Institute

different *Candida spp*. show diverse virulence traits [21] and antifungal susceptibility profiles, and clearly this heterogeneity complicates decision making for the clinician.

To summarise, systemic *Candida* infections remain a significant medical problem. The important impact of non-*albicans Candida* species observed today requires particular consideration in the development of modern diagnostic and therapeutic tools.

Molecular and genomic analyses in pathogenic *Candida* species

The past two decades have seen dramatic advances in our academic understanding of some, but not all of the pathogenic *Candida* species described above. In particular, research into the most prevalent of these pathogens, *C. albicans*, has moved firmly into the post-genomics era. The *C. albicans* genome sequence was published in 2004 [22] and genome annotations were published shortly thereafter [23,24]. However, comprehensive *C. albicans* genome sequence datasets were released early thanks to the generosity of the Stanford Genome Database (Table 1). This helped to accelerate the molecular dissection of the pathogenicity of *C. albicans* and facilitated the development of genomic technologies such as microarrays and proteomics. These global technologies opened the door to unbiased global searches for subsets of genes and/or proteins that are regulated, for example, during yeast-hypha morphogenesis [4], biofilm formation [25] or following contact with host cells [5]. The roles of specific genes and/or proteins arising from these searches have then been investigated using an increasingly sophisticated molecular toolbox. Recent advances to this toolbox include the development of improved cassettes and strains for gene disruption, fluorescent reporters that facilitate analysis of fungal gene regulation during infections, and a tetracycline-regulatable promoter that can be used to examine conditional mutants in classical infection models [26]. This powerful combination of genomic and reductionist approaches is helping to reveal ways in which this pathogenic fungus responds to its host during disease establishment. Many research groups are focussing on the regulation of virulence attributes. However, genomic data are telling us that, in addition to regulating virulence genes, *C. albicans* fine tunes its metabolism and modulates its stress responses in specific host niches (e.g. [5]). This provides a timely reminder that, for this fungus to establish an infection, its physiological fitness is as important as the expression of virulence factors. As described below, most antifungals target functions that are essential for growth, not virulence factors.

The *C. glabrata* research community is catching up rapidly with the *C. albicans* community in terms of the available genomic and molecular tools. The genomic sequence was published in 2004 [27] and this is facilitating the development of *C. glabrata* proteomics and microarray platforms [28,29]. Although gene disruption technologies are less straightforward than for *C. albicans*, the haploid nature of *C. glabrata* facilitates comprehensive genetic screens for virulence traits [30]. This is likely to make *C. glabrata* a powerful model system for the analysis of fungal pathogenicity in the future.

The genome sequencing of other pathogenic *Candida* species is underway, including *C. dubliniensis*, *C. parapsilosis*, *C. tropicalis, C. guilliermondii* and *C. lusitaniae* (Table 1). Molecular transformation systems have been developed for some of these species, but their molecular analysis is less well advanced compared with *C. albicans* and *C. glabrata*. Therefore, a major challenge for the future will be to translate this information into improved diagnostic approaches and antifungal drugs. Even in the absence of sophisticated molecular toolboxes, the bioinformatic comparison of these genomes will provide valuable clues about potentially useful markers and targets. No doubt pharmaceutical companies will be interested in essential functions that are highly conserved in these *Candida* species (and other pathogenic fungi). Those interested in the development of novel diagnostics might want to focus on species- or genus-specific proteins or other 'signatures' that are expressed at detectable levels early in an infection.

Therapy: new antifungals

The antimycotics currently in clinical use for the treatment of *Candida* infections target a limited number of fungal macromolecules. The polyenes, the azoles and the allylamines all interfere with the fungal membrane. A comprehensive review about the precise mechanisms of action of clinically used antifungals can be found in a previous *Trends in Microbiology* issue [31].

Various new antifungal agents for the treatment of life-threatening candidiasis are currently being developed or tested in clinical trials by the industry. It is worth noting that they can be categorised in two major groups: (i) the cell wall active echinocandins (anidulafungin, caspofungin, micafungin) and (ii) extended spectrum azoles which inhibit ergosterol synthesis (posaconazole, ravuconazole and voriconazole).

Echinocandins interfere with a relatively new target, the synthesis of β-1,3-glucan, which is a carbohydrate backbone of the fungal cell wall (Figure 1). This group of antifungals is attractive for the treatment of severe candidasis because of their activity against *Candida spp*. in biofilms [32] and an improved post-antifungal effect as compared with

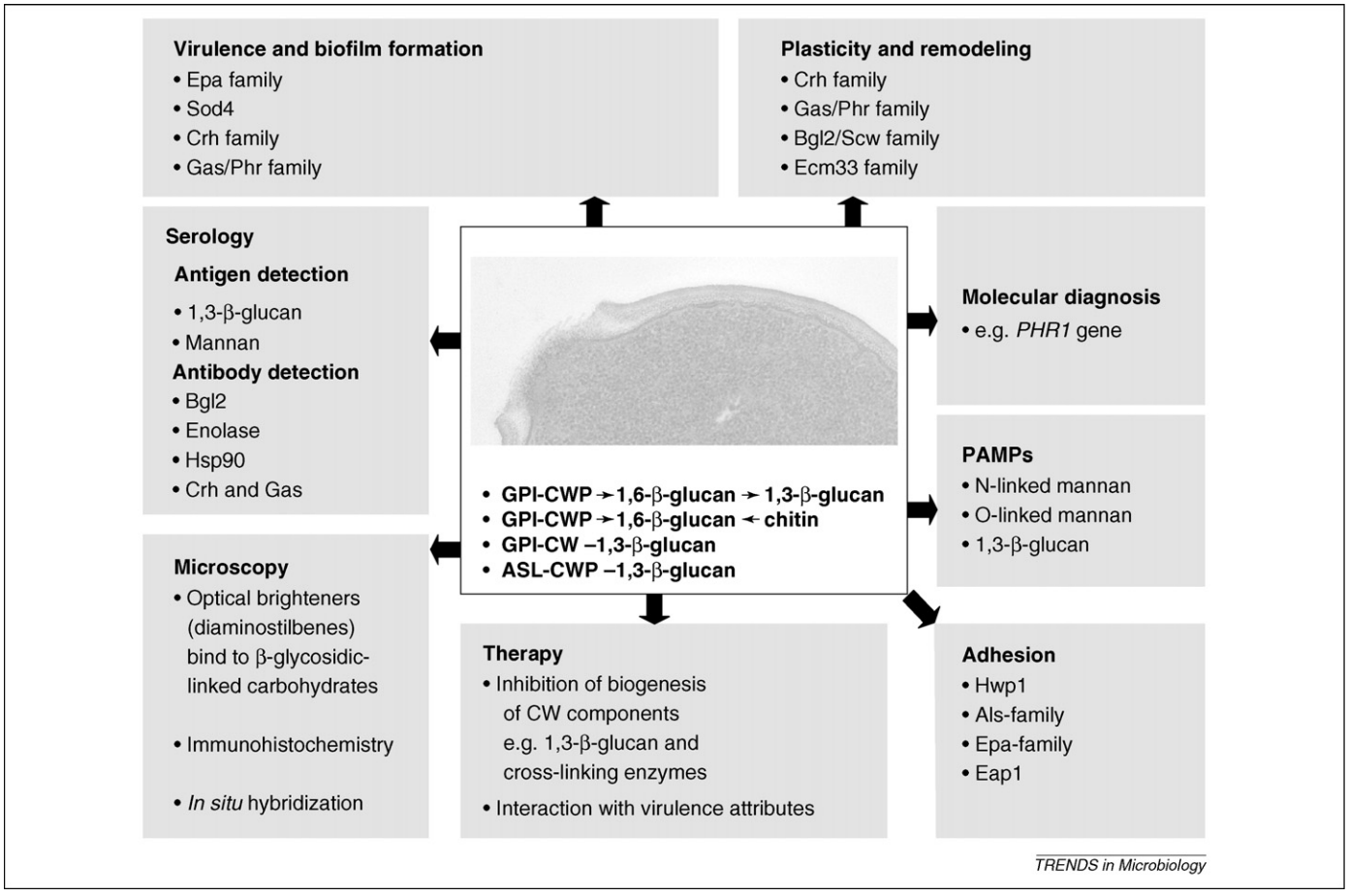

Figure 1. The clinical significance of the *Candida* cell wall. The cell wall is essential for fungal viability and therefore its components are attractive targets for antifungal drug development and for diagnostic tests. The cell walls of pathogenic *Candida spp.*, which account for 10–25% of their mass, consist of a platform of carbohydrates (chitin, 1,3-β-glucan, 1,6-β-glucan,) networked to surface mannoproteins. Arrows represent glycosidic linkages and point from the reducing end of a macromolecule to a non-reducing end of the acceptor polysaccharide. Mannoproteins are divisible into GPI-dependent cell wall proteins (GPI-CWPs) and alkali-sensitive cell wall proteins (ASL-CWPs). During human infection, the cell wall is a dynamic and adaptive structure. Mannoproteins facilitate the assembly and the remodelling of cell wall components, enable morphological plasticity, serve as adhesion molecules and are involved in other aspects of fungal pathogenicity. Examples of cell surface molecules that are relevant to these aspects of the cell wall are indicated, including some non-signal-peptide proteins that have been reported at the cell surface. Abbreviations: CW, cell wall; PAMP, pathogen associated molecular pattern.

fluconazole [33]. However, echinocandins appear to be less effective against *C. guilliermondii*, *C. parapsilosis* and possibly *C. famata*. Notably, the clinical relevance of this observation remains unclear, as interpretative breakpoints are not yet available for the echinocandins.

Voriconazole and posaconazole constitute new extended spectrum triazoles with potent fungistatic activity against many *Candida spp*. Voriconazole, which has been tested *in vitro* against more than 80 000 isolates [34], is less active against *C. glabrata* and *C. krusei* as compared with other species [35]. There are some indications that, compared with voriconazole, posaconazole shows higher minimum inhibitory concentration (MIC)-values against *C. albicans* and lower MIC-values against *C. guilliermondii*. A comprehensive review of the performance of these new antifungal agents *in vitro, in vivo* studies and in animal models can be found in a recent paper by Aperis *et al*. [34].

All these new antifungals have been generated through classical routes, rather than via genomics.

Understanding and testing resistance

The emergence of drug resistant *Candida* isolates has serious implications for the outcome of the disease. Although technically challenging for yeasts, collaborative investigations and consensus agreements have been achieved by the Clinical and Laboratory Standards Institute [CLSI, formerly National Committee for Clinical Laboratory Standards (NCCLS)], European Committee on Antibiotic Susceptibility Testing (EUCAST) and the Deutsches Institut für Normung (DIN) [36–38] to standardise susceptibility testing of *Candida spp*. towards antifungal drugs. These methods have allowed the reproducible and comparable determination of fungal resistance between laboratories. Still these methods have technical shortcomings (e.g. the M-27A method separates poorly the rates of resistance of *Candida spp*. to amphotericin B), and conclusive breakpoints for the echinocandins and the newer broad spectrum triazoles have not yet been determined. In addition, although *in vitro* susceptibility is a basic and valuable variable, it should be emphasised that clinical treatment failure (clinical resistance) using an antifungal agent is the result of a complex interaction between *Candida* and its host [39].

Genomics is proving useful in defining the emergence and the molecular basis of drug resistance in *Candida* species [7,40]. These studies reinforce the importance of

mutations affecting ABC transporters (*CDR1* and *CDR2*), major facilitators (*MDR1*), drug target enzymes (*ERG11)*, or transcription factors *(TAC1*) in mediating drug resistance. These genomic studies have also highlighted roles for stress and metabolic functions during fungal adaptation to antifungals. A useful summary of molecular drug resistance mechanisms in *Candida spp*. is given by Sanglard and Odds, 2002 [41]. However, this new knowledge has not yet been translated into sufficient improvements in the management of invasive candidiasis.

Therefore, combating antifungal resistance is a complex task, as the requirements differ between fungal species, between isolates, and in individual patients. So far, the clinician has benefited more from the development of relatively robust methods for the measurement of antifungal resistance than from a genome-wide understanding of the mechanisms of this resistance. However, genomics is providing a greater understanding of antifungal resistance and/or tolerance, and this should facilitate the development of improved therapeutic regimes in the future. For example, combination therapies could use one drug to target a particular process and a second drug to inhibit mechanisms of adaptation to the first drug.

Identification of new targets through bioinformatics

The availability of genome sequence data for some *Candida* species has allowed novel potential antifungal targets to be selected using bioinformatic approaches. These potential targets are often identified on the basis that these functions are essential for viability, they are conserved amongst fungal pathogens, and they have diverged significantly or are absent in humans. Initial assumptions about gene essentiality are often based on the behaviour of *S. cerevisiae* mutants [42]. In some cases, these assumptions have been followed up by testing the essentiality of the corresponding genes in *C. albicans* and/or other pathogenic fungi (e.g. [26]) or by testing the effects of inactivating the target gene upon virulence. Navarro-Garcia *et al.* have reviewed those genes that influence the virulence of *C. albicans* [43].

Potential targets involved in processes such as cell wall biosynthesis or ergosterol biosynthesis are attractive partly because they are fungus specific and because there are strong precedents for successful antifungal drugs that target these processes (as described above). Recent progress in exploring the cell wall composition, architecture and proteome of *C. albicans* and *C. glabrata* [44–46] seems promising in this respect [47]. The public release of genome sequence data for these two organisms enabled comprehensive *in silico* predictions of the entire cell wall proteome [28,48]. Elegant proteomic approaches to identify the cell wall incorporation of highly N- und O-glycosylated proteins [8] combined with the molecular analysis of the identified proteins confirmed predictions that diverse functional protein families are covalently linked to the cell wall glycan backbone [48,49] (Table 2). In this way, cell wall modules have been characterised that are involved in many fungus-specific physiological processes, environmental protection, morphogenesis and virulence [46,50–55] (Figure 1). The fact that the function of 75 (66%) of the glycosylphosphatidylinositol (GPI)-dependent cell surface proteins of *C. albicans* is still unexplored might be especially relevant for the future discovery of *C. albicans*-specific and essential genes involved in pathogenicity [56].

Other types of drug target have been suggested, some of which break the 'rule' that the target molecule should not be conserved in humans. There are examples of highly conserved functions that could ultimately prove to be useful antifungal targets. These include translation initiation factors and even central metabolic enzymes [57,58]. A good example is heat shock protein 90 (Hsp90). This conserved molecule is the target of the therapeutic antibody, Mycograb® [59]. Initially, academic studies revealed that patients with invasive candidiasis often express antibodies against *C. albicans* Hsp90. Anti-Hsp90 antibodies were then found to be immunoprotective against *C. albicans* challenges. These studies led to the development of Mycograb®, a recombinant antibody against fungal Hsp90, the therapeutic efficacy of which is being tested in single and combination trials [59,60]. Mycograb® might not have evolved through genomics, but this story does illustrate how conserved proteins can be valid antifungal targets, and shows how tight links between academia and the clinic can generate medically useful tools (Figure 2).

Alternative routes to new targets

Putative drug targets identified using bioinformatic approaches might not have yielded clinically useful

Table 2. Covalently linked cell wall proteins in pathogenic *Candida spp.* and in yeast[a]

Species	Number of putative GPI-proteins	Examples of GPI-dependent CW-agglutinins and adhesins	Examples of GPI-dependent CW-glycoside hydrolases[b]	Examples of GPI-dependent non-enzymatic CWPs	Examples of ASL-CWPs
C. albicans	115 [56]	Als1, Als2, Als3, Als4, Als5, Als6, Als7, Als9 [52], Pga24 [8], Eap1 [75], Hwp1 [53]	Cht2 (GH18) [76], Crh11 (GH16), Pga4 (GH72), Phr1 (GH72) [8]	Pga29 [8]	Pir1 [8,77], Scw1 (GH17) [8]
C. glabrata	106 [28]	Epa1 [30], Epa6, Epa7 [51]	Crh1 (GH16) [28]	Cwp1.1, Cwp1.2 [28]	Not analysed
C. krusei	Not analysed	Not analysed	Not analysed	Not analysed	Not analysed
C. parapsilosis	Not analysed	Not analysed	Not analysed	Not analysed	Not analysed
C. tropicalis	Not analysed	Not analysed	Not analysed	Not analysed	Not analysed
S. cerevisiae	66 [48]	Flo1, Flo5, Flo9, Flo10, Flo11 [78], Awa1 [79], Sag1 [80]	Crh1 (GH16), Utr2 (GH16), Gas1 (GH72), Gas3 (GH72), Gas5 (GH72) [81]	Cwp1, Cwp2, Tir1, Tip1 [81,82], Dan1 [83]	Pir1, Pir2, Pir3, Pir4, Scw4 (GH17), Scw10 (GH17), Tos1 [81]

For *C. albicans, C. glabrata* and *S. cerevisiae* comprehensive *in silico* predictions of the cell wall proteome have been performed [28,48]. The cell wall incorporation has been confirmed experimentally for numerous of these proteins and an ampleness of functions could be assigned.

[a]Abbreviations: ASL, alkali sensitive; CW, cell wall; CWP, cell wall protein.

[b]GH, glycoside hydrolase, classification according to the CAZy (carbohydrate-active enzymes) database (http://afmb.cnrs-mrs.fr/CAZY/).

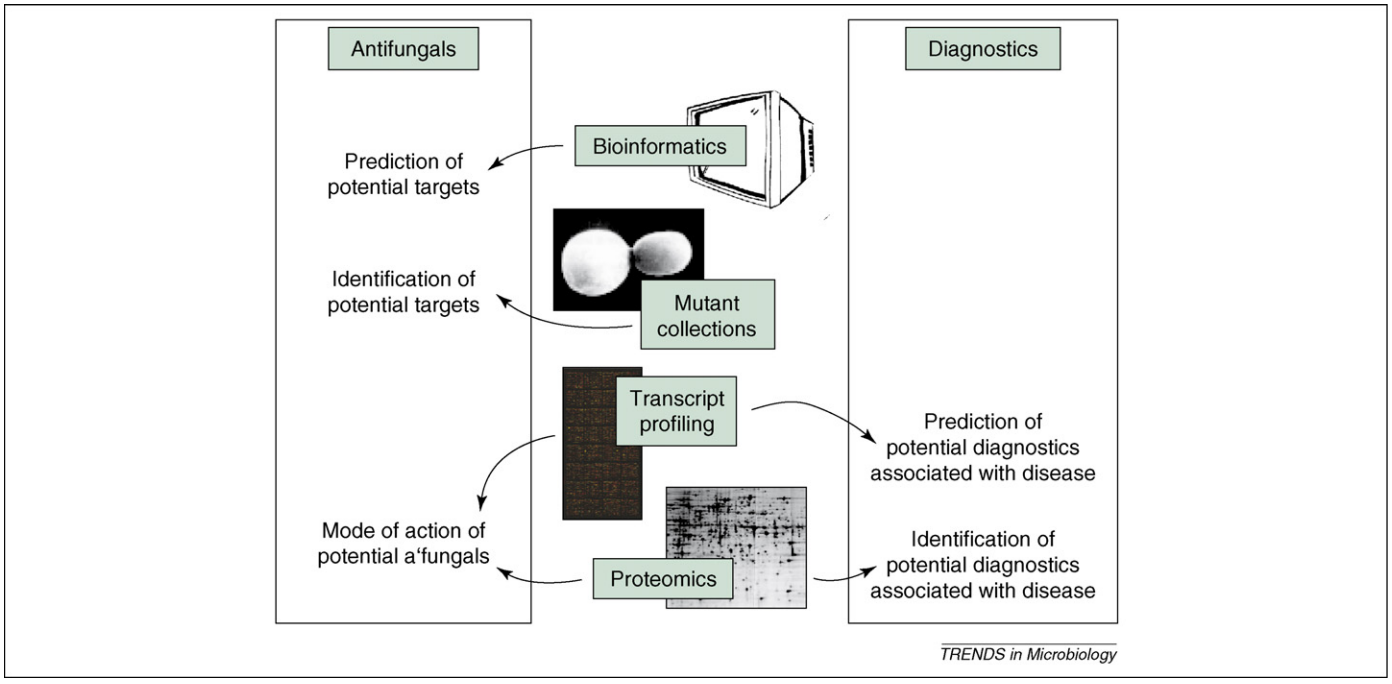

Figure 2. Genomics is contributing to the development of diagnostics and antifungals. Although genomics has not led directly to a new antifungal drug or diagnostic tool so far, genomic strategies (bioinformatics, genome-wide mutant collections, transcript profiling and proteomics) are already contributing to the development of novel antifungals and new diagnostic tools in a number of ways.

antifungal drugs as yet; however, functional genomics is proving useful in helping to define the mode of action of drugs. The key point is that genomic screens provide relatively unbiased views of the impact of a drug upon the fungal cell. One important approach involves the phenotypic screening of genome-wide mutant collections. The availability of collections of heterozygous and homozygous gene knockouts for *S. cerevisiae* [42] is helping to reveal mutations that affect drug sensitivity (e.g. through haploinsufficiency screens). The nature of the defects in these drug-sensitive mutants provides important insights into the cellular processes that are affected by the drug in question (e.g. [61]). The same approach can be applied in *C. albicans* and *C. glabrata* using mutant collections created in the Cormack–Falkow, Johnson and Mitchell laboratories [30,62,63] or, in principle, the GRACE™ (gene replacement and conditional expression) collection of doxycycline-conditional mutants generated by Elitra [26].

The application of doxycycline-conditional mutants is potentially very powerful. Exogenous doxycycline inhibits the expression of the target *C. albicans* gene that has been placed under the control of the *tet* promoter in a particular GRACE mutant, even in the mouse model of systemic candidiasis. Elitra examined 1152 target genes using this approach (roughly 20% of the genome), which were preselected on the basis that they might be essential for growth. Of these, 574 proved essential for growth *in vitro* on minimal agar, and about half of these were essential for viability [26]. The latter genes encode attractive antifungal targets because a drug that blocks their action is likely to kill the fungal cell. Furthermore, GRACE mutants can be used to identify prophylactic and remedial targets. For example, by applying doxycycline at the start of an animal infection one can identify those fungal genes that are required to establish an infection and hence might be useful for prophylactic treatment. Alternatively, by administering doxycycline to animals late in an infection, one can select potential antifungal targets, the inactivation of which can lead to the clearance of an established infection.

A second approach involves the analysis of the global effects of a drug upon gene expression. In *C. albicans* this has been addressed at the level of the transcriptome and the proteome [9,64]. For example, transcript profiling showed that an azole (ketoconazole) affects the expression of *C. albicans* genes involved in lipid, fatty acid and sterol metabolism, and that a β-1,3-glucan synthase inhibitor (caspofungin) influences genes involved in cell wall biosynthesis [9]. These genomic datasets have largely reinforced previous assumptions about the mode of action of these well-characterised drugs. More to the point, these experiments have confirmed that genomic approaches can provide valuable information about the mode of action of a new antifungal by revealing which genes are regulated in response to the drug.

Nevertheless, in the foreseeable future, the treatment of systemic candidiasis will be limited mainly to drugs that interfere with the cell surface (plasma membrane and cell wall). The extensive scientific data on putative novel fungal-specific drug targets that have been generated using genomic and proteomic approaches remain to be translated into target-based drug development (Figure 2). However, having identified a potential antifungal agent that inhibits the activity of a target *in vitro*, this substance must be optimised, its efficacy and toxicity tested in cell culture and animal models, and Food and Drug Administration (FDA) or European Medicines Agency (EMEA) supervised clinical trials performed to compare this new antifungal drug with existing drugs in terms of efficacy, pharmacokinetics, dynamics and side effects. Hence the process of *de novo*

drug development typically lasts a decade or longer and costs at the least $500 million before the drug reaches the market [65]. Therefore, it might be too early to expect a range of antifungal classes to have emerged on the back of genomics-led research programmes.

The importance of strain typing

Strain typing can provide useful information about the sources and transmission of strains, relationships between isolates, and their virulence properties. Furthermore, strain typing can potentially forewarn the clinician of infections involving drug resistant *Candida* strains.

The recent consensus work for Multi-Locus Sequence Typing (MLST) of *C. albicans* represents a major improvement in strain typing. This collaborative MLST is based on fragments of seven housekeeping genes [66]. At present, MLST-databases can be found on the web at http://calbicans.mlst.net (*C. albicans*, *C. glabrata*, *C. tropicalis*) and http://pubmlst.org/ckrusei/ (*C. krusei, C. tropicalis*) [67]. Data from existing strain typing studies reinforce the view of persistent strain carriage in individuals and the idea that *C. albicans* reproduces predominantly in a clonal manner. These studies also confirmed the phenomenon of 'microevolution' of single strains in individual patients. Genetic variations can arise by events, such as recombination, gene replacement or even cryptic mating [68].

Diagnostic approaches

Automated blood culture systems, such as the BacT/ALERT 3D (bioMérieux) or the BACTEC 9240 (Becton Dickinson) and chromogenic *Candida* agars, such as BBL™ CHROMagar™ Candida (Becton Dickinson), Candida ID2 agar (bioMérieux) or Oxoid Chromogenic Candida agar (OCCA) have facilitated the culture-based diagnosis of invasive candidiasis. In addition, a variety of DNA targets have been proposed for the molecular species identification of cultured fungi.

Evidentiary culture-based invasive diagnostic procedures are often precluded and therefore rarely helpful for critically ill patients. Yet the successful treatment of these infections depends largely upon timely and appropriate antifungal therapy [69]. It is disappointing to report that, despite rapid advances in *Candida* genomics and molecular biology, reliable, sensitive and specific non-culture based diagnostic tools have not yet been developed.

A majority of clinical laboratories offer serological *Candida* diagnosis. The commercially available CandTEC® antigen test (Ramco, Houston, Texas) and the monoclonal antibodies-based Platelia®-*Candida* sandwich ELISA (BIO-RAD, München, Germany) are frequently applied. However, both systems have important shortcomings. Other antigens that were proposed for serodiagnosis so far include the cell wall carbohydrate β-1,3-glucan, the metabolite D-Arabinitol, Enolase and a 47 KDa degradation product of Hsp90 (Figure 1).

Most anti-*Candida* antibody detection systems in clinical use are based on crude and undefined antigen preparations. As a consequence, at present the value of IgG-, IgM- or IgA-specific antibody detection is not well established. In this context, recent genomics-based work might offer useful new diagnostic possibilities. Pitarch and coworkers have combined modern proteomic and bioinformatics approaches to analyze the *C. albicans* cell wall-immunome (i.e. proteins at the *C. albicans* cell surface that react with the sera of patients with systemic candidiasis). This group reported significantly elevated antibodies against a β-1,3-glucosidase (Bgl2) in these patients compared to control groups. Hence they suggest that anti-Bgl2 antibodies in the serum might represent a novel and useful marker for the diagnosis of systemic candidiasis. They also report that antibodies against cell wall-associated enolase confer protection against invasive disease and might serve as novel prognostic indicators [6].

Although conclusive clinical evaluations are lacking so far, hypha-specific molecules such as Hwp1 might shape up as specific diagnostic indicators for *C. albicans* infections [53]. Some hypha-specific molecules have been identified by genomic approaches [4]. Hence genomics is identifying potentially useful diagnostic and prognostic markers (Figure 2) [6].

Numerous papers report encouraging data on the molecular biology-based diagnosis of invasive candidiasis directly from clinical material, such as whole blood, serum, plasma, blood culture bottles, sterile body fluids or tissue samples (e.g. [70,71]) compared with conventional culture-based methods. However, PCR-based methods are susceptible for cross-contamination, resulting in false positives. Other methodological problems include the rigid cell wall of *Candida spp*. (which demands harsh DNA-extraction procedures), the low number of circulating yeasts during systemic infection, and *Candida* colonisation that can contaminate clinical samples. These problems and the absence of standardised approaches for specimen selection and handling, DNA-extraction, DNA-target or amplicon detection have led to divergent results. As a consequence, molecular results are not yet recognised as consensual diagnostic criteria.

The recent implementation of real-time technologies such as the Light Cycler (Roche Molecular Systems) or the TaqMan (Perkin Elmer) approaches (e.g. [72]) offers new opportunities as these methods do not rely on post-amplification manipulations, reduce the risk of false positive results and deliver quantitative results. However, a comprehensive interlaboratory evaluation of published approaches, a methodological consensus and quality management strategies are urgently needed [73,74].

We conclude that genomic approaches such as proteomics are identifying potentially useful diagnostic markers (Figure 2). However, methodological standardisation and sophisticated clinical evaluations are as important for improving molecular diagnosis as the identification of novel diagnostic targets using genomics and bioinformatics.

Conclusions and future perspectives

To summarise, no antifungal drugs that are in clinical use have been developed through genomics. Therefore, one could argue that the jury is still out with regard to the impact of genomics on the development of novel anti-*Candida* drugs. However, we have only recently entered the *Candida* genomics era, and it takes many years to develop, validate and test a new antifungal drug. Hence, it is still too early to decide on the impact of genomics on

antifungal development. Furthermore, it is clear that genomics is proving useful by defining the global effects of antifungal drugs upon the fungal cell, and the mechanisms of cellular adaptation to antifungals. These data will inform the honing of therapeutic strategies. Genomic approaches are also helping to define potentially useful tools for the diagnosis and prognosis of systemic *Candida* infections, but have not yet had a major impact in this area either. Despite the fact that major pharmaceutical companies have reduced their investments in antifungal research, many in the academic community are continuing their efforts to translate their research into the clinic. Therefore, we are confident that, in the not too distant future, the experimental power of genomics will be realised in significant improvements in antifungal diagnosis and therapy. Most probably this will be through the intelligent use of genome-wide profiling and mutant collections, and in combination with well-focussed, clinically relevant molecular and cellular approaches.

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