Drug Resistance Updates 42 (2019) 22–34

Contents lists available at ScienceDirect

Drug Resistance Updates

journal homepage: www.elsevier.com/locate/drup

Can *Saccharomyces cerevisiae* keep up as a model system in fungal azole susceptibility research?

Liesbeth Demuyser[a,b], Patrick Van Dijck[a,b,*]

[a] VIB-KU Leuven Center for Microbiology, Flanders, Belgium
[b] Laboratory of Molecular Cell Biology, Institute of Botany and Microbiology, KU Leuven, Leuven, Belgium

ARTICLE INFO

Keywords:
Saccharomyces cerevisiae
Yeast
Azole
Resistance
Model
Fungi

ABSTRACT

The difficulty of manipulation and limited availability of genetic tools for use in many pathogenic fungi hamper fast and adequate investigation of cellular metabolism and consequent possibilities for antifungal therapies. *S. cerevisiae* is a model organism that is used to study many eukaryotic systems. In this review, we analyse the potency and relevance of this model system in investigating fungal susceptibility to azole drugs. Although many of the concepts apply to multiple pathogenic fungi, for the sake of simplicity, we will focus on the validity of using *S. cerevisiae* as a model organism for two *Candida* species, *C. albicans* and *C. glabrata*. Apart from the general benefits, we explore how *S. cerevisiae* can specifically be used to improve our knowledge on azole drug resistance and enables fast and efficient screening for novel drug targets in combinatorial therapy. We consider the shortcomings of the model system, yet conclude that it is still opportune to use *S. cerevisiae* as a model system for pathogenic fungi in this era.

The use of azole drugs in antifungal therapy

The relatively higher frequencies with which *Candida* species are being isolated from the blood can, at least partially, be ascribed to the growing population of immunocompromised patients and the use of antibiotics or invasive medical material (reviewed in (Yapar, 2014)). The high inherent antifungal drug resistance in combination with the increased onset of resistance during infection and treatment, make that the currently-used drugs are insufficiently effective. Antifungal drug development has evolved slowly, generating only few classes of potential therapies.

One of the three main drug classes used in a clinical setting today, are the azoles. Fluconazole and itraconazole were developed in the 1990s, representing a second generation of azoles (Como and Dismukes, 1994). Their significantly reduced toxicity, good efficacy and the possibility to administer them orally, greatly improved the success of these azoles as therapeutics. Fluconazole nowadays even represents the most-frequently prescribed antifungal drug against *C. albicans* infections (Whaley et al., 2016). Recently developed azole drugs are voriconazole, posaconazole and isavuconazole, representing the third generation of azoles. They have been shown to be more active against less common *Candida* pathogens and have improved pharmacological properties, although being costlier as well (Nett and Andes, 2012; Miceli and Kauffman, 2015).

The target of all azole antifungals is the fungal cytochrome P450 member lanosterol-14α-demethylase encoded by *ERG11* (Vanden Bossche, 1985; Kelly et al., 1993). This enzyme is necessary for the biosynthesis of ergosterol, the primary sterol in the fungal cell membrane. Apart from *Sc*Erg11, it has been shown that *Sc*Erg5 mediates susceptibility and potentially represents a target of azole antifungals in *S. cerevisiae* (Skaggs et al., 1996). Ergosterol is important for the cell to maintain correct levels of membrane viscosity and elasticity in order to allow membrane trafficking, membrane transport and to resist osmotic lysis. Ergosterol and precursors from its biosynthesis have also been shown to play a role in additional processes, such as respiration, and protein, lipid and transfer (t)RNA modification (Parks and Casey, 1995). Pyruvate is a simple α-keto acid involved in several processes inside the cell, mainly acting as an energy donor through input in the Krebs cycle. This molecule undergoes oxidative decarboxylation, catalysed by a pyruvate dehydrogenase, to form acetyl-coenzyme A (CoA) (Parks and Casey, 1995). Through a number of intermediate molecules, acetyl-CoA is converted into squalene and further into lanosterol. This sterol is converted into 4,4-dimethyl-cholesta-8,14,24-triene-3β-ol by Erg11 and further metabolized into ergosterol. By targeting Erg11 and inhibiting further flux through the pathway towards ergosterol, azoles diminish the level of ergosterol in the cell. Under these conditions, lanosterol is increasingly converted into 14-methylfecosterol and ultimately into the toxic 14-methylergosta-8,24(28)-dien-3β,6α-diol,

* Corresponding author at: VIB-KU Leuven Center for Microbiology, Flanders, Belgium.
E-mail address: Patrick.vandijck@kuleuven.vib.be (P. Van Dijck).

https://doi.org/10.1016/j.drup.2019.02.002
Received 29 October 2018; Received in revised form 30 January 2019; Accepted 11 February 2019

which represents an important aspect of the mode-of-action of the drug (Kelly et al., 1995). The altered membrane sterol composition leads to altered membrane fluidity and permeability as well as decreased activity of several membrane-bound enzymes, followed by cell growth arrest (Cowen et al., 2002).

Positive attributes of azole antifungals are their low cost and easy oral administration. The toxicity of the drug towards the host is low, although it is not completely negligible. The P450 cytochromes are also present in humans, presenting a risk of cross-reactivity and host toxicity (Mast et al., 2013). The major side-effects reported, are linked to the gastrointestinal tract, eyesight and liver. Furthermore, azoles are fungistatic drugs, only inhibiting growth yet not killing the fungi. The residual growth perceived at drug concentrations above the minimal inhibitory concentration (MIC), is known as tolerance or FoG for fraction of growth (Rosenberg et al., 2018). As we published recently, novel insights in the mechanisms employed by fungi to survive in the presence of existing antifungal drugs, could generate opportunities for more effective combinatorial therapies (Demuyser et al., 2017).

Why we need a model system

Working with pathogenic fungi can be quite challenging. *C. albicans* belongs to the CTG clade, implicating that the leucine CUG codon is translated as a serine, in most cases (Sugita and Nakase, 1999). It is reasonable to state that this slows down genetic manipulation and general experimentation in the fungus to a substantial extent. Most of the established tools that are available for *S. cerevisiae*, cannot be used directly in *C. albicans*. Codon optimization is necessary in many cases. *C. glabrata* does not belong to this CTG clade and achieving genetic alterations in this fungus is thus more straightforward. However, homologous recombination is not very efficient in *C. glabrata*, contrary to *S. cerevisiae*. Non-homologous end-joining is very efficient in the pathogen, hampering targeted modifications of the genome. In general, for *C. albicans* as well as for *C. glabrata*, the array of tools available for genetic manipulation is considerably smaller and less established, compared to *S. cerevisiae*. Genome-wide deletion collections, such as the ones available for this model organism (Dujon, 1996), are not present for any of the *Candida* species, although recent efforts have been made in this direction, as described further.

Another important difference between *C. albicans* and *C. glabrata* versus *S. cerevisiae*, is the absence of a normal sexual cycle in the first two organisms. In *S. cerevisiae*, haploid cells of the opposite mating type (**a** or α) can mate and form diploid cells. These produce haploid cells again through meiosis and sporulation. Both haploid and diploid cells can proliferate via budding. *C. albicans* has a parasexual cycle, where diploid (**a**/α) cells lose either of the two *CaMTL* alleles and undergo white-opaque switching. Opaque cells with different mating types can mate to form tetraploid cells. These can lose (part of) a diploid genome, to become diploid themselves, possibly retaining some aneuploidy chromosomes (Noble et al., 2017). Since *C. albicans* doesn't seem to undergo meiosis, it was long believed to be an obligate diploid. Recently, however, haploid cells were detected (Hickman et al., 2013). For *C. glabrata*, no sexual cycle has been observed to date (Shin et al., 2007). Both species predominantly reproduce through clonal division. The lack of a (normal) sexual cycle for *Candida* species hampers fast genetic experimentation.

Finally, *C. albicans* and *C. glabrata* have genomes with a high level of plasticity. Translocations, truncations and ploidy changes are commonly observed. Practical implications are, for instance, that integration of a plasmid in the genome of *C. albicans* happens at a highly variable frequency. Several experimental conditions can induce genomic alterations, such as treatment with antifungal drugs, e.g. fluconazole (Selmecki et al., 2010).

The general relevance of *S. cerevisiae* as a model system

The two main advantages of using *S. cerevisiae* as a model organism today, are the relative ease of manipulation and experimentation, and the immense amount of data that is available. Both assets reinforce each other. Most importantly, it is an enormous source of knowledge on gene and protein functions. The ease of gene manipulation and mutation in *S. cerevisiae*, has led to the generation of many genome-wide mutant collections. With these tools, making connections between genes, proteins and their function has become easier. The majority of the information available for *S. cerevisiae* genes and gene ontology (GO) is brought together in the *Saccharomyces* genome database (SGD) (Cherry et al., 2012). This information, as well as the functional networks that were built based on these interactions, present a very valuable source of information for other eukaryotes (Botstein and Fink, 2011). Furthermore, important techniques that are used regularly nowadays, were first used and optimized in yeast. The simplicity of this organism, compared to other eukaryotes, and the vast amount of information available for validation, make tool development relatively easy, especially on the genomic scale. Examples of tools first developed for *S. cerevisiae* are chromatin immunoprecipitation-sequencing (ChIP-seq) for transcriptional analysis, the yeast-two-hybrid system for protein-protein interaction detection and computational techniques to analyse large data sets (Fields and Song, 1989; Lefrancois et al., 2009). General advantages over other model organisms are the small size, fast generation time, low costs for purchase and culturing, absence of ethical issues, etc.

The specific relevance of *S. cerevisiae* as a model system for fungal research

Is it justifiable to use *S. cerevisiae* as a model organism for fungi, such as *C. albicans* and *C. glabrata*? There are advantages and disadvantages to replacing the organism under study with a 'simpler' or more well-known model system. The attributes of yeast that make it an ideal organism, mentioned in the previous paragraph, also apply here. The vast amount of knowledge and relatively easy experimentation possible in this organism, especially when it comes to mating and consequent genetic manipulation, advocate its usefulness. The fact that this yeast is rarely pathogenic adds to this, since no special precautions have to be taken when handling it.

Does *S. cerevisiae* resemble *C. albicans* and *C. glabrata*? The three organisms are unicellular eukaryotic organisms sharing quite a lot of features. In 2004, Jones et al. indicated that for about 75% of the open reading frames (ORFs) in *C. albicans*, matches can be found in *S. cerevisiae* (Jones et al., 2004). Although most genes are thus shared between both species, rewiring events have been shown to occur, for instance in transcriptional regulatory networks (Khamooshi et al., 2014). Especially those ORFs that do not seem to match between both species can be interesting for virulence studies. An obvious example is the lack of true hyphae in *S. cerevisiae*. Table 1 shows an overview of *S. cerevisiae* proteins relevant for azole research and their closest orthologues in *C. glabrata* and *C. albicans*. As an indication of homology, the percentage of identical and positive residues is depicted alongside the orthologues. Apart from the virulence factors and responsible genes described above, other remarkable differences are the oxidative metabolism, cell cycle factors and specific alterations for sensing and adapting to the environment and acquiring nutrients (Jones et al., 2004). Despite these differences, *S. cerevisiae* is actively used as a model for *Candida* species. One remarkable example is the use of this yeast as a model system for biofilm formation. Quite a lot of the features of pathogenic fungi important in adhesion and biofilm maturation, e.g. extracellular matrix formation and drug resistance, are largely conserved (Bojsen et al., 2012).

Taking a look at the evolutionary tree of life, we see that the divergence between *C. albicans* and *S. cerevisiae* happened more than 700

Table 1
Overview of proteins involved in azole susceptibility in *S. cerevisiae* and their respective orthologues in *C. glabrata* and *C. albicans*. Along with the protein names, the percentages of identical amino acid residues and positively matching residues, as defined by BLAST analysis, are shown (Skrzypek et al., 2017). The colour code ranges from green, lower percentage, to red, higher percentage. Within each class, proteins were ranged from least to most sequence identity. (For interpretation of the references to colour in this table legend, the reader is referred to the web version of this article.)

	S. cerevisiae	*C. glabrata*	*% identities*	*% positives*	*C. albicans*	*% identities*	*% positives*
Sterol metabolism	Erg8	Erg8	46,8	66,4	Erg8	41,9	59,5
	Erg12	CAGL0F03861g	55,1	71,2	Erg12	42,7	59,1
	Hmg2	CAGL0L11506g	59,9	74,6	Hmg1	51,1	66,2
	Hmg1	CAGL0L11506g	66,1	81,7	Hmg1	50	64,3
	Erg3	Erg3	70,8	83,9	Erg3	55,6	67,9
	Erg9	Erg9	70,9	82,5	Erg9	56,5	71,6
	Erg19	CAGL0C03630g	72,1	83	Mvd1	64,3	74,5
	Erg28	CAGL0J02684g	73,6	87,8	Erg28	56,8	74,4
	Erg27	CAGL0M11506g	73,9	87,5	Erg27	58,6	73,5
	Erg2	Erg2	75,9	86,2	Erg6	58,7	69,7
	Erg24	CAGL0I02970g	75,9	89	Erg24	58,3	73,1
	Idi1	CAGL0J06952g	76,1	85,1	Idi1	55,4	71,1
	Erg13	Erg13	76,6	87,6	Erg13	67,5	80,8
	Erg20	CAGL0L00319g	78,2	87,7	Erg20	73,4	84,3
	Erg7	CAGL0J10824g	79,7	88,8	Erg7	62,9	75,4
	Erg10	Erg10	81,2	91,5	Erg10	68,3	79,9
	Erg6	Erg6	81,5	92,2	Erg6	65,7	78,4
	Erg26	CAGL0G00594g	82,8	91,1	Erg26	69,7	81
	Erg4	Erg4	82,9	90	Erg4	61	75,3
	Erg11	CAGL0E04334g	83	91,7	Erg11	65,1	78,9
	Erg5	Erg5	84,1	93,5	Erg5	70,7	82,7
	Erg1	Erg1	84,6	93	Erg1	51,8	68,3
	Erg25	CAGL0K04477g	89	93,9	Erg25	64,6	77,8
Regulation of sterol metabolism	Hap1	Hap1	37,6	52,7	Mrr1	21,7	39,9
	Ecm22	Upc2a	53	64,9	Upc2	65,5	78,6
	Nsg2	CAGL0J05918g	54,8	68,9	Nsg2	25,8	45,5
	Ncp1	Ncp1	65,9	81,1	Ncp1	50,4	66,9
	Upc2	Upc2a	74,6	86,8	Upc2	65,7	77,6
	Hem1	Hem1	82,9	90,1	Hem1	64,5	77
Drug efflux	Qdr3	Dtr1	37,9	61,1	Qdr3	44,3	63,9
	Ste6	CAGL0K00363g	41,7	62,5	Hst6	32,4	54,1
	Azr1	CAGL0B02079g	54,5	70,7	C1_09210C_A	33	54,1
	Flr1	Flr1	59,3	74,2	Mdr1	44,1	63,3
	Qdr1	Qdr2	63,2	79,8	Qdr1	37,7	58,4
	Pdr11	Aus1	66,2	78	Snq2	36,4	53,7
	Pdr10	Pdh1	67,1	80,7	Cdr1	52,6	69,6
	Qdr2	Qdr2	69,5	82,5	Qdr1	37,1	57
	Pdr18	Snq2	69,6	84,3	Snq2	55,2	71,7
	Aus1	Aus1	71,5	82,9	Snq2	35,7	54,5
	Yor1	Yor1	71,5	83,1	Yor1	49	66,3
	Pdr5	Cdr1	74	86,3	Cdr1	56,1	73,3
	Snq2	Snq2	74,6	86,7	Snq2	54,6	71,7
	Ycf1	Ycf1	75	87	Ycf1	56,4	72,9
	Pdr15	Pdh1	75,7	86,3	Cdr4	53,5	70,6
	Tpo1	Tpo1_1	76,1	86,1	Flu1	45	63,3
	Pdr12	CAGL0M07293g	85,3	92,4	Snq2	45,9	64,1
Regulation of drug efflux	Pdr3	Pdr1	32,2	53,8	Mrr1	30,9	41
	Yap1	Ap1	36,6	49,8	Cap1	53,9	77,6
	Pdr1	Pdr1	37,1	58,1	Cta7	42	53,6
	Yrr1	CAGL0L04576g	37,2	58,6	Zcf29	30,1	44,7
	Yrm1	CAGL0L04576g	39,3	64,2	Zcf29	39,6	62,3
	Rdr1	Pdr1	40,4	54,4	Cta7	21	37,1
Mitochondrial metabolism	Isa2	CAGL0D01496g	53,9	69,7	Isa2	43,9	58,3
	Pgs1	Pgs1	58	76,4	Pel1	48,5	63,7
	Isa1	CAGL0G03905g	58,4	69,9	Isa1	63,6	76,6
	Yfh1	Yfh1	59,1	77,4	Yfh1	51,7	73,7
	Mdl1	CAGL0E00385g	60	74,1	Mdl1	47,4	65,4
	Arh1	CAGL0A03014g	61,3	73,9	Arh2	51,3	69,7
	Oxa1	CAGL0I06534g	61,9	75,1	C7_01600W_A	36,9	57,6
	Psd1	CAGL0J06226g	63,7	80,5	Psd1	46,5	60,2
	Yah1	CAGL0H00660g	72,6	82,3	Yah1	66,9	77,3
	Atm1	Atm1	74,2	84,1	Atm1	67,1	79,2
	Mdl2	CAGL0A01133g	77	86,7	Mdl2	42,2	60,1
	Isu1	CAGL0M02629g	83,7	89,5	Isu1	75,7	87,5
	Nfs1	CAGL0H03817g	84,2	92,6	Spl1	77	88,8
Regulation of mitochondrial processes	Rtg3	Rtg3	38,9	54,3	Rtg3	44,7	63,2
	Rtg1	Rtg1	54,8	66,8	Rtg1	46,7	65,2
	Ssq1	CAGL0G04917g	65,1	80,7	Ssq1	51,8	72
	Rtg2	Rtg2	66,8	81,3	Pgi1	25	44,6
	Mge1	Mge1	67,1	78,2	Mge1	48,9	64,4
	Grx5	CAGL0M07271g	81,7	87,8	C1_07630W_A	72	87,2

million years ago (Hedges et al., 2004) while the deviation between baker's yeast and *C. glabrata* occurred 100 to 300 million years ago (Dujon et al., 2004). This illustrates that *C. albicans* differs from *S. cerevisiae* to a larger extent than does *C. glabrata*, which can also be concluded from the differing homology levels shown in Table 1. The use of yeast as a model system for *C. glabrata* is thus even more reasonable. Important differences between both organisms, such as the variable number of adhesins or stress resistance, show that the relatively shorter evolutionary distance can, however, still impede comparison (Roetzer et al., 2011). We conclude that *S. cerevisiae* can be used as a model system for *Candida* species, although care should be taken with extrapolation.

Using the unique properties of *S. cerevisiae* to accelerate fungal azole research

S. cerevisiae as a screening platform in chemogenomics

Chemogenomics can be described as the systematic analysis of cellular chemical-biological interactions, and has been applied frequently to elucidate the mode-of-action of the effect of chemical compounds on cells. Several types of screening assays have been developed for application in *S. cerevisiae*, each benefitting from alternative characteristics of the model organism (Giaever et al., 2004). First, the easy readout of compound effectiveness on cell proliferation or fitness allows parallel analysis of thousands of mutants (Boone et al., 2007). Further, inspired by the fully sequenced yeast genome, large deletion mutant collections containing strains with kanamycin cassette-replaced ORFs are available (Goffeau et al., 1996; Giaever et al., 2002). These collections contain homozygous and heterozygous diploid as well as haploid deletion strains from both mating types covering the entire protein encoding genome (Giaever and Nislow, 2014). The use of temperature sensitivity as well as repressible promoters has allowed for the generation of essential gene mutant collections (Mnaimneh et al., 2004; Dohmen and Varshavsky, 2005). Having exploited these screening systems for drug interaction analysis, researchers found many processes involved in the susceptibility of *S. cerevisiae* to azole antifungals. Anderson et al. used such a chemogenomic setup, where they assessed growth of the *S. cerevisiae* haploid *MATa* deletion collection of non-essential genes on medium containing fluconazole. They found 13 deletion mutants, disturbed in lipid and sterol metabolism, to be resistant to the drug (Anderson et al., 2003). Using a similar *MATa* deletion collection, other researchers showed the involvement of peroxisomal and mitochondrial organization in resistance of biofilms against miconazole (Vandenbosch et al., 2013). Recently, by screening the haploid *MATα* deletion collection for increased susceptibility to fluconazole, we found that vesicular transport plays an important role in the regulation of this process (Demuyser, Van Dyck et al., AAC, resubmission). After initial observations and mode-of-action studies in *S. cerevisiae*, we successfully confirmed our findings in both *C. albicans* and *C. glabrata*. The observed synergism between fluconazole and sortin2, a compound inhibiting vesicular transport, demonstrates the potential value of using genome-wide screenings as an accelerator of drug susceptibility research. More precise profiling of the cellular drug response can be achieved, using HIPHOP (Lee et al., 2014). This technique combines drug induced haploinsufficiency profiling (HIP) and homozygous profiling (HOP). During HIP, one screens for fitness defects in heterozygous deletion strains and as such identifies potential drug targets (Giaever et al., 1999; Lum et al., 2004). During HOP, fitness defects indicate genes required to buffer the targeted pathway. Combination of both screens yields a cellular response profile specific for a certain drug. The mode-of-action of compounds affecting fungal growth can effectively be determined using HIPHOP analysis (Hoepfner et al., 2012; Robbins et al., 2015). A database has been established with the results of many such chemogenomic screens (Lee et al., 2014). Researchers used the HIP strategy in *S. cerevisiae*, for instance, to analyse the antifungal mechanism of the novel drug candidate AR-12, initially developed as an anticancer compound yet now in preclinical development for treatment of cryptococcosis (Pianalto and Alspaugh, 2016). It was shown that the chemical affects the acetyl-CoA synthetase (*Sc*Acs2) leading to severe cellular defects. Genome-wide synthetic genetic arrays (SGA) are another interesting example of how deletion collections can be used to study chemogenetic interactions (Baryshnikova et al., 2010). Very recently, Mount et al. used this technique to uncover genetic interaction partners of *ScERG3* (Mount et al., 2018). Resistance to azole drugs can be caused by mutations in *ScERG3*, since the gene product is responsible for converting ergosterol precursor molecules into toxic sterols. The identification of conditional genetic interaction partners of *ScERG3* was achieved by screening double deletion strains, with the query strain *Scerg3*Δ and the haploid gene deletion collection, for growth in the presence of an azole drug. Nine genes were identified for which deletion suppressed *Scerg3*-mediated resistance to azoles. Finding genetic interactions using this setup could allow for the discovery of drug targets, leading to compounds applied in combination therapy. Another interesting feature of the deletion collections is the presence of 20-bp barcodes in the kanamycin deletion cassette, unique to the individual

Table 2
Screenings involved in the meta-analysis of azole susceptibility regulation. The type of azole drug used, is showed, together with the type of screening and the reference article. HIP, haploinsufficiency profiling; HOP, homozygous profiling; R, resistant deletion or mutant strains isolated; S, susceptible deletion or mutant strains isolated.

Azole drug	Type of screening	Reference
Fluconazole	HOP^{R}	(Anderson et al., 2003)
Fluconazole	$HOP^{S,R}$	(Demuyser, Van Dyck et al., AAC accepted for publication)
Fluconazole	$Transposon^{S,R}$	(Kontoyiannis, 1999)
Fluconazole	HIP^{S}	(Giaever et al., 2004)
Miconazole	HIP^{S}	(Giaever et al., 2004)
Clotrimazole	HIP^{S}	(Lum et al., 2004)
Fluconazole	$HOP^{S,R}$	(Hoepfner et al., 2014)
Fluconazole	$HIP^{S,R}$	(Hoepfner et al., 2014)
Clotrimazole	$HOP^{S,R}$	(Hoepfner et al., 2014)
Clotrimazole	$HIP^{S,R}$	(Hoepfner et al., 2014)
Fluconazole	$HOP^{S,R}$	(Spitzer et al., 2011)
Fluconazole	$HIP^{S,R}$	(Spitzer et al., 2011)
Fluconazole	$HOP^{S,R}$	(Lee et al., 2014)
Fluconazole	$HIP^{S,R}$	(Lee et al., 2014)
Itraconazole	$HOP^{S,R}$	(Lee et al., 2014)
Itraconazole	$HIP^{S,R}$	(Lee et al., 2014)
Ketoconazole	$HOP^{S,R}$	(Lee et al., 2014)
Ketoconazole	$HIP^{S,R}$	(Lee et al., 2014)
Miconazole	$HOP^{S,R}$	(Lee et al., 2014)
Miconazole	$HIP^{S,R}$	(Lee et al., 2014)
Clotrimazole	$HOP^{S,R}$	(Lee et al., 2014)
Clotrimazole	$HIP^{S,R}$	(Lee et al., 2014)

Anderson et al. (2003) Mode of selection and experimental evolution of antifungal drug resistance in *Saccharomyces cerevisiae*. *Genetics* **163**:1287–1298.
Giaever et al. (2004) Chemogenomic profiling: identifying the functional interactions of small molecules in yeast. *Proc Natl Acad Sci U S A* **101**:793–798.
Hoepfner et al. (2014) High-resolution chemical dissection of a model eukaryote reveals targets, pathways and gene functions. *Microbiol Res* **169**:107–120.
Kontoyiannis DP (1999) Genetic analysis of azole resistance by transposon mutagenesis in *Saccharomyces cerevisiae*. *Antimicrob Agents Chemother* **43**:2731–2735.
Lee et al. (2014) Mapping the cellular response to small molecules using chemogenomic fitness signatures. *Science* **344**:208–211.
Lum et al. (2004) Discovering modes of action for therapeutic compounds using a genome-wide screen of yeast heterozygotes. *Cell* **116**:121–137.
Skrzypek et al. (2017) The Candida Genome Database (CGD): incorporation of Assembly 22, systematic identifiers and visualization of high throughput sequencing data. *Nucleic Acids Res* **45**: D592-D596.
Spitzer et al. (2011) Cross-species discovery of syncretic drug combinations that potentiate the antifungal fluconazole. *Mol Syst Biol* **7**: 499.

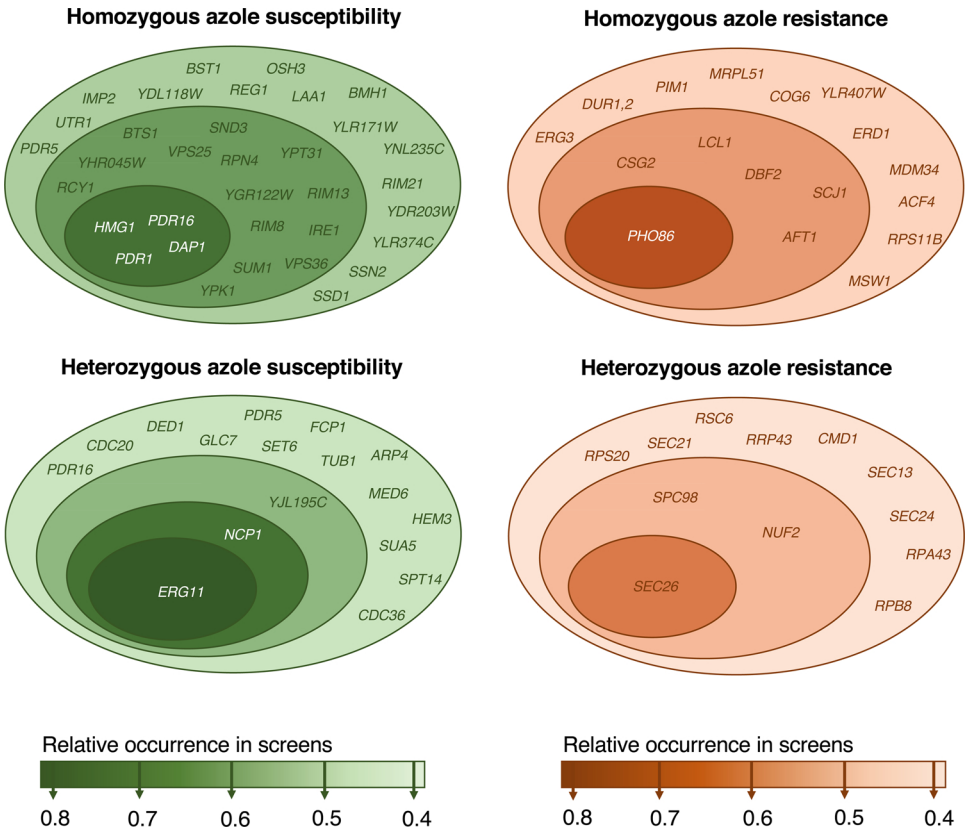

Fig. 1. Schematic overview of genes recovered in azole susceptibility and resistance screenings. Both results from HOP and HIP analyses are depicted, with genes involved in azole resistance and susceptibility. The genes for which the deletion or mutant strains show reduced resistance to the azole are depicted in green, the genes for which the deletion or mutant strains show improved resistance to the azole are depicted in orange. The colour scale at the bottom represents the percentage of screens the particular genes are identified in, ranging from 40 to 80%. Genes isolated in under 40% of the screenings are not depicted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

deletion strain. This allows for parallel analysis of many strains in competition assays, which is applied frequently in pharmacogenomics (Pierce et al., 2007, Delneri, 2010). The potential of this technique to find the target of a drug or factors involved in the mode-of-action has been illustrated for clotrimazole and confirmed for fluconazole (Hillenmeyer et al., 2008; Smith et al., 2009; Robbins et al., 2015). Chemical-genetic profiles of synergistic drug combinations with fluconazole were established, using this technique as well (Spitzer et al., 2011). Instead of using large collections of deletion mutants, one can also perform mutagenesis to examine the role of genes in processes related to azole susceptibility (Kontoyiannis, 1999; Hallstrom and Moye-Rowley, 2000).

Observing the effect of gene overexpression on fitness in the presence of an azole drug can also be applied to study interacting factors. Overexpression collections have been generated, using either a strong promoter, such as the *ScGAL1* promoter, or by using multicopy plasmids (Boone et al., 2007). By overexpressing a cDNA library, it was initially shown that overexpression of *ScERG11* in *S. cerevisiae* confers resistance to fluconazole (Kontoyiannis, 1999; Fiori and Van Dijck, 2012). In earlier work and using a similar multicopy overexpression collection, we found *Sc*Mge1 to be a suppressor of fluconazole susceptibility, leading to the discovery of Fe-S cluster and iron regulon involvement in this process. We were able to show that this correlation also applies to the pathogenic fungi, *C. albicans* and *C. glabrata* (Demuyser et al., 2017). The overlap between the results of several screening reports proves the validity of the setup. Slight differences in strain background and experimental conditions allow for the discovery of alternate processes involved in the phenotype.

Genetic screening of deletion or overexpression collections allows for identification of molecular drug targets. Yet, apart from identifying the major drug target, they also enable identification of off-targets. These are molecules not directly linked to the target pathway, but also represent part of the mode-of-action of the drug under study. Screening collections of heterozygous deletion mutants typically yields information on such off-targets. As several research groups already performed screenings of *S. cerevisiae* mutant collections in the presence of azole antifungals, we attempted to generate an overview of genes that are most often identified as regulators of azole resistance or susceptibility. We selected five azole drugs, fluconazole, miconazole, itraconazole, ketoconazole and clotrimazole, based on the available data. The reference databases used in this study are depicted in Table 2. Fig. 1 schematically shows the genes that were recovered in a minimal number of screening setups (Kontoyiannis, 1999; Anderson et al., 2003; Giaever et al., 2004; Lum et al., 2004; Spitzer et al., 2011; Hoepfner et al., 2014; Lee et al., 2014). While known determinants of azole resistance were clearly recovered, such as *Sc*Erg11, *Sc*Pdr5 and *Sc*Erg3, also potential off-targets are shown. Performing meta-analysis of these widely-available datasets thus proofs to be an efficient method to search for unknown regulators of drug resistance.

Finally, *S. cerevisiae* gene collections have been generated where the translated protein is modified with some type of tag. To study protein-protein interactions, protein arrays were generated for yeast two-hybrid analysis (Uetz et al., 2000). This platform has been applied to uncover the interaction of the primary regulatory protein of multidrug resistance, *Sc*Pdr1, and chromatin modifying factors (Martens et al., 1996). Further, collections were generated where each ORF is linked to a sequence encoding a tag to allow easy purification, such as a tandem affinity purification (TAP) tag or a glutathione S-transferase polyhistidine (GST-HisX6) tag (Zhu et al., 2001; Ghaemmaghami et al., 2003). The latter method was used recently to elucidate that azole resistance conferred by *Sc*Pdr16 is dependent on the protein's lipid binding properties (Holic et al., 2014). Systematic analysis of protein localization can be achieved by using the green fluorescence protein (GFP) tagged *S. cerevisiae* collection (Huh et al., 2003). The data can be accessed from the YeastGFP website (https://yeastgfp.yeastgenome.org). It needs to be pointed out here, however, that verification of localization is necessary when of particular interest, as it has been shown that reported information is not always reliable (Demuyser et al., 2017).

S. cerevisiae as a heterologous expression system

S. cerevisiae has been used as a system for heterologous expression of genes for many years, both for large scale production of proteins and for research purposes (Mokdad-Gargouri et al., 2012). Especially, the well-established genetic tools and consequent simplicity of experimentation allow for straightforward heterologous expression in this organism. Researchers have used the system for validation of the role of genes from alternative organisms in azole susceptibility. As such, it was shown that the wheat pathogen *Mycosphaerella graminicola* CYP51 protein complements the function of its orthologue in *S. cerevisiae* (Cools et al., 2010). They, furthermore, provide evidence that a mutation in the *MgCYP51* gene, is causal of the decreased azole susceptibility in the *M. graminicola* population. Likewise, it was shown in *Aspergillus fumigatus* that both the *Af*Erg11A and *Af*Erg11B isoenzymes functionally complement *S. cerevisiae* Erg11, yet *Af*Erg11A is more important for fluconazole resistance compared to *Af*Erg11B (Martel et al., 2010). The system of heterologous expression in *S. cerevisiae* is also used frequently to verify the effect of *ERG11* mutations in azole susceptibility (Lamb et al., 1997). A strategy to assess the role of target altering mutations in azole resistance in clinical *C. albicans* isolates has been set up by Sanglard and coworkers (Sanglard et al., 1998). The *CaERG11* genes from several isolates were amplified and cloned in a galactose-inducible yeast expression plasmid and transformed in the YKKB-13 *S. cerevisiae* strain, lacking the major multidrug efflux gene *ScPDR5*, as such yielding a more susceptible strain. The endogenous *ScERG11* gene was disrupted to reduce the background signal. Assessing the azole susceptibility by disk diffusion assays or an alternative method, generates information on the affinity of the Erg11 enzyme to the azole tested and thus, the clinical relevance of the allele under study. This strategy has since been used extensively to correlate *ERG11* mutations from clinical isolates from different pathogenic species to phenotypes (Favre et al., 1999; Cools et al., 2010; Alcazar-Fuoli et al., 2011; Xiang et al., 2013).

Apart from Erg11 functionality testing, also other genes can be expressed in *S. cerevisiae* to determine their role in drug susceptibility. A *C. albicans* genomic library can be constructed and integrated in *S. cerevisiae* plasmids. Transformation of these plasmids in a hypersusceptible *S. cerevisiae* strain and screening of these clones for increased drug resistance, can yield information on additional factors involved in the regulation of this process in the organism under study. Using such a genomic assay, the *CaERG6* gene was isolated by complementing the concomitant *S. cerevisiae* deletion strain and monitoring of ergosterol synthesis (Jensen-Pergakes et al., 1998). By expressing the *C. albicans* genome in a *Scpdr5*Δ strain, researchers identified the major *C. albicans* multidrug transporters, *Ca*Cdr1 and *Ca*Cdr2 (Prasad et al., 1995; Sanglard et al., 1997). In 1997, Sanglard and co-workers isolated *CaCDR2*, encoding a major *C. albicans* drug efflux pump (Sanglard et al., 1997). Similarly, the multidrug efflux transporters *Ca*Flu1 and *Ca*Mdr1 were identified (Fling et al., 1991; Calabrese et al., 2000). Other labs used this strategy to identify additional factors involved in azole susceptibility. Screening a *C. albicans* genomic DNA library for complementation of a *S. cerevisiae Scpdr1*Δ *Scpdr3*Δ mutant strain yielded three genes, *CaFCR1*, *CaFCR2* and *CaFCR3*. The *CaFCR1* gene was shown to encode a protein containing a zinc finger motif, as found in *ScPDR1* and *ScPDR3* (Talibi and Raymond, 1999). *CaFCR3* was shown to encode a protein containing a basic leucine zipper domain, as encountered in the *ScYAP3* gene (Yang et al., 2001). Likewise, the proteins *Ca*Cta4, *Ca*Asg1 and *Ca*Ctf1 were identified as transcription factors regulating drug resistance by functional complementation of a *S. cerevisiae* strain lacking *ScPDR1* and *ScPDR3* (Coste et al., 2008). One can also opt to search for factors influencing the known drug efflux pump encoding genes by monitoring expression of a reporter gene, such as *lacZ* placed behind the drug efflux gene promoter. *CaNdt80* was discovered to be positively involved in resistance by regulating *CaCDR1*, using this setup (Chen et al., 2004a, 2004b, 2004c).

However, despite the homology between several of these *C. albicans* and *S. cerevisiae* transcription factors, their characteristics do not always seem to overlap (Coste et al., 2008). It thus appears that some aspects of drug susceptibility regulation underwent significant rewiring. This divergence is nicely illustrated by Mount and co-workers. Using a genomic screening in *S. cerevisiae*, they identified nine determinants of azole susceptibility, while only two of these seemed to play a role in *C. albicans* (Mount et al., 2018). Apart from regulation of drug susceptibility and cellular response to stress, other processes also underwent rewiring events, such as nutrient metabolism (Cowen et al., 2006; Sarda and Hannenhalli, 2015; Dalal et al., 2016; Tebung et al., 2016).

Further optimization of the heterologous drug efflux expression strategy by the lab of Richard Cannon lead to the establishment of the yeast membrane protein hyperexpression system (Niimi et al., 2005; Lamping and Cannon, 2010). The gene encoding a (putative) drug efflux pump is cloned in a plasmid containing the strong *ScPDR5* promoter, a multiple cloning site, a *ScPGK1* terminator, a marker gene and the 3′ end of the *ScPDR5* ORF for homologous recombination. The transformation cassette obtained by restriction digest is transformed in a *S. cerevisiae* strain lacking seven major ABC drug efflux transporters and the *ScPDR3* transcriptional regulator gene, rendering the strain more sensitive to drugs. In addition, the *ScPDR1* gene contains a gain-of-function mutation allowing for strong expression from the *ScPDR5* promoter. Using this approach, efflux pumps from various fungal pathogens can be structurally and functionally analysed (Nakamura et al., 2001; Shukla et al., 2003; Lamping et al., 2007; Basso et al., 2015; Panapruksachat et al., 2016; Sanglard and Coste, 2016; Watanasrisin et al., 2016; Lamping et al., 2017). One can compare the MIC values of the strains, the substrate specificity of the pumps and their efflux activity by rhodamine 6 G assays. Tthe role of post-translational modifications of the pumps, such as phosphorylation, in efflux activity has been determined, using this setup (Wada et al., 2002, 2005).

Another promising application of heterologous expression of drug efflux pumps in *S. cerevisiae*, are chemosensitization assays. Compounds that inhibit drug efflux could be used in combination therapy, potentiating the existing yet limitedly efficient azole drugs (Maesaki et al., 1998; Lamping et al., 2007). The inhibitory effect of established drugs, such as the immunosuppressants FK506 and tacrolimus, the antifungal drug terbinafine, the anthelminthic milbemycin and the anticancer drug curcumin, on *Ca*CDR-mediated efflux has successfully been established using the above-mentioned platform (Maesaki et al., 1998; Schuetzer-Muehlbauer et al., 2003; Lamping et al., 2007; Sharma et al., 2009). One can, however, also screen compound libraries for inhibition of drug efflux. Niimi et al. screened a 1.8-million-member collection of surface-active D-octapeptides for increased susceptibility against fluconazole of the *S. cerevisiae* expressing *CaCDR1* strain and identified the compound RC21v3 as a potent inhibitor of this drug efflux pump (Niimi et al., 2004, 2012). It was shown that potentiating fluconazole with this compound is effective in eliminating azole-resistant oral candidiasis (Hayama et al., 2012). Screening a library of 1200 marketed drugs in a similar system, Holmes et al. identified the monoamine oxidase A inhibitor clorgyline as an inhibitor of both *Ca*Cdr1 and *Ca*Cdr2. Synergy with multiple azoles was established against resistant *C. albicans* and *C. glabrata* isolates (Holmes et al., 2012). It was shown recently, however, that the compound also decreases susceptibility to polyenes and echinocandins (Nagayoshi et al., 2017). Multiple other assays have been carried out using different target efflux pumps or alternative compound collections (Li et al., 2006; Keniya et al., 2015). Even targets not directly related to drug efflux yet promising in combinatorial therapy with azoles have been screened using the established platforms (Tebbets et al., 2012; Keniya et al., 2013).

Finally, other screening setups in pharmacology can also benefit from heterologous expression in *S. cerevisiae*. One can verify that a novel drug is not a substrate for efflux in the targeted fungal species, as such avoiding efflux-mediated resistance (Holmes et al., 2016). Secondly, off-target effects of a novel drug can be identified early on using heterologous expressed human genes in *S. cerevisiae*. Parker et al.

generated a humanized yeast strain, containing the *HsERG11* gene, to allow screening of off-target effects of novel drugs targeting Erg11 (Parker et al., 2008). This strategy allows for early identification of non-specific compounds, potentially causing severe side-effects.

S. cerevisiae as a hub of information on sterol metabolism and cellular signalling

Apart from the major tools designed specifically for *S. cerevisiae*, one can also take advantage of the wealth of knowledge available for this model organism. Several factors have been implicated in fungal azole susceptibility, such as sterol metabolism, drug efflux and mitochondrial signalling. For each of these factors, significantly more is known in *S. cerevisiae* compared to any other fungus.

Sterol metabolism is particularly well-studied in *S. cerevisiae*, as reviewed in (Lv et al., 2016), since it is not only involved in resistance against azole antifungals, but also in fungal generation of products of high economic value, such as provitamin D_2 (Ma et al., 2018). Recently, a potential role of ergosterol was established in generation of anticancer drugs (Subbiah and Abplanalp, 2003). Optimization of ergosterol production through metabolic engineering is performed frequently (Nahlik et al., 2017). Genetic engineering altering fluxes through the sterol biosynthesis pathway has been applied to increase the thermotolerance of ethanol production in *S. cerevisiae* (Caspeta et al., 2014). As *S. cerevisiae* is also used as a model system for higher eukaryotes, sterol metabolism has been subject of investigation in disease models, such as Niemann-Pick disease, a cholesterol storage disorder (Reiner et al., 2005). The high interest in the sterol synthesis pathway in various research fields, has boosted the knowledge we have on this process in yeast compared to other fungi (Lv et al., 2016). Historically, almost all genes encoding enzymes involved in sterol biosynthesis were discovered first in *S. cerevisiae*, creating a head start with respect to other fungi (Karst and Lacroute, 1977; Yoshida and Aoyama, 1987; Lees et al., 1995). Based on homology, orthologues in other organisms were found. Table 1 shows an overview of the *S. cerevisiae* proteins involved in ergosterol synthesis and regulation. It can be observed that the degree of homology between the proteins involved in these processes and their respective orthologues in *C. glabrata* or *C. albicans* is relatively high compared to proteins involved in other processes, thus deeming it relevant to use *S. cerevisiae* as a model for this particular cellular process. For the *ERG11* gene, encoding the lanosterol-14α-demethylase, the *C. albicans* orthologue was found using the *ScERG11* sequence as a hybridization probe against a library of *C. albicans* nucleotide sequences (Kirsch et al., 1988). It was confirmed that the hit gene encoded a cytochrome P450 enzyme and conferred resistance against imidazole drugs. Other ergosterol biosynthesis genes were annotated based on homology with the *S. cerevisiae* genome as well and their role in sterol synthesis was confirmed by heterologous expression in the model organism, deleted for the respective gene (Roessner et al., 1993, Jensen-Pergakes et al., 1998; Miyazaki et al., 1999; Kennedy et al., 2000; Aaron et al., 2001; Jia et al., 2002; Pierson et al., 2004). Further, structural analysis of lanosterol-14α-demethylase has been pioneered in *S. cerevisiae* (Lewis et al., 1999; Monk et al., 2014). The Monk lab has established crystal structures of the enzyme complexed with its substrate or azole antifungals (Monk et al., 2014; Sagatova et al., 2015; Tyndall et al., 2016). They also mapped the interactions between the azole drugs and the enzyme containing relevant mutations that render the drug less effective (Sagatova et al., 2018). Recently, they published the Erg11 crystal structures of other species, such as *C. albicans* and *C. glabrata* (Caramalho et al., 2017; Keniya et al., 2018). Finally, factors interacting up- and downstream with sterol metabolism are studied more intensely in *S. cerevisiae*. It was shown that mutants affected in sterol metabolism are more sensitive to stress factors, exhibit a significantly increased doubling time and are more susceptible to a wide array of drugs, possibly due to the reduced *Sc*Pdr5 drug efflux activity (Kodedova and Sychrova, 2015; Bhattacharya et al., 2018). Most recently, other researchers identified ergosterol as a trigger of macrophage pyroptosis, or inflammatory, programmed cell death in an initial phase of the innate immune response (Koselny et al., 2018). These newly-established aspects of sterol metabolism acknowledge its important status as a drug target and support therapy strategies that aim to potentiate azole antifungals. As nicely reviewed in (Hu et al., 2017), sterol metabolism is regulated by many environmental factors, such as presence of iron, ethanol and oxygen, and a multitude of biosynthesis enzymes. Recent advances in this field include the elucidation of the importance and regulation of sterol transport in the cell (Roelants et al., 2018; Tian et al., 2018).

Drug efflux in *S. cerevisiae* is mainly achieved by *Sc*Pdr5, the most-known member of the Pdr5 subfamily of asymmetric ATP-binding cassette (ABC) transporters (Golin and Ambudkar, 2015). Although this subfamily only exists in fungi and moulds, various aspects related to function and structure of *Sc*Pdr5 can also be applied to mammalian asymmetric ABC transporters, many of which are of medical importance (Dean et al., 2001). Examples are the *Hs*CFTR protein, a chloride channel that when mutated can cause cystic fibrosis, and the *Hs*Tap1 transporter that is involved in specific types of diabetes (Jackson and Capra, 1993; Quinton, 1999). Furthermore, several of the *S. cerevisiae* specific tools described above have been used to study anticancer drugs, since resistance to chemotherapy is often caused by induction of multidrug transporters, such as *Hs*Mdr1 and *Hs*ABCB5, that resemble *Sc*Pdr5 significantly (Porcu et al., 2010; Keniya et al., 2014; Demir and Koc, 2015). Due to their clinical importance in the above-mentioned diseases as well as in fungal pathogenesis, ABC transporters, with *Sc*Pdr5 as model molecule, have been studied quite extensively. Several articles report on the link between specific point mutations, or regions, and transport function, thereby increasing understanding of the structure-function relationship of ABC proteins (Chen et al., 2004a, 2004b, 2004c, Gupta et al., 2014; Dou et al., 2016). Another way to support our understanding of the function and mode-of-action of ABC transporters, is the membrane yeast two-hybrid or MYTH technology. In this setup *S. cerevisiae* is used to heterologously or endogenously (in this case, iMYTH) express an ABC transporter and a split-ubiquitin system is employed to detect interaction between the transporter and prey proteins (Snider et al., 2010). Interactomes of several ABC transporters have been established using this technique (Paumi et al., 2009; Snider et al., 2013). Other efforts have been made as well to uncover *Sc*Pdr5 involved signal transduction in *S. cerevisiae* and investigate it as a potential drug target (Conseil et al., 2003; Hiraga et al., 2005; Yamamoto et al., 2005; Lotti et al., 2012; Nishida et al., 2014; Rahman et al., 2018). Table 1 shows an overview of the *S. cerevisiae* proteins involved in drug efflux and regulation thereof. Although the degree of homology between the proteins involved in the actual efflux and their respective orthologues in *C. glabrata* or *C. albicans* seems average compared to other processes, regulation of drug efflux is clearly not conserved to a large extent. It can thus be noted that usage of *S. cerevisiae* for the study of drug efflux regulation in *Candida* species is limited.

Several studies have shown that the mitochondria are important regulators of drug susceptibility in fungi (Shingu-Vazquez and Traven, 2011). In some species, such as *S. cerevisiae* and *C. glabrata*, loss of the mitochondrial DNA and concomitant mitochondrial dysfunction is viable, while in *C. albicans* it is not. This hampers investigation of mitochondrial functioning and drug susceptibility in the latter. In *S. cerevisiae*, complete or partial loss of the mitochondrial DNA, generating rho° or rho$^-$ cells, respectively, is viable and increases resistance to azole antifungals (Traven et al., 2001). It has been shown that this process is mediated by *Sc*Pdr5, an ABC multidrug transporter. Although the exact mechanism is not known, it is presumed that enzymes involved in mitochondrial signalling towards the nucleus, such as the transcription factor *Sc*Rtg1, are important in increasing expression of *ScPDR5* via the transcription factor *Sc*Pdr3 (Hallstrom and Moye-Rowley, 2000; Mutlu et al., 2014). It was shown as well, that *Sc*Erg3

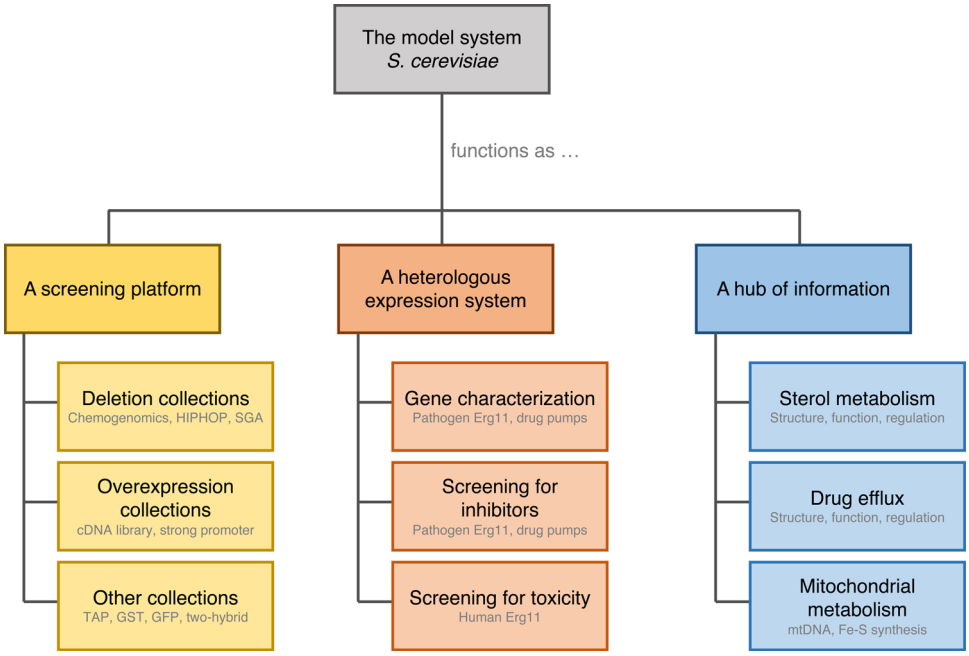

Fig. 2. Schematic overview of the fields and subfields wherein *S. cerevisiae* can be of particular use as a model organism for research on azole susceptibility.

plays an important role in rho° or rho$^-$ mediated azole resistance since an *Scerg3* rho$^-$ mutant strain appears susceptible to the drug (Kontoyiannis, 2000). The same phenomenon of rho°-induced drug resistance has been reported for *C. glabrata*. Increased efflux through *Cg*Cdr1 and *Cg*Cdr2, two efflux pumps of the ABC family, has been observed in rho° cells (Brun et al., 2004). Although rarely witnessed, clinically-resistant *C. glabrata* rho° isolates have been reported, with the parental susceptible isolate being rho$^+$. Remarkably, these resistant isolates showed increased virulence as well (Ferrari et al., 2011). Other fungal pathogens such as *C. albicans*, *Cryptococcus neoformans* and *A. fumigatus* are petite-negative, indicating that loss of mitochondrial DNA is lethal. A *C. albicans* strain with uncoupled oxidative phosphorylation was reported to exhibit upregulation of *CaMDR1* expression, encoding a multidrug efflux pump, indicating that mitochondrial dysfunction can also play a role in drug susceptibility in this petite-negative yeast (Cheng et al., 2007). Apart from the effect of mitochondrial DNA loss on drug efflux in *S. cerevisiae* and *C. glabrata*, changes in membrane lipid composition have also been suggested to contribute to the resistance phenotype (Shingu-Vazquez and Traven, 2011). For *C. albicans*, azole-resistant isolates have been described where the lipid composition of the mitochondrial membrane was clearly altered (Singh et al., 2012). Recently, using *S. cerevisiae* as a model system, we identified *Sc*Mge1, a cochaperone involved in Fe-S cluster metabolism and protein import into the mitochondria, as a multicopy suppressor of fluconazole susceptibility (Demuyser et al., 2017). We showed, however, that neither loss of the mitochondrial DNA nor drug efflux through *Sc*Pdr5 can solely account for the increased growth of the *ScMGE1* overexpression strain on fluconazole. *Sc*Mge1 positively affects the level of ergosterol remaining after fluconazole treatment and negatively affects the production of toxic sterols. Additionally, we found that activation of the iron regulon is necessary, yet not sufficient, for *Sc*Mge1 to lower susceptibility to fluconazole. A link between Fe-S cluster metabolism and this iron regulon had been suggested, as a yet-unknown Fe-S cluster-containing factor negatively regulates transcription of the genes belonging to the iron regulon thereby signalling the sufficiency of iron in the cell or mitochondria (Chen et al., 2004a, 2004b, 2004c). Interestingly, we have established a similar role for Mge1 in drug susceptibility in the pathogenic fungi *C. glabrata* and *C. albicans*. Most recently, another study showed a relation between sterol metabolism and mitochondrial Fe-S cluster synthesis (Ward et al., 2018). Table 1 shows an overview of the *S. cerevisiae* proteins involved in Fe-S cluster formation as well as other mitochondrial processes and regulation thereof. The degree of homology between the proteins involved in these processes and their respective orthologues in *C. glabrata* or *C. albicans* seems average compared to other processes. A relation between mitochondrial functioning and azole resistance has also been established in *A. fumigatus*, where mutations in the genes encoding the subunits of the mitochondrial complex I caused resistance to azole drugs (Bromley et al., 2016). Interestingly, depending on the precise mechanism and conditions, mitochondrial dysfunction can lead to azole susceptibility as well as resistance. For instance, clinically-isolated *C. glabrata* strains affected in aerobic growth were hypersusceptible to azoles, while the strains co-isolated that retained complete mitochondrial functioning, were resistant (Vandeputte et al., 2009).

Balancing the benefits and shortcomings

Although the benefits of using *S. cerevisiae* as a model system to study fungal azole resistance are numerous, certain shortcomings and limitations need to be addressed and accounted for as well. First, in the era of high-throughput sequencing, genomes of most important human fungal pathogens are known (Denning et al., 2002; Dujon et al., 2004; Jones et al., 2004; Janbon et al., 2014). This allows for genomic engineering and more targeted functional annotation. The ease of sequencing also allows for fast characterization of clinical isolates and determination of virulence or drug resistance related mutations (Bougnoux et al., 2002; Abdolrasouli et al., 2015). Apart from genomics, also transcriptomics, proteomics and metabolomics techniques have been applied to fungal pathogens, frequently in relation to azole susceptibility (Rogers et al., 2006, Hoehamer et al., 2010, Sun et al., 2013; Katragkou et al., 2016; Li et al., 2018; Salazar et al., 2018). The availability of genome-wide mutant collections in *S. cerevisiae* allows for determination of the mode-of-action of a drug or the profiling of genetic interactions. Many initiatives aim to generate similar collections for pathogenic fungi (Roemer et al., 2003; Liu et al., 2008; Noble et al., 2010; Schwarzmuller et al., 2014). Although these tools have been used to understand azole susceptibility, the limited genome coverage of these collections prevent complete replacement of the *S. cerevisiae* counterparts (Schwarzmuller et al., 2014; Mount et al., 2018). A great effort that has recently been made in this respect, was the

generation of the *C. albicans* ORFeome collection encompassing more than 5000 ORFs (Legrand et al., 2018). This collection in combination with a versatile series of destination vectors, containing optional promoters, tags and markers, will allow for in-depth exploration of the organism's ORFeome. Our lab contributed to this endeavour by setting up a two-hybrid approach as a proof-of-concept application. Apart from the establishment of mutant collections, also many state-of-the-art techniques have been developed for specific use in pathogenic fungi. The most important evolution in genomic engineering of the last years is the introduction of CRISPR-Cas9 in several research fields. This technique allows fast and precise alteration of genetic sequences and has been optimized for use in a plethora of fungal pathogens, such as *C. albicans*, *C. glabrata*, *A. fumigatus* and *C. neoformans* (Fuller et al., 2015; Vyas et al., 2015; Arras et al., 2016; Enkler et al., 2016). Apart from the *Candida*-two-hybrid approach introduced above, we have also optimized a bimolecular fluorescence complementation (BiFC) setup for detection of protein-protein interactions in *C. albicans* (Subotić et al., 2017). Finally, we recently published the optimization and use of two fluorescence resonance energy transfer (FRET)-based biosensors for *in vivo* determination of protein kinase A (PKA) signalling in *C. glabrata* (Demuyser et al., 2018). The specific optimization of collections and techniques for pathogenic fungi diminishes the need for a model organism.

A shortcoming of *S. cerevisiae* as a model organism one needs to bear in mind, is its relatively limited resemblance to pathogenic fungi. As this organism is generally not pathogenic, at least not to a similar extent as for instance *C. albicans* or *C. glabrata*, essential processes related to growth or virulence are not necessarily shared between them. A simple deduction of similarity is thus not always as straightforward. Apart from virulence, also certain aspects of azole susceptibility are not shared between these pathogens and *S. cerevisiae*, as is illustrated in Table 1. As mentioned before, *C. albicans* and *C. neoformans* are petite-negative and cannot survive without their mitochondrial DNA nor show the accompanying azole resistance. Mount et al. also concluded from a comparison of a synthetic genetic analysis in *S. cerevisiae* with a *C. albicans* homozygous deletion mutant screen, that there is significant divergence in genes involved in azole resistance between both organisms, thereby limiting the relevance of using *S. cerevisiae* as a model for this purpose (Mount et al., 2018). Further, the target of the azoles, Erg11, is essential in *S. cerevisiae*, yet not necessary for viability in *C. albicans*, indicating another important point of divergence between both organisms (Bard et al., 1993). One can appreciate that the majority of dissimilarities between the model organism and pathogenic fungi are yet to be uncovered. It is, thus, of uttermost importance that findings in *S. cerevisiae* are verified in the organism-of-interest in an early stadium of research or drug development.

Finally, it can be discussed whether we want to develop new antifungal drugs that also act against non-pathogenic fungi, such as *S. cerevisiae*. Over the last few years, the role of the mycobiome in health and disease is appreciated more. As several reports link the presence of certain pathogenic fungi with gut diseases such as Crohn's disease, diarrhoea and pouchitis, and the presence of *S. cerevisiae* with a healthy gut status, the specificity of antifungal drugs appears an important feature to be considered during drug development (Kuhbacher et al., 2006; Hoarau et al., 2016; Sangster et al., 2016; Sokol et al., 2017). Wouldn't it, thus, be better if we would search for antifungal drug targets that are specific to a certain pathogen instead of general to all fungi? An approach that is suggested to do exactly that, is targeting virulence instead of viability. Virulence factors, such as the yeast-to-hyphae transition in *C. albicans*, adherence in *C. glabrata*, melanin production in *C. neoformans* or biofilm formation can be inhibited without disturbing growth and creating selective pressure for drug resistance (Gauwerky et al., 2009). The major disadvantage of such a strategy is its largely preventive instead of curative potential, yet preventing an outbreak may be enough for the immune system or other members of the microbiota to take over again.

Conclusions

In this review, we addressed the question whether *S. cerevisiae* can keep up as a model organism for studying azole susceptibility in pathogenic fungi. We conclude that this organism can indeed still be play a significant role in research related to various human fungal pathogens, especially in terms of high-throughput mutant screening or heterologous expression (Fig. 2). The enormous amount of knowledge available concerning sterol metabolism, drug efflux or mitochondrial metabolism will play a major role in advancing drug development in the following years. Special caution is needed, however, when inferring conclusions made in *S. cerevisiae* to other fungi. We recommend to test findings obtained with the model system, as soon as possible in the organism under study. *S. cerevisiae* should be seen as a catalyser of antifungal research, where it can speed up research instead of taking actual part in it.

Acknowledgment

FWO Research Community on Biofilms - Flanders-Belgium (grant number WO.009.16N).

References

Aaron, K.E., Pierson, C.A., Lees, N.D., Bard, M., 2001. The *Candida albicans ERG26* gene encoding the C-3 sterol dehydrogenase (C-4 decarboxylase) is essential for growth. FEMS Yeast Res. 1, 93–101.

Abdolrasouli, A., Rhodes, J., Beale, M.A., Hagen, F., Rogers, T.R., Chowdhary, A., Meis, J.F., Armstrong-James, D., Fisher, M.C., 2015. Genomic context of azole resistance mutations in *Aspergillus fumigatus* determined using whole-genome sequencing. MBio 6, e00536.

Alcazar-Fuoli, L., Mellado, E., Cuenca-Estrella, M., Sanglard, D., 2011. Probing the role of point mutations in the *cyp51A* gene from *Aspergillus fumigatus* in the model yeast *Saccharomyces cerevisiae*. Med. Mycol. 49, 276–284.

Anderson, J.B., Sirjusingh, C., Parsons, A.B., Boone, C., Wickens, C., Cowen, L.E., Kohn, L.M., 2003. Mode of selection and experimental evolution of antifungal drug resistance in *Saccharomyces cerevisiae*. Genetics 163, 1287–1298.

Arras, S.D., Chua, S.M., Wizrah, M.S., Faint, J.A., Yap, A.S., Fraser, J.A., 2016. Targeted genome editing via CRISPR in the pathogen *Cryptococcus neoformans*. PLoS One 11, e0164322.

Bard, M., Lees, N.D., Turi, T., Craft, D., Cofrin, L., Barbuch, R., Koegel, C., Loper, J.C., 1993. Sterol synthesis and viability of erg11 (cytochrome P450 lanosterol demethylase) mutations in Saccharomyces cerevisiae and Candida albicans. Lipids 28, 963–967.

Baryshnikova, A., Costanzo, M., Dixon, S., Vizeacoumar, F.J., Myers, C.L., Andrews, B., Boone, C., 2010. Synthetic genetic array (SGA) analysis in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Methods Enzymol. 470, 145–179.

Basso Jr., L.R., Gast, C.E., Bruzual, I., Wong, B., 2015. Identification and properties of plasma membrane azole efflux pumps from the pathogenic fungi *Cryptococcus gattii* and *Cryptococcus neoformans*. J. Antimicrob. Chemother. 70, 1396–1407.

Bhattacharya, S., Esquivel, B.D., White, T.C., 2018. Overexpression or deletion of ergosterol biosynthesis genes alters doubling time, response to stress agents, and drug susceptibility in *Saccharomyces cerevisiae*. MBio 9.

Bojsen, R.K., Andersen, K.S., Regenberg, B., 2012. *Saccharomyces cerevisiae*–a model to uncover molecular mechanisms for yeast biofilm biology. FEMS Immunol. Med. Microbiol. 65, 169–182.

Boone, C., Bussey, H., Andrews, B.J., 2007. Exploring genetic interactions and networks with yeast. Nat. Rev. Genet. 8, 437–449.

Botstein, D., Fink, G.R., 2011. Yeast: an experimental organism for 21st Century biology. Genetics 189, 695–704.

Bougnoux, M.E., Morand, S., d'Enfert, C., 2002. Usefulness of multilocus sequence typing for characterization of clinical isolates of *Candida albicans*. J. Clin. Microbiol. 40, 1290–1297.

Bromley, M., Johns, A., Davies, E., et al., 2016. Mitochondrial complex I is a global regulator of secondary metabolism, virulence and azole sensitivity in Fungi. PLoS One 11, e0158724.

Brun, S., Berges, T., Poupard, P., Vauzelle-Moreau, C., Renier, G., Chabasse, D., Bouchara, J.P., 2004. Mechanisms of azole resistance in petite mutants of Candida glabrata. Antimicrob. Agents Chemother. 48, 1788–1796.

Calabrese, D., Bille, J., Sanglard, D., 2000. A novel multidrug efflux transporter gene of the major facilitator superfamily from Candida albicans (FLU1) conferring resistance to fluconazole. Microbiology 146 (Pt 11), 2743–2754.

Caramalho, R., Tyndall, J.D.A., Monk, B.C., Larentis, T., Lass-Florl, C., Lackner, M., 2017. Intrinsic short-tailed azole resistance in mucormycetes is due to an evolutionary conserved aminoacid substitution of the lanosterol 14alpha-demethylase. Sci. Rep. 7, 15898.

Caspeta, L., Chen, Y., Ghiaci, P., Feizi, A., Buskov, S., Hallstrom, B.M., Petranovic, D., Nielsen, J., 2014. Biofuels. Altered sterol composition renders yeast thermotolerant.

Science 346, 75–78.
Chen, C.G., Yang, Y.L., Shih, H.I., Su, C.L., Lo, H.J., 2004a. CaNdt80 is involved in drug resistance in Candida albicans by regulating CDR1. Antimicrob. Agents Chemother. 48, 4505–4512.
Chen, O.S., Crisp, R.J., Valachovic, M., Bard, M., Winge, D.R., Kaplan, J., 2004b. Transcription of the yeast iron regulon does not respond directly to iron but rather to iron-sulfur cluster biosynthesis. J. Biol. Chem. 279, 29513–29518.
Chen, Z., Li, J., Wang, W., Guo, X., Li, Y., Mao, X., Chen, X., Guan, W., 2014c. Mutations adjacent to the end of transmembrane helices 6 and 7 independently affect drug efflux capacity of yeast ABC transporter Pdr5p. Biochim. Biophys. Acta 1838, 932–939.
Cheng, S., Clancy, C.J., Nguyen, K.T., Clapp, W., Nguyen, M.H., 2007. A Candida albicans petite mutant strain with uncoupled oxidative phosphorylation overexpresses MDR1 and has diminished susceptibility to fluconazole and voriconazole. Antimicrob. Agents Chemother. 51, 1855–1858.
Cherry, J.M., Hong, E.L., Amundsen, C., et al., 2012. Saccharomyces Genome Database: the genomics resource of budding yeast. Nucleic Acids Res. 40, D700–705.
Como, J.A., Dismukes, W.E., 1994. Oral azole drugs as systemic antifungal therapy. N. Engl. J. Med. 330, 263–272.
Conseil, G., Perez-Victoria, J.M., Renoir, J.M., Goffeau, A., Di Pietro, A., 2003. Potent competitive inhibition of drug binding to the Saccharomyces cerevisiae ABC exporter Pdr5p by the hydrophobic estradiol-derivative RU49953. Biochim. Biophys. Acta 1614, 131–134.
Cools, H.J., Parker, J.E., Kelly, D.E., Lucas, J.A., Fraaije, B.A., Kelly, S.L., 2010. Heterologous expression of mutated eburicol 14alpha-demethylase (CYP51) proteins of *Mycosphaerella graminicola* to assess effects on azole fungicide sensitivity and intrinsic protein function. Appl. Environ. Microbiol. 76, 2866–2872.
Coste, A.T., Ramsdale, M., Ischer, F., Sanglard, D., 2008. Divergent functions of three Candida albicans zinc-cluster transcription factors (CTA4, ASG1 and CTF1) complementing pleiotropic drug resistance in Saccharomyces cerevisiae. Microbiology 154, 1491–1501.
Cowen, L.E., Anderson, J.B., Kohn, L.M., 2002. Evolution of drug resistance in *Candida albicans*. Annu. Rev. Microbiol. 56, 139–165.
Cowen, L.E., Carpenter, A.E., Matangkasombut, O., Fink, G.R., Lindquist, S., 2006. Genetic architecture of Hsp90-dependent drug resistance. Eukaryot. Cell 5, 2184–2188.
Dalal, C.K., Zuleta, I.A., Mitchell, K.F., Andes, D.R., El-Samad, H., Johnson, A.D., 2016. Transcriptional rewiring over evolutionary timescales changes quantitative and qualitative properties of gene expression. Elife 5.
Dean, M., Rzhetsky, A., Allikmets, R., 2001. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res. 11, 1156–1166.
Delneri, D., 2010. Barcode technology in yeast: application to pharmacogenomics. FEMS Yeast Res. 10, 1083–1089.
Demir, A.B., Koc, A., 2015. High-copy overexpression screening reveals *PDR5* as the main doxorubicin resistance gene in yeast. PLoS One 10, e0145108.
Demuyser, L., Swinnen, E., Fiori, A., Herrera-Malaver, B., Vestrepen, K., Van Dijck, P., 2017. Mitochondrial cochaperone Mge1 is involved in regulating susceptibility to fluconazole in *Saccharomyces cerevisiae* and *Candida* species. MBio 8.
Demuyser, L., Van Genechten, W., Mizuno, H., Colombo, S., Van Dijck, P., 2018. Introducing fluorescence resonance energy transfer-based biosensors for the analysis of cAMP-PKA signalling in the fungal pathogen Candida glabrata. Cell. Microbiol., e12863.
Denning, D.W., Anderson, M.J., Turner, G., Latge, J.P., Bennett, J.W., 2002. Sequencing the *Aspergillus fumigatus* genome. Lancet Infect. Dis. 2, 251–253.
Dohmen, R.J., Varshavsky, A., 2005. Heat-inducible degron and the making of conditional mutants. Methods Enzymol. 399, 799–822.
Dou, W., Zhu, J., Wang, T., Wang, W., Li, H., Chen, X., Guan, W., 2016. Mutations of charged amino acids at the cytoplasmic end of transmembrane helix 2 affect transport activity of the budding yeast multidrug resistance protein Pdr5p. FEMS Yeast Res. 16.
Dujon, B., 1996. The yeast genome project: what did we learn? Trends Genet. 12, 263–270.
Dujon, B., Sherman, D., Fischer, G., et al., 2004. Genome evolution in yeasts. Nature 430, 35–44.
Enkler, L., Richer, D., Marchand, A.L., Ferrandon, D., Jossinet, F., 2016. Genome engineering in the yeast pathogen *Candida glabrata* using the CRISPR-Cas9 system. Sci. Rep. 6, 35766.
Favre, B., Didmon, M., Ryder, N.S., 1999. Multiple amino acid substitutions in lanosterol 14alpha-demethylase contribute to azole resistance in *Candida albicans*. Microbiology 145 (Pt 10), 2715–2725.
Ferrari, S., Sanguinetti, M., De Bernardis, F., Torelli, R., Posteraro, B., Vandeputte, P., Sanglard, D., 2011. Loss of mitochondrial functions associated with azole resistance in Candida glabrata results in enhanced virulence in mice. Antimicrob. Agents Chemother. 55, 1852–1860.
Fields, S., Song, O., 1989. A novel genetic system to detect protein-protein interactions. Nature 340, 245–246.
Fiori, A., Van Dijck, P., 2012. Potent synergistic effect of doxycycline with fluconazole against *Candida albicans* is mediated by interference with iron homeostasis. Antimicrob. Agents Chemother. 56, 3785–3796.
Fling, M.E., Kopf, J., Tamarkin, A., Gorman, J.A., Smith, H.A., Koltin, Y., 1991. Analysis of a Candida albicans gene that encodes a novel mechanism for resistance to benomyl and methotrexate. Mol. Gen. Genet. 227, 318–329.
Fuller, K.K., Chen, S., Loros, J.J., Dunlap, J.C., 2015. Development of the CRISPR/Cas9 system for targeted gene disruption in *Aspergillus fumigatus*. Eukaryot. Cell 14, 1073–1080.
Gauwerky, K., Borelli, C., Korting, H.C., 2009. Targeting virulence: a new paradigm for antifungals. Drug Discov. Today 14, 214–222.
Ghaemmaghami, S., Huh, W.K., Bower, K., Howson, R.W., Belle, A., Dephoure, N., O'Shea, E.K., Weissman, J.S., 2003. Global analysis of protein expression in yeast. Nature 425, 737–741.
Giaever, G., Nislow, C., 2014. The yeast deletion collection: a decade of functional genomics. Genetics 197, 451–465.
Giaever, G., Shoemaker, D.D., Jones, T.W., Liang, H., Winzeler, E.A., Astromoff, A., Davis, R.W., 1999. Genomic profiling of drug sensitivities via induced haploinsufficiency. Nat. Genet. 21, 278–283.
Giaever, G., Chu, A.M., Ni, L., et al., 2002. Functional profiling of the *Saccharomyces cerevisiae* genome. Nature 418, 387–391.
Giaever, G., Flaherty, P., Kumm, J., Proctor, M., Nislow, C., Jaramillo, D.F., Chu, A.M., Jordan, M.I., Arkin, A.P., Davis, R.W., 2004. Chemogenomic profiling: identifying the functional interactions of small molecules in yeast. Proc. Natl. Acad. Sci. U. S. A. 101, 793–798.
Goffeau, A., Barrell, B.G., Bussey, H., et al., 1996. Life with 6000 genes. Science 274 (546) 563-547.
Golin, J., Ambudkar, S.V., 2015. The multidrug transporter Pdr5 on the 25th anniversary of its discovery: an important model for the study of asymmetric ABC transporters. Biochem. J. 467, 353–363.
Gupta, R.P., Kueppers, P., Hanekop, N., Schmitt, L., 2014. Generating symmetry in the asymmetric ATP-binding cassette (ABC) transporter Pdr5 from Saccharomyces cerevisiae. J. Biol. Chem. 289, 15272–15279.
Hallstrom, T.C., Moye-Rowley, W.S., 2000. Multiple signals from dysfunctional mitochondria activate the pleiotropic drug resistance pathway in Saccharomyces cerevisiae. J. Biol. Chem. 275, 37347–37356.
Hayama, K., Ishibashi, H., Ishijima, S.A., et al., 2012. A D-octapeptide drug efflux pump inhibitor acts synergistically with azoles in a murine oral candidiasis infection model. FEMS Microbiol. Lett. 328, 130–137.
Hedges, S.B., Blair, J.E., Venturi, M.L., Shoe, J.L., 2004. A molecular timescale of eukaryote evolution and the rise of complex multicellular life. BMC Evol. Biol. 4, 2.
Hickman, M.A., Zeng, G., Forche, A., et al., 2013. The' obligate diploid' *Candida albicans* forms mating-competent haploids. Nature 494, 55–59.
Hillenmeyer, M.E., Fung, E., Wildenhain, J., et al., 2008. The chemical genomic portrait of yeast: uncovering a phenotype for all genes. Science 320, 362–365.
Hiraga, K., Yamamoto, S., Fukuda, H., Hamanaka, N., Oda, K., 2005. Enniatin has a new function as an inhibitor of Pdr5p, one of the ABC transporters in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. 328, 1119–1125.
Hoarau, G., Mukherjee, P.K., Gower-Rousseau, C., et al., 2016. Bacteriome and mycobiome interactions underscore microbial dysbiosis in familial crohn's disease. MBio 7.
Hoehamer, C.F., Cummings, E.D., Hilliard, G.M., Rogers, P.D., 2010. Changes in the proteome of *Candida albicans* in response to azole, polyene, and echinocandin antifungal agents. Antimicrob. Agents Chemother. 54, 1655–1664.
Hoepfner, D., Karkare, S., Helliwell, S.B., et al., 2012. An integrated approach for identification and target validation of antifungal compounds active against Erg11p. Antimicrob. Agents Chemother. 56, 4233–4240.
Hoepfner, D., Helliwell, S.B., Sadlish, H., et al., 2014. High-resolution chemical dissection of a model eukaryote reveals targets, pathways and gene functions. Microbiol. Res. 169, 107–120.
Holic, R., Simova, Z., Ashlin, T., Pevala, V., Poloncova, K., Tahotna, D., Kutejova, E., Cockcroft, S., Griac, P., 2014. Phosphatidylinositol binding of *Saccharomyces cerevisiae* Pdr16p represents an essential feature of this lipid transfer protein to provide protection against azole antifungals. Biochim. Biophys. Acta 1842, 1483–1490.
Holmes, A.R., Keniya, M.V., Ivnitski-Steele, I., Monk, B.C., Lamping, E., Sklar, L.A., Cannon, R.D., 2012. The monoamine oxidase A inhibitor clorgyline is a broad-spectrum inhibitor of fungal ABC and MFS transporter efflux pump activities which reverses the azole resistance of *Candida albicans* and *Candida glabrata* clinical isolates. Antimicrob. Agents Chemother. 56, 1508–1515.
Holmes, A.R., Cardno, T.S., Strouse, J.J., Ivnitski-Steele, I., Keniya, M.V., Lackovic, K., Monk, B.C., Sklar, L.A., Cannon, R.D., 2016. Targeting efflux pumps to overcome antifungal drug resistance. Future Med. Chem. 8, 1485–1501.
Hu, Z., He, B., Ma, L., Sun, Y., Niu, Y., Zeng, B., 2017. Recent advances in Ergosterol Biosynthesis and regulation mechanisms in Saccharomyces cerevisiae. Indian J. Microbiol. 57, 270–277.
Huh, W.K., Falvo, J.V., Gerke, L.C., Carroll, A.S., Howson, R.W., Weissman, J.S., O'Shea, E.K., 2003. Global analysis of protein localization in budding yeast. Nature 425, 686–691.
Jackson, D.G., Capra, J.D., 1993. *TAP1* alleles in insulin-dependent diabetes mellitus: a newly defined centromeric boundary of disease susceptibility. Proc. Natl. Acad. Sci. U. S. A. 90, 11079–11083.
Janbon, G., Ormerod, K.L., Paulet, D., et al., 2014. Analysis of the genome and transcriptome of *Cryptococcus neoformans* var. *grubii* reveals complex RNA expression and microevolution leading to virulence attenuation. PLoS Genet. 10, e1004261.
Jensen-Pergakes, K.L., Kennedy, M.A., Lees, N.D., Barbuch, R., Koegel, C., Bard, M., 1998. Sequencing, disruption, and characterization of the *Candida albicans* sterol methyltransferase (*ERG6*) gene: drug susceptibility studies in erg6 mutants. Antimicrob. Agents Chemother. 42, 1160–1167.
Jia, N., Arthington-Skaggs, B., Lee, W., Pierson, C.A., Lees, N.D., Eckstein, J., Barbuch, R., Bard, M., 2002. Candida albicans sterol C-14 reductase, encoded by the ERG24 gene, as a potential antifungal target site. Antimicrob. Agents Chemother. 46, 947–957.
Jones, T., Federspiel, N.A., Chibana, H., et al., 2004. The diploid genome sequence of *Candida albicans*. Proc. Natl. Acad. Sci. U. S. A. 101, 7329–7334.
Karst, F., Lacroute, F., 1977. Ertosterol biosynthesis in *Saccharomyces cerevisiae:* mutants deficient in the early steps of the pathway. Mol. Gen. Genet. 154, 269–277.
Katragkou, A., Alexander, E.L., Eoh, H., Raheem, S.K., Roilides, E., Walsh, T.J., 2016. Effects of fluconazole on the metabolomic profile of *Candida albicans*. J. Antimicrob. Chemother. 71, 635–640.

Kelly, S.L., Arnoldi, A., Kelly, D.E., 1993. Molecular genetic analysis of azole antifungal mode of action. Biochem. Soc. Trans. 21, 1034–1038.
Kelly, S.L., Lamb, D.C., Corran, A.J., Baldwin, B.C., Kelly, D.E., 1995. Mode of action and resistance to azole antifungals associated with the formation of 14 alpha-methylergosta-8,24(28)-dien-3 beta,6 alpha-diol. Biochem. Biophys. Res. Commun. 207, 910–915.
Keniya, M.V., Cannon, R.D., Nguyen, A., Tyndall, J.D., Monk, B.C., 2013. Heterologous expression of *Candida albicans* Pma1p in *Saccharomyces cerevisiae*. FEMS Yeast Res. 13, 302–311.
Keniya, M.V., Holmes, A.R., Niimi, M., Lamping, E., Gillet, J.P., Gottesman, M.M., Cannon, R.D., 2014. Drug resistance is conferred on the model yeast *Saccharomyces cerevisiae* by expression of full-length melanoma-associated human ATP-binding cassette transporter ABCB5. Mol. Pharm. 11, 3452–3462.
Keniya, M.V., Fleischer, E., Klinger, A., Cannon, R.D., Monk, B.C., 2015. Inhibitors of the Candida albicans major facilitator superfamily transporter Mdr1p responsible for fluconazole resistance. PLoS One 10, e0126350.
Keniya, M.V., Sabherwal, M., Wilson, R.K., Woods, M.A., Sagatova, A.A., Tyndall, J.D.A., Monk, B.C., 2018. Crystal structures of full-length lanosterol 14alpha-demethylases of prominent fungal pathogens *Candida albicans* and *Candida glabrata* provide tools for antifungal discovery. Antimicrob. Agents Chemother.
Kennedy, M.A., Johnson, T.A., Lees, N.D., Barbuch, R., Eckstein, J.A., Bard, M., 2000. Cloning and sequencing of the *Candida albicans* C-4 sterol methyl oxidase gene (*ERG25*) and expression of an *ERG25* conditional lethal mutation in *Saccharomyces cerevisiae*. Lipids 35, 257–262.
Khamooshi, K., Sikorski, P., Sun, N., Calderone, R., Li, D., 2014. The Rbf1, Hfl1 and Dbp4 of *Candida albicans* regulate common as well as transcription factor-specific mitochondrial and other cell activities. BMC Genomics 15, 56.
Kirsch, D.R., Lai, M.H., O'Sullivan, J., 1988. Isolation of the gene for cytochrome P450L1A1 (lanosterol 14 alpha-demethylase) from *Candida albicans*. Gene 68, 229–237.
Kodedova, M., Sychrova, H., 2015. Changes in the sterol composition of the plasma membrane affect membrane potential, salt tolerance and the activity of multidrug resistance pumps in *Saccharomyces cerevisiae*. PLoS One 10, e0139306.
Kontoyiannis, D.P., 1999. Genetic analysis of azole resistance by transposon mutagenesis in *Saccharomyces cerevisiae*. Antimicrob. Agents Chemother. 43, 2731–2735.
Kontoyiannis, D.P., 2000. Modulation of fluconazole sensitivity by the interaction of mitochondria and erg3p in *Saccharomyces cerevisiae*. J. Antimicrob. Chemother. 46, 191–197.
Koselny, K., Mutlu, N., Minard, A.Y., Kumar, A., Krysan, D.J., Wellington, M., 2018. A genome-wide screen of deletion mutants in the filamentous *Saccharomyces cerevisiae* background identifies ergosterol as a direct trigger of macrophage pyroptosis. MBio 9.
Kuhbacher, T., Ott, S.J., Helwig, U., et al., 2006. Bacterial and fungal microbiota in relation to probiotic therapy (VSL#3) in pouchitis. Gut 55, 833–841.
Lamb, D.C., Kelly, D.E., Schunck, W.H., Shyadehi, A.Z., Akhtar, M., Lowe, D.J., Baldwin, B.C., Kelly, S.L., 1997. The mutation T315A in *Candida albicans* sterol 14alpha-demethylase causes reduced enzyme activity and fluconazole resistance through reduced affinity. J. Biol. Chem. 272, 5682–5688.
Lamping, E., Cannon, R.D., 2010. Use of a yeast-based membrane protein expression technology to overexpress drug resistance efflux pumps. Methods Mol. Biol. 666, 219–250.
Lamping, E., Monk, B.C., Niimi, K., Holmes, A.R., Tsao, S., Tanabe, K., Niimi, M., Uehara, Y., Cannon, R.D., 2007. Characterization of three classes of membrane proteins involved in fungal azole resistance by functional hyperexpression in *Saccharomyces cerevisiae*. Eukaryot. Cell 6, 1150–1165.
Lamping, E., Zhu, J.Y., Niimi, M., Cannon, R.D., 2017. Role of ectopic gene conversion in the evolution of a *Candida krusei* pleiotropic drug resistance transporter family. Genetics 205, 1619–1639.
Lee, A.Y., St Onge, R.P., Proctor, M.J., et al., 2014. Mapping the cellular response to small molecules using chemogenomic fitness signatures. Science 344, 208–211.
Lees, N.D., Skaggs, B., Kirsch, D.R., Bard, M., 1995. Cloning of the late genes in the ergosterol biosynthetic pathway of *Saccharomyces cerevisiae*–a review. Lipids 30, 221–226.
Lefrancois, P., Euskirchen, G.M., Auerbach, R.K., Rozowsky, J., Gibson, T., Yellman, C.M., Gerstein, M., Snyder, M., 2009. Efficient yeast ChIP-Seq using multiplex short-read DNA sequencing. BMC Genomics 10, 37.
Legrand, M., Bachellier-Bassi, S., Lee, K.K., et al., 2018. Generating genomic platforms to study *Candida albicans* pathogenesis. Nucleic Acids Res. 46, 6935–6949.
Lewis, D.F., Wiseman, A., Tarbit, M.H., 1999. Molecular modelling of lanosterol 14 alpha-demethylase (CYP51) from *Saccharomyces cerevisiae* via homology with CYP102, a unique bacterial cytochrome P450 isoform: quantitative structure-activity relationships (QSARs) within two related series of antifungal azole derivatives. J. Enzyme Inhib. Med. Chem. 14, 175–192.
Li, X.C., Jacob, M.R., Ding, Y., Agarwal, A.K., Smillie, T.J., Khan, S.I., Nagle, D.G., Ferreira, D., Clark, A.M., 2006. Capisterones A and B, which enhance fluconazole activity in *Saccharomyces cerevisiae*, from the marine green alga Penicillus capitatus. J. Nat. Prod. 69, 542–546.
Li, L., Liao, Z., Yang, Y., Lv, L., Cao, Y., Zhu, Z., 2018. Metabolomic profiling for the identification of potential biomarkers involved in a laboratory azole resistance in *Candida albicans*. PLoS One 13, e0192328.
Liu, O.W., Chun, C.D., Chow, E.D., Chen, C., Madhani, H.D., Noble, S.M., 2008. Systematic genetic analysis of virulence in the human fungal pathogen *Cryptococcus neoformans*. Cell 135, 174–188.
Lotti, C., Piccinelli, A.L., Arevalo, C., Ruiz, I., Migliani De Castro, G.M., Figueira Reis De Sa, L., Tessis, A.C., Ferreira-Pereira, A., Rastrelli, L., 2012. Constituents of Honduran propolis with inhibitory effects on *Saccharomyces cerevisiae* multidrug resistance protein Pdr5p. J. Agric. Food Chem. 60, 10540–10545.
Lum, P.Y., Armour, C.D., Stepaniants, S.B., et al., 2004. Discovering modes of action for therapeutic compounds using a genome-wide screen of yeast heterozygotes. Cell 116, 121–137.
Lv, Q.Z., Yan, L., Jiang, Y.Y., 2016. The synthesis, regulation, and functions of sterols in *Candida albicans*: well-known but still lots to learn. Virulence 7, 649–659.
Ma, B.X., Ke, X., Tang, X.L., Zheng, R.C., Zheng, Y.G., 2018. Rate-limiting steps in the Saccharomyces cerevisiae ergosterol pathway: towards improved ergosta-5,7-dien-3beta-ol accumulation by metabolic engineering. World J. Microbiol. Biotechnol. 34, 55.
Maesaki, S., Marichal, P., Hossain, M.A., Sanglard, D., Vanden Bossche, H., Kohno, S., 1998. Synergic effects of tacrolimus and azole antifungal agents against azole-resistant *Candida albicans* strains. J. Antimicrob. Chemother. 42, 747–753.
Martel, C.M., Parker, J.E., Warrilow, A.G., Rolley, N.J., Kelly, S.L., Kelly, D.E., 2010. Complementation of a *Saccharomyces cerevisiae ERG11*/CYP51 (sterol 14alpha-demethylase) doxycycline-regulated mutant and screening of the azole sensitivity of *Aspergillus fumigatus* isoenzymes CYP51A and CYP51B. Antimicrob. Agents Chemother. 54, 4920–4923.
Martens, J.A., Genereaux, J., Saleh, A., Brandl, C.J., 1996. Transcriptional activation by yeast PDR1p is inhibited by its association with NGG1p/ADA3p. J. Biol. Chem. 271, 15884–15890.
Mast, N., Zheng, W., Stout, C.D., Pikuleva, I.A., 2013. Antifungal azoles: structural insights into undesired tight binding to cholesterol-metabolizing CYP46A1. Mol. Pharmacol. 84, 86–94.
Miceli, M.H., Kauffman, C.A., 2015. Isavuconazole: a new broad-spectrum triazole antifungal agent. Clin. Infect. Dis. 61, 1558–1565.
Miyazaki, Y., Geber, A., Miyazaki, H., Falconer, D., Parkinson, T., Hitchcock, C., Grimberg, B., Nyswaner, K., Bennett, J.E., 1999. Cloning, sequencing, expression and allelic sequence diversity of *ERG3* (C-5 sterol desaturase gene) in *Candida albicans*. Gene 236, 43–51.
Mnaimneh, S., Davierwala, A.P., Haynes, J., et al., 2004. Exploration of essential gene functions via titratable promoter alleles. Cell 118, 31–44.
Mokdad-Gargouri, R., Abdelmoula-Soussi, S., Hadiji-Abbes, N., Amor, I.Y., Borchani-Chabchoub, I., Gargouri, A., 2012. Yeasts as a tool for heterologous gene expression. Methods Mol. Biol. 824, 359–370.
Monk, B.C., Tomasiak, T.M., Keniya, M.V., et al., 2014. Architecture of a single membrane spanning cytochrome P450 suggests constraints that orient the catalytic domain relative to a bilayer. Proc. Natl. Acad. Sci. U. S. A. 111, 3865–3870.
Mount, H.O., Revie, N.M., Todd, R.T., Anstett, K., Collins, C., Costanzo, M., Boone, C., Robbins, N., Selmecki, A., Cowen, L.E., 2018. Global analysis of genetic circuitry and adaptive mechanisms enabling resistance to the azole antifungal drugs. PLoS Genet. 14, e1007319.
Mutlu, N., Garipler, G., Akdogan, E., Dunn, C.D., 2014. Activation of the pleiotropic drug resistance pathway can promote mitochondrial DNA retention by fusion-defective mitochondria in *Saccharomyces cerevisiae*. G3 Bethesda (Bethesda) 4, 1247–1258.
Nagayoshi, Y., Miyazaki, T., Shimamura, S., et al., 2017. Unexpected effects of azole transporter inhibitors on antifungal susceptibility in *Candida glabrata* and other pathogenic *Candida* species. PLoS One 12, e0180990.
Nahlik, J., Hrncirik, P., Mares, J., Rychtera, M., Kent, C.A., 2017. Towards the design of an optimal strategy for the production of ergosterol from *Saccharomyces cerevisiae* yeasts. Biotechnol. Prog. 33, 838–848.
Nakamura, K., Niimi, M., Niimi, K., Holmes, A.R., Yates, J.E., Decottignies, A., Monk, B.C., Goffeau, A., Cannon, R.D., 2001. Functional expression of *Candida albicans* drug efflux pump Cdr1p in a *Saccharomyces cerevisiae* strain deficient in membrane transporters. Antimicrob. Agents Chemother. 45, 3366–3374.
Nett, J.E., Andes, D.R., 2012. Antifungals: drug class, mechanisms of action, pharmacokinetics/pharmacodynamics, drug-drug interactions, toxicity, and clinical use. In: Calderone, R., Clancy, C. (Eds.), Candida and Candidiasis. ASM Press.
Niimi, K., Harding, D.R., Parshot, R., et al., 2004. Chemosensitization of fluconazole resistance in *Saccharomyces cerevisiae* and pathogenic fungi by a D-octapeptide derivative. Antimicrob. Agents Chemother. 48, 1256–1271.
Niimi, M., Wada, S., Tanabe, K., et al., 2005. Functional analysis of fungal drug efflux transporters by heterologous expression in *Saccharomyces cerevisiae*. Jpn. J. Infect. Dis. 58, 1–7.
Niimi, K., Harding, D.R., Holmes, A.R., Lamping, E., Niimi, M., Tyndall, J.D., Cannon, R.D., Monk, B.C., 2012. Specific interactions between the *Candida albicans* ABC transporter Cdr1p ectodomain and a D-octapeptide derivative inhibitor. Mol. Microbiol. 85, 747–767.
Nishida, N., Jing, D., Kuroda, K., Ueda, M., 2014. Activation of signaling pathways related to cell wall integrity and multidrug resistance by organic solvent in *Saccharomyces cerevisiae*. Curr. Genet. 60, 149–162.
Noble, S.M., French, S., Kohn, L.A., Chen, V., Johnson, A.D., 2010. Systematic screens of a *Candida albicans* homozygous deletion library decouple morphogenetic switching and pathogenicity. Nat. Genet. 42, 590–598.
Noble, S.M., Gianetti, B.A., Witchley, J.N., 2017. *Candida albicans* cell-type switching and functional plasticity in the mammalian host. Nat. Rev. Microbiol. 15, 96–108.
Panapruksachat, S., Iwatani, S., Oura, T., Vanittanakom, N., Chindamporn, A., Niimi, K., Niimi, M., Lamping, E., Cannon, R.D., Kajiwara, S., 2016. Identification and functional characterization of *Penicillium marneffei* pleiotropic drug resistance transporters ABC1 and ABC2. Med. Mycol. 54, 478–491.
Parker, J.E., Merkamm, M., Manning, N.J., Pompon, D., Kelly, S.L., Kelly, D.E., 2008. Differential azole antifungal efficacies contrasted using a *Saccharomyces cerevisiae* strain humanized for sterol 14 alpha-demethylase at the homologous locus. Antimicrob. Agents Chemother. 52, 3597–3603.
Parks, L.W., Casey, W.M., 1995. Physiological implications of sterol biosynthesis in yeast. Annu. Rev. Microbiol. 49, 95–116.

Paumi, C.M., Chuk, M., Snider, J., Stagljar, I., Michaelis, S., 2009. ABC transporters in *Saccharomyces cerevisiae* and their interactors: new technology advances the biology of the ABCC (MRP) subfamily. Microbiol. Mol. Biol. Rev. 73, 577–593.
Pianalto, K.M., Alspaugh, J.A., 2016. New horizons in antifungal therapy. J. Fungi (Basel) 2.
Pierce, S.E., Davis, R.W., Nislow, C., Giaever, G., 2007. Genome-wide analysis of barcoded *Saccharomyces cerevisiae* gene-deletion mutants in pooled cultures. Nat. Protoc. 2, 2958–2974.
Pierson, C.A., Jia, N., Mo, C., Lees, N.D., Sturm, A.M., Eckstein, J., Barbuct, R., Bard, M., 2004. Isolation, characterization, and regulation of the *Candida albicans ERG27* gene encoding the sterol 3-keto reductase. Med. Mycol. 42, 461–473.
Porcu, G., Wilson, C., Di Giandomenico, D., Ragnini-Wilson, A., 2010. A yeast-based genomic strategy highlights the cell protein networks altered by FTase inhibitor peptidomimetics. Mol. Cancer 9, 197.
Prasad, R., De Wergifosse, P., Goffeau, A., Balzi, E., 1995. Molecular cloning and characterization of a novel gene of *Candida albicans*, *CDR1*, conferring multiple resistance to drugs and antifungals. Curr. Genet. 27, 320–329.
Quinton, P.M., 1999. Physiological basis of cystic fibrosis: a historical perspective. Physiol. Rev. 79, S3–S22.
Rahman, H., Carneglia, J., Lausten, M., Robertello, M., Choy, J., Golin, J., 2018. Robust, pleiotropic drug resistance 5 (Pdr5)-mediated multidrug resistance is vigorously maintained in *Saccharomyces cerevisiae* cells during glucose and nitrogen limitation. FEMS Yeast Res. 18.
Reiner, S., Micolod, D., Schneiter, R., 2005. *Saccharomyces cerevisiae*, a model to study sterol uptake and transport in eukaryotes. Biochem. Soc. Trans. 33, 1186–1188.
Robbins, N., Spitzer, M., Yu, T., Cerone, R.P., Averette, A.K., Bahn, Y.S., Heitman, J., Sheppard, D.C., Tyers, M., Wright, G.D., 2015. An antifungal combination matrix identifies a rich pool of adjuvant molecules that enhance drug activity against diverse fungal pathogens. Cell Rep. 13, 1481–1492.
Roelants, F.M., Chauhan, N., Muir, A., Davis, J.C., Menon, A.K., Levine, T.P., Thorner, J., 2018. TOR complex 2-regulated protein kinase Ypk1 controls sterol distribution by inhibiting StARkin domain-containing proteins located at plasma membrane-endoplasmic reticulum contact sites. Mol. Biol. Cell 29, 2128–2136.
Roemer, T., Jiang, B., Davison, J., et al., 2003. Large-scale essential gene identification in *Candida albicans* and applications to antifungal drug discovery. Mol. Microbiol. 50, 167–181.
Roessner, C.A., Min, C., Hardin, S.H., Harris-Haller, L.W., McCollum, J.C., Scott, A.I., 1993. Sequence of the *Candida albicans erg7* gene. Gene 127, 149–150.
Roetzer, A., Gabaldon, T., Schüller, C., 2011. From *Saccharomyces cerevisiae* to *Candida glabrata* in a few easy steps: important adaptations for an opportunistic pathogen. FEMS Microbiol. Lett. 314, 1–9.
Rogers, P.D., Vermitsky, J.P., Edlind, T.D., Hilliard, G.M., 2006. Proteomic analysis of experimentally induced azole resistance in *Candida glabrata*. J. Antimicrob. Chemother. 58, 434–438.
Rosenberg, A., Ene, I.V., Bibi, M., Zakin, S., Segal, E.S., Ziv, N., Dahan, A.M., Colombo, A.L., Bennett, R.J., Berman, J., 2018. Antifungal tolerance is a subpopulation effect distinct from resistance and is associated with persistent candidemia. Nat. Commun. 9, 2470.
Sagatova, A.A., Keniya, M.V., Wilson, R.K., Monk, B.C., Tyndall, J.D., 2015. Structural insights into binding of the antifungal drug fluconazole to *Saccharomyces cerevisiae* lanosterol 14alpha-Demethylase. Antimicrob. Agents Chemother. 59, 4982–4989.
Sagatova, A.A., Keniya, M.V., Tyndall, J.D.A., Monk, B.C., 2018. Impact of homologous resistance mutations from pathogenic yeast on *Saccharomyces cerevisiae* lanosterol 14alpha-Demethylase. Antimicrob. Agents Chemother. 62.
Salazar, S.B., Wang, C., Munsterkotter, M., Okamoto, M., Takahashi-Nakaguchi, A., Chibana, H., Lopes, M.M., Guldener, U., Butler, G., Mira, N.P., 2018. Comparative genomic and transcriptomic analyses unveil novel features of azole resistance and adaptation to the human host in *Candida glabrata*. FEMS Yeast Res. 18.
Sanglard, D., Coste, A.T., 2016. Activity of Isavuconazole and other Azoles against *Candida* clinical isolates and yeast model systems with known azole resistance mechanisms. Antimicrob. Agents Chemother. 60, 229–238.
Sanglard, D., Ischer, F., Monod, M., Bille, J., 1997. Cloning of Candida albicans genes conferring resistance to azole antifungal agents: characterization of CDR2, a new multidrug ABC transporter gene. Microbiology 143 (Pt 2), 405–416.
Sanglard, D., Ischer, F., Koymans, L., Bille, J., 1998. Amino acid substitutions in the cytochrome P-450 lanosterol 14alpha-demethylase (CYP51A1) from azole-resistant *Candida albicans* clinical isolates contribute to resistance to azole antifungal agents. Antimicrob. Agents Chemother. 42, 241–253.
Sangster, W., Hegarty, J.P., Schieffer, K.M., Wright, J.R., Hackman, J., Toole, D.R., Lamendella, R., Stewart, D.B., Sr, 2016. Bacterial and fungal microbiota changes distinguish C. Difficile infection from other forms of diarrhea: results of a prospective inpatient study. Front. Microbiol. 7, 789.
Sarda, S., Hannenhalli, S., 2015. High-throughput identification of cis-regulatory rewiring events in yeast. Mol. Biol. Evol. 32, 3047–3063.
Schuetzer-Muehlbauer, M., Willinger, B., Egner, R., Ecker, G., Kuchler, K., 2003. Reversal of antifungal resistance mediated by ABC efflux pumps from *Candida albicans* functionally expressed in yeast. Int. J. Antimicrob. Agents 22, 291–300.
Schwarzmuller, T., Ma, B., Hiller, E., et al., 2014. Systematic phenotyping of a large-scale *Candida glabrata* deletion collection reveals novel antifungal tolerance genes. PLoS Pathog. 10, e1004211.
Selmecki, A., Forche, A., Berman, J., 2010. Genomic plasticity of the human fungal pathogen *Candida albicans*. Eukaryot. Cell 9, 991–1008.
Sharma, M., Manoharlal, R., Shukla, S., Puri, N., Prasad, T., Ambudkar, S.V., Prasad, R., 2009. Curcumin modulates efflux mediated by yeast ABC multidrug transporters and is synergistic with antifungals. Antimicrob. Agents Chemother. 53, 3256–3265.
Shin, J.H., Chae, M.J., Song, J.W., Jung, S.I., Cho, D., Kee, S.J., Kim, S.H., Shin, M.G., Suh, S.P., Ryang, D.W., 2007. Changes in karyotype and azole susceptibility of sequential bloodstream isolates from patients with *Candida glabrata* candidemia. J. Clin. Microbiol. 45, 2385–2391.
Shingu-Vazquez, M., Traven, A., 2011. Mitochondria and fungal pathogenesis: drug tolerance, virulence, and potential for antifungal therapy. Eukaryot. Cell 10, 1376–1383.
Shukla, S., Saini, P., Smriti, Jha, S., Ambudkar, S.V., Prasad, R., 2003. Functional characterization of *Candida albicans* ABC transporter Cdr1p. Eukaryot. Cell 2, 1361–1375.
Singh, A., Yadav, V., Prasad, R., 2012. Comparative lipidomics in clinical isolates of Candida albicans reveal crosstalk between mitochondria, cell wall integrity and azole resistance. PLoS One 7, e39812.
Skaggs, B.A., Alexander, J.F., Pierson, C.A., Schweitzer, K.S., Chun, K.T., Koegel, C., Barbuch, R., Bard, M., 1996. Cloning and characterization of the Saccharomyces cerevisiae C-22 sterol desaturase gene, encoding a second cytochrome P-450 involved in ergosterol biosynthesis. Gene 169, 105–109.
Skrzypek, M.S., Binkley, J., Binkley, G., Miyasato, S.R., Simison, M., Sherlock, G., 2017. The Candida Genome Database (CGD): incorporation of Assembly 22, systematic identifiers and visualization of high throughput sequencing data. Nucleic Acids Res. 45, D592–D596.
Smith, A.M., Heisler, L.E., Mellor, J., Kaper, F., Thompson, M.J., Chee, M., Roth, F.P., Giaever, G., Nislow, C., 2009. Quantitative phenotyping via deep barcode sequencing. Genome Res. 19, 1836–1842.
Snider, J., Kittanakom, S., Curak, J., Stagljar, I., 2010. Split-ubiquitin based membrane yeast two-hybrid (MYTH) system: a powerful tool for identifying protein-protein interactions. J. Vis. Exp.
Snider, J., Hanif, A., Lee, M.E., et al., 2013. Mapping the functional yeast ABC transporter interactome. Nat. Chem. Biol. 9, 565–572.
Sokol, H., Leducq, V., Aschard, H., et al., 2017. Fungal microbiota dysbiosis in IBD. Gut 66, 1039–1048.
Spitzer, M., Griffiths, E., Blakely, K.M., et al., 2011. Cross-species discovery of syncretic drug combinations that potentiate the antifungal fluconazole. Mol. Syst. Biol. 7, 499.
Subbiah, M.T., Abplanalp, W., 2003. Ergosterol (major sterol of baker's and brewer's yeast extracts) inhibits the growth of human breast cancer cells in vitro and the potential role of its oxidation products. Int. J. Vitam. Nutr. Res. 73, 19–23.
Subotić, A., Swinnen, E., Demuyser, L., De Keersmaecker, H., Mizuno, H., Tournu, H., Van Dijck, P., 2017. A Bimolecular Fluorescence Complementation Tool for Identification of protein-protein Interactions in Candida albicans. G3. .
Sugita, T., Nakase, T., 1999. Non-universal usage of the leucine CUG codon and the molecular phylogeny of the genus Candida. Syst. Appl. Microbiol. 22, 79–86.
Sun, N., Fonzi, W., Chen, H., She, X., Zhang, L., Zhang, L., Calderone, R., 2013. Azole susceptibility and transcriptome profiling in *Candida albicans* mitochondrial electron transport chain complex I mutants. Antimicrob. Agents Chemother. 57, 532–542.
Talibi, D., Raymond, M., 1999. Isolation of a putative Candida albicans transcriptional regulator involved in pleiotropic drug resistance by functional complementation of a pdr1 pdr3 mutation in Saccharomyces cerevisiae. J. Bacteriol. 181, 231–240.
Tebbets, B., Stewart, D., Lawry, S., Nett, J., Nantel, A., Andes, D., Klein, B.S., 2012. Identification and characterization of antifungal compounds using a *Saccharomyces cerevisiae* reporter bioassay. PLoS One 7, e36021.
Tebung, W.A., Choudhury, B.I., Tebbji, F., Morschhauser, J., Whiteway, M., 2016. Rewiring of the Ppr1 zinc cluster transcription factor from purine catabolism to pyrimidine biogenesis in the Saccharomycetaceae. Curr. Biol. 26, 1677–1687.
Tian, S., Ohta, A., Horiuchi, H., Fukuda, R., 2018. Oxysterol-binding protein homologs mediate sterol transport from the endoplasmic reticulum to mitochondria in yeast. J. Biol. Chem. 293, 5636–5648.
Traven, A., Wong, J.M., Xu, D., Sopta, M., Ingles, C.J., 2001. Interorganellar communication. Altered nuclear gene expression profiles in a yeast mitochondrial dna mutant. J. Biol. Chem. 276, 4020–4027.
Tyndall, J.D., Sabherwal, M., Sagatova, A.A., Keniya, M.V., Negroni, J., Wilson, R.K., Woods, M.A., Tietjen, K., Monk, B.C., 2016. Structural and functional elucidation of yeast lanosterol 14alpha-Demethylase in complex with agrochemical antifungals. PLoS One 11, e0167485.
Uetz, P., Giot, L., Cagney, G., et al., 2000. A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. Nature 403, 623–627.
Vanden Bossche, H., 1985. Biochemical targets for antifungal azole derivatives: hypothesis on the mode of action. Curr. Top. Med. Mycol. 1, 313–351.
Vandenbosch, D., De Canck, E., Dhondt, I., Rigole, P., Nelis, H.J., Coenye, T., 2013. Genomewide screening for genes involved in biofilm formation and miconazole susceptibility in *Saccharomyces cerevisiae*. FEMS Yeast Res. 13, 720–730.
Vandeputte, P., Tronchin, G., Rocher, F., Renier, G., Berges, T., Chabasse, D., Bouchara, J.P., 2009. Hypersusceptibility to azole antifungals in a clinical isolate of Candida glabrata with reduced aerobic growth. Antimicrob. Agents Chemother. 53, 3034–3041.
Vyas, V.K., Barrasa, M.I., Fink, G.R., 2015. A *Candida albicans* CRISPR system permits genetic engineering of essential genes and gene families. Sci. Adv. 1, e1500248.
Wada, S., Niimi, M., Niimi, K., Holmes, A.R., Monk, B.C., Cannon, R.D., Uehara, Y., 2002. *Candida glabrata* ATP-binding cassette transporters Cdr1p and Pdh1p expressed in a *Saccharomyces cerevisiae* strain deficient in membrane transporters show phosphorylation-dependent pumping properties. J. Biol. Chem. 277, 46809–46821.
Wada, S., Tanabe, K., Yamazaki, A., Niimi, M., Uehara, Y., Niimi, K., Lamping, E., Cannon, R.D., Monk, B.C., 2005. Phosphorylation of candida glabrata ATP-binding cassette transporter Cdr1p regulates drug efflux activity and ATPase stability. J. Biol. Chem. 280, 94–103.
Ward, D.M., Chen, O.S., Li, L., Kaplan, J., Bhuiyan, S.A., Natarajan, S.K., Bard, M., Cox, J.E., 2018. Altered sterol metabolism in budding yeast affects mitochondrial iron-sulfur (Fe-S) cluster synthesis. J. Biol. Chem. 293, 10782–10795.
Watanasrisin, W., Iwatani, S., Oura, T., et al., 2016. Identification and characterization of

Candida utilis multidrug efflux transporter CuCdr1p. FEMS Yeast Res. 16.
Whaley, S.G., Berkow, E.L., Rybak, J.M., Nishimoto, A.T., Barker, K.S., Rogers, P.D., 2016. Azole antifungal resistance in *Candida albicans* and emerging non-*albicans Candida* species. Front. Microbiol. 7, 2173.
Xiang, M.J., Liu, J.Y., Ni, P.H., Wang, S., Shi, C., Wei, B., Ni, Y.X., Ge, H.L., 2013. Erg11 mutations associated with azole resistance in clinical isolates of *Candida albicans*. FEMS Yeast Res. 13, 386–393.
Yamamoto, S., Hiraga, K., Abiko, A., Hamanaka, N., Oda, K., 2005. A new function of isonitrile as an inhibitor of the Pdr5p multidrug ABC transporter in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. 330, 622–628.
Yang, X., Talibi, D., Weber, S., Poisson, G., Raymond, M., 2001. Functional isolation of the Candida albicans FCR3 gene encoding a bZip transcription factor homologous to Saccharomyces cerevisiae Yap3p. Yeast 18, 1217–1225.
Yapar, N., 2014. Epidemiology and risk factors for invasive candidiasis. Ther. Clin. Risk Manag. 10, 95–105.
Yoshida, Y., Aoyama, Y., 1987. Interaction of azole antifungal agents with cytochrome P-45014DM purified from *Saccharomyces cerevisiae* microsomes. Biochem. Pharmacol. 36, 229–235.
Zhu, H., Bilgin, M., Bangham, R., et al., 2001. Global analysis of protein activities using proteome chips. Science 293, 2101–2105.