Divergent functions of three *Candida albicans* zinc-cluster transcription factors (*CTA4*, *ASG1* and *CTF1*) complementing pleiotropic drug resistance in *Saccharomyces cerevisiae*

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One of the mediators of pleiotropic drug resistance in *Saccharomyces cerevisiae* is the ABC-transporter gene *PDR5*. This gene is regulated by at least two transcription factors with Zn(2)-Cys(6) finger DNA-binding motifs, Pdr1p and Pdr3p. In this work, we searched for functional homologues of these transcription factors in *Candida albicans*. A *C. albicans* gene library was screened in a *S. cerevisiae* mutant lacking *PDR1* and *PDR3* and clones resistant to azole antifungals were isolated. From these clones, three genes responsible for azole resistance were identified. These genes (*CTA4*, *ASG1* and *CTF1*) encode proteins with Zn(2)-Cys(6)-type zinc finger motifs in their N-terminal domains. The *C. albicans* genes expressed in *S. cerevisiae* could activate the transcription of a *PDR5-lacZ* reporter system and this reporter activity was PDRE-dependent. They could also confer resistance to azoles in a *S. cerevisiae* strain lacking *PDR1*, *PDR3* and *PDR5*, suggesting that *CTA4*-, *ASG1*- and *CTF1*-dependent azole resistance can be caused by genes other than *PDR5* in *S. cerevisiae*. Deletion of *CTA4*, *ASG1* and *CTF1* in *C. albicans* had no effect on fluconazole susceptibility and did not alter the expression of the ABC-transporter genes *CDR1* and *CDR2* or the major facilitator gene *MDR1*, which encode multidrug transporters known as mediators of azole resistance in *C. albicans*. However, additional phenotypic screening tests on the *C. albicans* mutants revealed that the presence of *ASG1* was necessary to sustain growth on non-fermentative carbon sources (sodium acetate, acetic acid, ethanol). In conclusion, *C. albicans* possesses functional homologues of the *S. cerevisiae* Pdr1p and Pdr3p transcription factors; however, their properties in *C. albicans* have been rewired to other functions.

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INTRODUCTION

Multidrug transporters are widely distributed proteins in living organisms and are responsible for the transport of a large variety of compounds. Two types of transporters, the ATP-binding cassette (ABC) and the major facilitator superfamily (MFS) transporters have been described in lower eukaryotes such as *Saccharomyces cerevisiae* and have several functions, including phospholipid translocation in the plasma membrane, pheromone transport and the efflux of different classes of antifungal agents. Multidrug transporters are important for the human pathogen *Candida albicans*, as they have been found to be responsible

for the development of resistance to azoles, which are important antifungal agents.

In *C. albicans*, two ABC transporters, Cdr1p and Cdr2p (for *Candida* drug resistance) and one MFS transporter, Mdr1p (multidrug resistance) were shown to be upregulated in several azole-resistant isolates (Sanglard *et al.*, 1995). Each of the genes encoding these proteins can be upregulated in distinct clinical azole-resistant strains. The transcription of the gene encoding Mdr1p, *MDR1*, is almost absent in azole-susceptible isolates, but is measurable in specific azole-resistant cells. The gene encoding Cdr1p, *CDR1*, shows basal transcriptional activity in azole-susceptible isolates, but higher transcription levels in azole-resistant isolates. *CDR1* is usually co-ordinately upregulated in such isolates with *CDR2*, a gene with no detectable transcriptional activity in azole-susceptible isolates.

Abbreviations: ABC, ATP-binding cassette; DRE, drug-responsive element; MFS, major facilitator superfamily; PDRE, pleiotropic drug resistance responsive element.

Interestingly, these transporters can also be induced transiently by treating cells with other drugs such as oestradiol and fluphenazine for *CDR1* and *CDR2*, and benomyl or H_2O_2 for *MDR1* (De Micheli *et al.*, 2002; Gupta *et al.*, 1998; Karababa *et al.*, 2004), mimicking their expression in azole-resistant cells. Recently we demonstrated the crucial role of Tac1p (transcriptional activator of *CDR* genes), a Zn(2)-Cys(6) transcription factor, in the regulation of *CDR1* and *CDR2* in clinical azole-resistant isolates and during other drug treatments (Coste *et al.*, 2004, 2007). We showed that Tac1p acts through the previously described drug-responsive element (DRE) located in the promoters of *CDR1* and *CDR2* (Coste *et al.*, 2004; De Micheli *et al.*, 2002). The presence of gain-of-function mutations in *TAC1* is one of the mechanisms permitting development of antifungal drug resistance (Coste *et al.*, 2006).

In *S. cerevisiae*, multidrug transporter genes are regulated by a network of transcription factors with leucine zipper dimerization domains (*YAP1* and *YAP2*) or with Zn(2)-Cys(6) DNA-binding motifs (*PDR1*, *PDR3*). Alteration of the transcription factor *PDR1* is associated with increased transcription of the ABC transporters *PDR5*, *SNQ2* and *YOR1* (Balzi *et al.*, 1994; Carvajal *et al.*, 1997). The Pdr1p and Pdr3p binding site has been delimited to a consensus [5'-TCCG(C/T)GGA-3'] known as a PDRE (pleiotropic drug resistance responsive element) (Katzmann *et al.*, 1996) and is also present in the promoters of additional ABC-transporter genes, for example *PDR10* and *PDR15* (Decottignies *et al.*, 1998; Wolfer *et al.*, 1997). It is possible that similar transcription factor networks operate by transactivating multidrug transporters genes in *C. albicans* isolates resistant to azole antifungals. Such transcription factors may be involved in the regulation of *MDR1* or the basal level of *CDR1* transcription, neither of which is dependent on Tac1p (Coste *et al.*, 2004).

In this paper we describe the isolation of such factors by functional complementation of a *C. albicans* library in a *S. cerevisiae* *PDR1/PDR3* mutant. Using such an approach, Talibi & Raymond (1999) and Yang *et al.* (2001) previously isolated two distinct transcription factors (*FCR1* and *FCR3*) that were able to restore *PDR5* regulation. Using a similar approach we have isolated a further set of three genes that complement *PDR1/PDR3* mutants and confer resistance to azoles in *S. cerevisiae*. Although these genes could restore *PDR5* transcription in *S. cerevisiae*, their involvement in regulating *CDR1*, *CDR2* and *MDR1* expression in *C. albicans* could not be established, thus leading to a wider examination of their roles.

METHODS

Strains and media. The *C. albicans* and *S. cerevisiae* strains used in this study are listed in Table 1. These strains were grown either in complete medium YEPD, containing 1% Bacto peptone (Difco), 0.5% yeast extract (Difco) and 2% glucose (Fluka), or in minimal medium YNB (Yeast Nitrogen Base, Difco) and the required carbon

source (Fluka). Strain DSY1654 was obtained from DSY669 by inactivation of the *PDR5* ORF using the *kanMX6* module as outlined by Longtine *et al.* (1998). For solid media, 2% agar (Difco) was added to the media. Oleate-containing medium was composed of 0.12% sodium oleate and 0.2% Tween 40. Other carbon sources (sodium acetate, ethanol, glycerol and sodium lactate) were adjusted to 1% (w/v) and acetic acid to 0.05% (v/v). *Escherichia coli* DH5 α (Hanahan, 1985) was used as a host for plasmid constructions and propagation. It was grown in LB (Luria-Bertani broth) or on LB plates, supplemented with ampicillin (0.1 mg ml⁻¹) when required.

Yeast transformations. *S. cerevisiae* was transformed by the standard protocol (Gietz *et al.*, 1992). For *C. albicans* transformations, cells from 0.2 ml stationary-phase cultures were resuspended in 0.1 ml of a solution containing 200 mM lithium acetate (pH 7.5), 40% (w/v) PEG 8000, 15 mg DTT ml⁻¹, 250 μ g denatured salmon sperm DNA ml⁻¹. Transforming DNA (1–5 μ g) was added to the yeast suspension, which was then incubated for 60 min at 43.5 °C. Transformation mixtures were plated directly on to selective plates.

Measurement of β -galactosidase activities. To measure β -galactosidase activities in *S. cerevisiae* strain PB4, 100 μ l crude protein extract was incubated for 30 min at 30 °C with 400 μ l Z buffer (60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 10 mM KCl, 1 mM MgSO₄, 50 mM β -mercaptoethanol). Next, 150 μ l ONPG (Roche) previously diluted to a final concentration of 1 mg ml⁻¹ in filter-sterilized 0.1 M KH₂PO₄ (pH 7.0) was added to the mixture, which was further incubated for 30 min at 30 °C. The reaction was stopped by adding 400 μ l 1 M Na₂CO₃. Reactions were then mixed by inversion and centrifuged for 1 min at 13 000 r.p.m. A₄₂₀ was measured using a spectrophotometer (Spectrometer Lambda 18, Perkin Elmer). Plasmids containing the PDRE-CYC1 and PDREmut-CYC1 chimeric constructions (pDK52 and pDK53, respectively) were a generous gift from S. Moye-Rowley (University of Iowa, Iowa City, USA) and were introduced into *S. cerevisiae* PB4. These plasmids were introduced simultaneously with pDS139, pDS173 and pS174 (containing *CTA4*, *ASG1* and *CTF1*, respectively) or the parent vector (YE24) alone. The *PDR5-lacZ* (pDS450) fusion was produced by inserting the *PDR5* promoter (–1246 bp) with 89 bp of coding sequence as a *SmaI*–*HindIII* fragment into YE2367 and was a kind gift of A. Delahodde and C. Jacq (Ecole Normale Supérieure, Paris, France). The β -galactosidase activities were calculated in Miller units as recommended by Ausubel *et al.* (1987).

Plasmids pDS1161 and pDS1181, which are derived from Yip353 and contain a *CDR2*-(DRE) promoter element, were described previously (Coste *et al.*, 2004). As compared to pDS1161, pDS1181 contains *TAC1* from *C. albicans*.

Drug susceptibility testing. Yeast cultures were grown overnight in YEPD and diluted to a density of 1.5×10^7 cells ml⁻¹ and 10-fold dilutions were performed. Cell suspensions in 4 μ l aliquots were spotted onto YEPD plates containing drugs (see Fig. 3 for compound concentrations used). Plates were incubated for 48 h at 30 °C for *S. cerevisiae* and at 35 °C for *C. albicans*.

Immunoblots. *C. albicans* protein extracts for immunoblotting were prepared by alkaline extraction of cells grown to mid-exponential phase. Briefly, 2 ml of cells at 1.5×10^7 cells ml⁻¹ was resuspended in an Eppendorf tube with 500 μ l water and 150 μ l of a solution containing 1.85 M NaOH and 7.5% β -mercaptoethanol, before incubating on ice for 10 min. Proteins were then precipitated with 150 μ l of a 50% trichloroacetic acid solution and the suspension was left on ice for another 10 min. Precipitated proteins were sedimented by centrifugation at maximum speed in a microfuge for 15 min. The sediment was resuspended in 50 μ l loading buffer (40 mM Tris/HCl pH 6.8, 8 M urea, 5% SDS, 0.1 M EDTA, 1% β -mercaptoethanol

Table 1. Strains used in this study

Strain	Genotype	Parent strain	Reference
<i>C. albicans</i>			
CAF2-1	<i>ura3Δ::imm434/URA3</i>	SC5314	Fonzi & Irwin (1993)
CAF4-2	<i>ura3Δ::imm434/ura3Δ::imm434</i>	CAF2-1	Fonzi & Irwin (1993)
GPY115	<i>ura3Δ::imm434/ura3Δ::imm434 RPS10/RPS10::Clp10</i>	CAF4-2	This study
DSY1269	<i>CTA4/cta4Δ::hisG-URA3-hisG</i>	CAF4-2	This study
DSY1276	<i>CTA4/cta4Δ::hisG</i>	DSY1269	This study
DSY1306	<i>cta4Δ::hisG/cta4Δ::hisG-URA3-hisG</i>	DSY1276	This study
DSY3699	<i>cta4Δ::hisG/cta4Δ::hisG</i>	DSY1306	This study
DSY1711	<i>ASG1/asg1Δ::hisG-URA3-hisG</i>	CAF4-2	This study
DSY1712	<i>ASG1/asg1Δ::hisG</i>	DSY1711	This study
DSY1760	<i>asg1Δ::hisG/asg1Δ::hisG-URA3-hisG</i>	DSY1712	This study
DSY1762	<i>asg1Δ::hisG/asg1Δ::hisG</i>	DSY1760	This study
DSY1687	<i>CTF1/ctf1Δ::hisG-URA3-hisG</i>	CAF4-2	This study
DSY1698	<i>CTF1/ctf1Δ::hisG</i>	DSY1687	This study
DSY1714	<i>ctf1Δ::hisG/ctf1Δ::hisG-URA3-hisG</i>	DSY1698	This study
DSY3700	<i>ctf1Δ::hisG/ctf1Δ::hisG</i>	DSY1714	This study
DSY3765	<i>cta4Δ::hisG/cta4Δ::hisG [pDS1538]</i>	DSY3699	This study
DSY3761	<i>asg1Δ::hisG/asg1Δ::hisG [pDS1536]</i>	DSY1712	This study
DSY3763	<i>ctf1Δ::hisG/ctf1Δ::hisG [pDS1537]</i>	DSY3700	This study
DSY3703	<i>cta4Δ::hisG/cta4Δ::hisG RPS10/RPS10::Clp10</i>	DSY3699	This study
DSY3704	<i>asg1Δ::hisG/asg1Δ::hisG RPS10/RPS10::Clp10</i>	DSY1712	This study
DSY3705	<i>ctf1Δ::hisG/ctf1Δ::hisG RPS10/RPS10::Clp10</i>	DSY3700	This study
<i>S. cerevisiae</i>			
DSY669	<i>MATa pdr1Δ::TRP1 pdr3Δ::HIS3 PDR5 ura3-52 leu2-Δ1 GAL2</i>	FY1679-28C	Delaveau <i>et al.</i> (1994)
DSY1654	<i>MATa pdr1Δ::TRP1 pdr3Δ::HIS3 pdr5Δ::kanMX6 ura3-52 leu2-Δ1 GAL2</i>	DSY669	This study
PB4	<i>MATα leu2-3,-112 ura3-52 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9 Mel⁻ pdr1-2::hisG pdr3-1::hisG</i>	SEY6210	Katzmann <i>et al.</i> (1996)

and 0.1 mg bromophenol blue ml⁻¹) and incubated at 37 °C for 10 min. Non-solubilized material was cleared by a second centrifugation step for 10 min. Solubilized yeast proteins (10 µl) were separated by 10% SDS-PAGE and transferred by Western blotting onto a nitrocellulose membrane. Immunodetection of Cdr1p, Cdr2p and Mdr1p was performed with polyclonal rabbit anti-Cdr1p, anti-Cdr2p and anti-Mdr1p antibodies as previously described (De Micheli *et al.*, 2002; Hiller *et al.*, 2006a) by chemoluminescence with an ECL kit according to the recommendations of the manufacturer (Amersham Biosciences).

Construction of *C. albicans* mutant strains. Restriction maps for each gene investigated in this study and the positions of deletions are given in Fig. 1(A). For the disruption of *CTA4*, a 2.2 kb *Bam*HI–*Eco*RV fragment containing part of the *CTA4* ORF was first cloned into pBluescript KS+ to yield pDS516. A 3.7 kb *Sph*I–*Bgl*II fragment with the *URA3*-blaster cassette from pMB7 was cloned into pDS516 previously digested with the same enzymes, thus removing an internal 0.5 kb from *CTA4*, to yield pDS522. The linear fragment obtained after digestion of pDS522 with *Apa*I and *Sac*I was used to transform *C. albicans*.

For the disruption of *ASG1* and *CTF1*, a region containing the totality or a portion of the ORF (–535 to +1385 for *ASG1* and +1241 to +3296 for *CTF1*) was amplified from genomic DNA using the cloning primer pairs ASG1-Xba/ASG1-Xho and CTF1-Xba/CTF1-Xho (Table 2), respectively. The PCR fragments were cloned in the *Xho*I–*Xba*I sites of pBluescript KS+ to yield pDS656 and pDS640, respectively. Deletions within cloned regions in these plasmids were carried out by PCR with deletion primer pairs ASG1-Bgl/ASG1-Pst (deletion in *ASG1* from –62 to +873) and CTF1-Bgl/CTF1-Pst

(deletion in *CTF1* from +1735 to +2740). The 3.7 kb *Pst*I–*Bgl*II fragment with the *URA3*-blaster cassette from pMB7 was cloned into the PCR fragment previously digested with *Pst*I and *Bgl*II to obtain disruption cassettes pDS654 and pDS644. For transformation in *C. albicans*, linear fragments were obtained by digestion of deletion constructs with *Apa*I and *Sac*I.

After generation of heterozygous mutants for each gene, the *URA3* marker was regenerated by plating Ura⁺ colonies on selective medium containing 5-fluoro-uracil acid (5-FOA) as published elsewhere (Sanglard *et al.*, 1996). Ura[–] colonies for each heterozygous mutant were next transformed with the appropriate linearized disruption cassettes. The verification of homozygous mutants was performed by PCR and Southern blot analysis (see Fig. 1).

Complementation of homozygous mutants was achieved by expression of *CTA4*, *ASG1* or *CTF1* under the control of the *ADH1* promoter. ORFs of *CTA4*, *ASG1* and *CTF1* were amplified by PCR with primer pairs CTA4-5/CTA4-3, ASG1-5/ASG1-3 and CTF1-5/CTF1-3, respectively (Table 2). PCR fragments were next cloned into the expression plasmid pYPB1-ADH1pL (Leberer *et al.*, 1996) digested by *Xho*I and *Bgl*II to yield plasmids pDS1538, pDS1536 and pDS1537, respectively. These plasmids were transformed into homozygous mutants into which the *URA3* marker had been regenerated as mentioned above (see Table 1).

Southern blots. DNA probes used in this study were generated by digestion of pDS139, pDS173 and pDS174 as described in Fig. 1(A). ³²P-DNA labelled probes were generated by random priming (Feinberg & Vogelstein, 1984). Southern blots were performed as described previously (Sanglard *et al.*, 1995). Radioactive signals were

RESULTS

Screening of a *C. albicans* genomic library in a *pdr1Δ pdr3Δ S. cerevisiae* mutant

In order to isolate such factors, we used a strategy reliant upon the complementation of the fluconazole hypersusceptibility of a *S. cerevisiae* mutant (DSY669), in which *PDR1* and *PDR3* were inactivated, with a *C. albicans* genomic library constructed in the vector YEp24 (Sanglard *et al.*, 1997). After screening of approximately 10^5 independent transformants on selective medium containing $5 \mu\text{g}$ fluconazole ml^{-1} , 34 fluconazole-resistant clones were obtained. After rescue from *S. cerevisiae*, the different plasmids were assayed for their ability to transactivate *PDR5* expression using a *lacZ* reporter system. The

Table 2. Primers used in this study

Primer	Nucleotide sequence*
ASG1-Xba	CGGCTCTAGACATTATCCATTGATCCATTTTACA
ASG1-Xho	GCCGCTCGAGGCACATCCAGAAGAACTTAATCGA
ASG1-Bgl	CGGCAGATCTTTCTATATTAGCATGTGGATCTTT
ASG1-Pst	CGAAACTGCAGGTGGTGGTAGTGGCAGCTCTGTTA
CTF1-Xba	CGGCTCTAGAGTACTAATGATTTGGCTAGTATAACTT
CTF1-Xho	GCCGCTCGAGGTGATGGTGCCACACTATCTCTCATGG
CTF1-Bgl	CGAAGATCTATTTCGGATTTAACAATATATTGATGTTGA
CTF1-Pst	CAACAACCTGCAGCAACAACATGCATTATCAAC
CTA4-5	AAAGCGCGGATCCAAAATGACATCTGAACATAAAGTATCA
CTA4-3	AAAGCGCTCGAGTTATACTCTATCCATAAAAATATCCATC
ASG1-5	AAAGCGCAGATCTAAAATGCCTAAAAGGGAAATTGAAG
ASG1-3	AAAGCGCTCGAGTTAAAATGGATCTAAATATTCTAATCC
CTF1-5	AAAGCGCAGATCTAAAATGACTGATCAAAATTTAAATTTAA
CTF1-3	AAAGGGCTCGAGTCAATTCATCATATGAAATAATGAATG

*Underlined nucleotides indicate the presence of a restriction site.

plasmids conferring *lacZ*-positive expression were analysed by restriction analysis, revealing plasmids with three different restriction patterns as shown in Fig. 1(A). *lacZ*-negative plasmids were not further analysed. Each *lacZ*-positive plasmid with a unique restriction pattern was transformed back into DSY669 and transformants tested in plates containing fluconazole to confirm drug resistance phenotypes.

Characteristics of the cloned genes

Plasmids pDS139, pDS173 and pDS174 conferred resistance to multiple compounds when compared to the *S.*

cerevisiae strain containing only the parent plasmid YEp24. As shown in Fig. 2, pDS139, pDS173 and pDS174 conferred resistance not only to fluconazole but also to itraconazole, rhodamine 6G and cycloheximide. The presence of pDS173 and pDS174 in DSY669 conferred enhanced resistance to cycloheximide as compared to pDS139. Nucleotide sequences of the insert flanking regions were determined and compared with the *C. albicans* genome data in order to obtain the entire sequence of the inserts. In each of the inserts distinct ORFs with Zn(2)-Cys(6) binuclear cluster domains could be located. The minimal complementing sizes for pDS139, pDS173 and pDS174 contained three ORFs which, according to the

Fig. 2. Drug susceptibility testing of *S. cerevisiae* isolates transformed with plasmids containing *C. albicans* genes. Serial dilution assays were performed in the background of DSY669 (*pdr1Δ, pdr3Δ*) (A) and DSY1654 (*pdr1Δ, pdr3Δ, pdr5Δ*) (B), respectively. Spotting assays were performed with serial dilutions of overnight cultures onto YEPD complex medium containing different drugs as indicated. Plates were incubated for 48 h at 30 °C. Graduation bars indicate 10-fold serial dilutions as described in Methods.

Fig. 3. Drug susceptibility testing of *C. albicans* mutants. Serial dilution assays were performed onto YEPD containing fluconazole (A) and onto YNB media with different carbon sources as indicated (B, D) and with glucose (C). Plates were incubated 48 h at 35 °C. Genotypes of each mutant are shown on the left. The following strains were used: wild-type, GPY115; $cta4\Delta/\Delta$, DSY3703; $cta4\Delta/\Delta + CTA4$, DSY3765; $asg1\Delta/\Delta$, DSY3704; $asg1\Delta/\Delta + ASG1$, DSY3761; $ctf1\Delta/\Delta$, DSY3705; $ctf1\Delta/\Delta + CTF1$, DSY3763.

Candida Genome Database (CGD), corresponded to orf19.7374 (*CTA4*), orf19.166 (*ASG1*) and orf19.1499 (*CTF1*), respectively.

Overall the proteins encoded by these *C. albicans* genes exhibited a low level of similarity with each other (14–18 % similarity) and were different from *FCR1*, a gene cloned with a similar approach to that used here. Blasts against the GenBank database highlighted best scores with genes of unknown functions from *Pichia stipitis* and *Lodderomyces elongisporus*. A partial gene sequence of *CTA4* was previously described and reported as a fragment with transcriptional activating properties in *S. cerevisiae* (Kaiser *et al.*, 1999). The best hit of *CTA4* with the *S. cerevisiae* genome is *OAF1*, an oleate-activated transcription factor, which acts alone and as a heterodimer with Pip2p and activates genes involved in β -oxidation of fatty acids and peroxisome organization and biogenesis (Karpichev & Small, 1998). Interestingly, *CTA4* is situated in an 11 kb genomic region of chromosome 3 containing two other genes with Zn(2)-Cys(6) motifs. Such gene 'clusters' have already been identified for similar transcription factors, including Tac1p, which is flanked by two other genes with the same DNA-binding motifs (Coste *et al.*, 2004, 2006, 2007). The closest homologue of orf19.166 in *S. cerevisiae* is *YIL130w* (*ASG1*), with no clear function yet. It was named *ASG1* in the Candida Genome Database due to this similarity. *CTF1* has strong hits with *GAL4* in *S. cerevisiae* and with transcription factors regulating cutinase activity in several other fungal species.

Pdr1p and Pdr3p in *S. cerevisiae* are the major Zn(2)-Cys(6) transcription factors interacting with PDRE. It can be expected that the *C. albicans* ORFs cloned here would also contain this conserved fungal DNA-binding domain. Since these genes complemented *PDR1* and *PDR3* defects and most likely *PDR5* expression in *S. cerevisiae*, a quantitative analysis of *lacZ* reporter activities was performed in *S. cerevisiae* carrying a *PDR5-lacZ* fusion. The analyses revealed that the presence of *ASG1* produced the highest reporter activities (215 ± 32 U), which were comparable to the values (250 ± 21 U) obtained with *PDR3*. *CTA4* and *CTF1* conferred only weak reporter

activities (4.7 ± 1.3 and 38 ± 5 U, respectively) (Table 3). To show that these activities were dependent on the presence of a PDRE, chimeric promoter constructs in the promoter of *CYC1* containing the wild-type PDRE or a mutated PDRE (PDREmut) were co-transformed into *S. cerevisiae* with the plasmids containing the *C. albicans* genes. *CYC1*-PDRE-dependent *lacZ* reporter activities were in general lower than those measured with the *PDR5-lacZ* fusion, and the *CTF1*-dependent activity was about 10-fold lower as compared to *CTA4* and *ASG1* or even *PDR3* (Table 3). The lower *CYC1*-PDRE-dependent *lacZ* reporter activities can be attributed to differences in the number of PDRE sequences or other regulatory elements existing between the chimeric *CYC1* promoter and the *PDR5* promoter. Mutations in PDRE resulted in a decrease of reporter activity as compared to the wild-type PDRE for the three transcription factors. The *CTA4*-, *ASG1*- and *CTF1*-dependent reporter activity decreased by approximately 4-, 11-fold and 10-fold, respectively, when the PDRE was mutated (Table 3). Although PDREs were not mutated in the *PDR5* promoter in this study because of their redundancy, these results suggest that the proteins encoded by *CTA4*, *ASG1* and *CTF1* could transactivate *PDR5* through PDRE.

Given that other Zn(2)-Cys(6) finger transcription factors exist in *C. albicans*, it is possible that, in addition to *CTA4*, *ASG1* and *CTF1*, other transcription factors of this family could activate the transcription of the *PDR5-lacZ* reporter. We addressed this possibility in *S. cerevisiae* by expressing *TAC1*, which is a Zn(2)-Cys(6) finger transcription factor involved in azole drug resistance in *C. albicans* (Coste *et al.*, 2004). No significant *PDR5-lacZ* reporter activity was measured upon *TAC1* expression in *S. cerevisiae* (see Table 3), thus suggesting that only a specific subset of *C. albicans* Zn(2)-Cys(6) finger transcription factors are able to interact with *PDR5*.

Role of *CTA4*, *ASG1* and *CTF1* in *C. albicans*

In order to get insight into the function of the genes cloned by functional complementation in *S. cerevisiae*, they were

Table 3. PDRE-dependent reporter activities mediated by *C. albicans* genes in *S. cerevisiae* PB4

Type of promoter- <i>lacZ</i> fusion (plasmid)	Reporter activity (Miller units)*					
	YEpl24	<i>CTA4</i> †	<i>ASG1</i> †	<i>CTF1</i> †	<i>PDR3</i> ‡	<i>TAC1</i> §
<i>PDR5-lacZ</i> (pDS450)	<0.1	4.7 ± 1.3 ‡	215 ± 32	38 ± 5	250 ± 21	<0.1
<i>CYC1</i> -PDRE (pDK52)	<0.1	32 ± 1.8	11.6 ± 0.2	1.2 ± 0.1	31 ± 2	ND
<i>CYC1</i> -PDREmut (pDK53)	<0.1	8.8 ± 0.6	0.9 ± 0.1	<0.1	12 ± 0.9	ND

*Values are mean values of three measurements. ND, Not determined.

†The *CTA4*, *ASG1* and *CTF1* genes were contained in pDS139, pDS173 and pDS174, respectively.

‡*PDR3* was expressed from plasmid pFL44-PP3 and is described by Delaveau *et al.* (1994)

§*TAC1*-dependent activity was calculated by subtracting *PDR5-lacZ* activities in PB4 transformed with pDS1181 (*TAC1*-containing plasmid) from activities obtained with the parent vector pDS1161 (no *TAC1*).

disrupted in *C. albicans*. The resulting mutants were analysed by Southern blotting to verify each disruption. As shown in Fig. 1(B), each mutant yielded restriction patterns expected from the disruption of each gene.

The *CTA4*, *ASG1* and *CTF1* mutants were tested in drug susceptibility assays performed on YEPD plates containing fixed drug concentrations. To avoid possible interference of the positioning of *URA3* in these assays, all mutants and the parent wild-type were restored to *Ura*⁺ prototrophy by integrating *URA3* at the *RPS10* locus (Brand *et al.*, 2004). Fig. 3(A) shows that the disruption of *CTA4*, *ASG1* and *CTF1* had no effect on fluconazole susceptibility. Other azoles including voriconazole and itraconazole were tested but no significant changes were observed (data not shown). The expression of the ABC transporters Cdr1p and Cdr2p, which are functional homologues of Pdr5p, was verified on cell extracts for each *C. albicans* mutant by Western blot analysis with Cdr1p and Cdr2p antibodies. Each mutant was also exposed to 10 µg fluphenazine ml⁻¹ for 20 min, which is a condition known to induce both *CDR1* and *CDR2* (Coste *et al.*, 2007). As observed in Fig. 4, Cdr1p and Cdr2p levels could still be increased after fluphenazine addition to all mutants, thus suggesting that *CTA4*, *ASG1* and *CTF1* played no role in the drug-dependent transcriptional activation of ABC transporters important for azole resistance in *C. albicans*. Slight variations were also observed in basal Cdr1p levels of the constructed mutants, especially in the *cta4Δ/Δ* and *ctf1Δ/Δ* mutants (Fig. 4). Given that these differences have no impact on the fluconazole susceptibility of these mutants (see Fig. 3A), it is likely that the variations in basal Cdr1p levels can be attributed to altered transfer efficiencies of the Western blot on the nylon membrane.

As Pdr1p and Pdr3p are known to modulate the expression of other types of transporters such as the hexose transporters *HTX9* and *HTX11* (Nourani *et al.*, 1997), which also belong to the MFS family, we also verified whether *CTA4*, *ASG1* and *CTF1* could regulate *MDR1*, which encodes an MFS transporter of *C. albicans* involved in resistance to fluconazole (Sanglard *et al.*, 1995). Western blot analysis with Mdr1p antibodies was performed on cell extracts of each mutant exposed to benomyl, a drug known to induce *MDR1* (Rognon *et al.*, 2006). This analysis revealed that the deletion of *CTA4*, *ASG1* and *CTF1* had no effect on the drug-induced production of Mdr1p (Fig. 4).

Taken together, the results suggest that *CTA4*, *ASG1* and *CTF1*, although capable of replacing the function of transcription factors involved in the regulation of multi-drug transporters in *S. cerevisiae*, are not fulfilling the same function in *C. albicans* – at least as far as *CDR1*, *CDR2* and *MDR1* upregulation is concerned.

Since it is known that transcription factors from different fungal species can share similar structural domains, but still display species-specific functions, we decided to perform additional phenotypic assays with the homozygous mutants. Mutant behaviour was examined on media containing different carbon sources (sodium acetate, ethanol, sodium citrate, sodium lactate, glycerol and sodium oleate) or different compounds that could help detect alterations in cell wall integrity pathways (SDS, Congo red) or response to different pHs (3–9). The phenotypic screening revealed reduced growth of the *asg1Δ/Δ* mutant on media containing sodium acetate, acetic acid, ethanol and sodium citrate (Fig. 3B). This phenotype could be reversed by overexpression of *ASG1*

Fig. 4. Immunodetection of Cdr1p, Cdr2p and Mdr1p in *C. albicans* mutants. Protein extracts of each strain were separated by 10% SDS-PAGE and immunoblotted with polyclonal rabbit anti-Cdr1p, anti-Cdr2p (A) and anti-Mdr1p (B) as described (De Micheli *et al.*, 2002; Hiller *et al.*, 2006a). *C. albicans* strains were grown on YEPD to mid-exponential phase and exposed (+) or not (-) to fluphenazine (10 µg ml⁻¹, 20 min) for Cdr1p and Cdr2p detection or to benomyl (35 µg ml⁻¹, 30 min) for Mdr1p detection. The polyclonal anti-Mdr1p detects a signal with decreased migration in SDS-PAGE. The signal was obtained in both benomyl-exposed and non-exposed conditions and thus served as an internal protein loading control. The *C. albicans* mutant strains were: *cta4Δ/CTA4*, DSY1276; *cta4Δ/Δ*, DSY1306; *asg1Δ/ASG1*, DSY1711; *asg1Δ/Δ*, DSY1760; *ctf1Δ/CTF1*, DSY1687; *ctf1Δ/Δ*, DSY1714.

under the control of the *ADHI* promoter. Additional phenotypic tests on media containing glucose and sodium acetate or acetic acid revealed growth defects only in the *ASG1* mutant (Fig. 3C), indicating that these metabolites might exert toxicity in *C. albicans*. The *asg1Δ/Δ* mutant was still able to assimilate glycerol, sodium lactate and sodium oleate (Fig. 3D), therefore suggesting that the growth defect could not be attributed to a general defect in carbon source assimilation through the TCA or glyoxylate cycles. Our results suggest rather that *ASG1* controls a specific metabolic step, and further studies are under way to elucidate the role of this gene in carbon source assimilation.

DISCUSSION

In this paper we report the isolation of several transcription factors involved in *S. cerevisiae* multidrug resistance. Our first approach involved using *S. cerevisiae* as a host for complementing with a *C. albicans* genomic library the loss of function of *PDR1* and *PDR3* when exposed to fluconazole. The three genes recovered (*CTA4*, *ASG1*, *CTF1*) were able to activate the expression of the ABC transporter *PDR5* in *S. cerevisiae* as judged by gene reporter assays (see Table 3). We addressed the possibility that *PDR5* could be a likely target of these *C. albicans* genes in *S. cerevisiae* and be responsible for the drug resistance phenotypes shown in Fig. 2 by introducing plasmids containing the *C. albicans* genes into a strain lacking *PDR5*, *PDR1* and *PDR3*. The absence of *PDR5* did not change (with the exception of the *S. cerevisiae* strain containing *CTF1* on cycloheximide-containing plates) the drug resistance phenotypes dependent on the *C. albicans* genes (Fig. 2B). The data therefore suggest that the *C. albicans* genes have additional gene targets in *S. cerevisiae*, probably including other PDRE-containing genes. The functional complementation procedure used to identify *C. albicans* genes also revealed, in separate studies, *FCR1* and *FCR3* (fluconazole resistance) (Talibi & Raymond, 1999; Yang *et al.*, 2001). *FCR1* encodes a transcription factor with a Zn(2)-Cy(6) DNA-binding motif as is the case for *CTA4*, *ASG1* and *CTF1*, while *FCR3* encodes a transcription factor with a bZip leucine zipper motif. The function of *FCR1* in *C. albicans* has been addressed by the construction of a mutant lacking both *FCR1* alleles; however, the mutant was more resistant to azoles than the wild-type, making *FCR1* a negative regulator of azole resistance. The role of *FCR1* in the regulation of *CDR* genes was not addressed in the study published by Talibi & Raymond (1999). A recent study reported that *FCR1* overexpression could however decrease *CDR1* upregulation upon exposure to fluconazole (Shen *et al.*, 2007). Our own investigations have not revealed *FCR1* as a key regulator of *CDR1* and *CDR2* (data not shown). The data presented here also did not reveal a functional link between the cloned *C. albicans* genes and the regulation of *CDR* genes or *MDR1* (Fig. 4). One likely explanation for these differences in species-specific gene activation is that the DNA-binding motifs of the *cis*-acting

elements responsible for the regulation of the *PDR5*, *CDR* and *MDR1* genes are different. Pdr1p and Pdr3p target a sequence motif or PDRE (5'-TCCG/aC/tGG/cA/G-3'), where variable bases in the consensus motif are shown in lower case (DeRisi *et al.*, 2000). This motif is not equivalent to the DRE [5'-CGGA(A/T)ATCGGATATTTT-3'] common to *CDR1* and *CDR2* (De Micheli *et al.*, 2002) or to the benomyl-responsive element (BRE) described recently in *MDR1* (Rognon *et al.*, 2006) and also by other studies (Harry *et al.*, 2005; Hiller *et al.*, 2006b; Riggle & Kumamoto, 2006). Even though *CTA4*, *ASG1* and *CTF1* (as well as *FCR1*) show little similarity to *PDR1* and *PDR3*, there may still be sufficient similarity to enable the activation of *PDR5* through a PDRE. These transcription factors all have in common a Zn(2)-Cys(6) DNA-binding domain, which targets sequences with direct, inverted or everted palindromic repeats containing a 5'-CGG-3' motif, with various numbers of nucleotides in the intervening spacer region (MacPherson *et al.*, 2006). It is possible therefore that when expressed in *S. cerevisiae*, the *C. albicans* proteins could interact with PDRE because of the presence of this 5'-CGG-3' motif. The exact DNA-binding motifs of Cta4p, Asg1p, Ctf1p and Fcr1p as well as their target genes remain to be established in *C. albicans*. Interestingly, a recent study reported that *CTA4* was critical for nitrosative stress response in *C. albicans*. Cta4p was shown to bind a palindrome sequence (5'-CCGTCGG-3') in the promoter of *YHB1*, encoding a flavohaemoglobin responsible for the conversion of nitric oxide to nitrate (Chiranand *et al.*, 2008). This sequence closely resembles the PDRE core sequence [5'-TCCG(C/T)GGA-3'] (Devaux *et al.*, 2001) and it is therefore not too surprising that *CTA4* is able to mediate *PDR5* transcription as shown in the present study.

Very recently, the transcription factor *MRR1*, which also possesses a Zn(2)-Cys(6) DNA-binding motif, was discovered as a major regulator of *MDR1* (Morschhauser *et al.*, 2007). Interestingly, *CTA4* is most similar among *C. albicans* ORFs to *MRR1* and is situated only 1 kb downstream of *MRR1*. Along with orf19.7371, these three genes constitute a cluster of transcription factors within a 10 kb region. Although we could not demonstrate any involvement of *CTA4* in *MDR1* regulation, the physical association of these genes is intriguing in the framework of drug resistance, since *TAC1*, the regulator of *CDR1* and *CDR2*, is also found in a cluster of three transcription factor genes with the same DNA-binding motifs. These two gene clusters, which both include genes involved in a similar function (drug resistance), suggest a common evolutionary process that probably resulted from gene duplication events associated with functional specialization. Synteny of these clusters among other sequenced yeast genomes, including *Candida tropicalis*, *Candida parapsilosis*, *Pichia stipitis* and *Debaryomyces hansenii*, is conserved and suggests the conservation of important functional traits (data not shown).

Since no association between azole resistance and *CTA4*, *ASG1* and *CTF1* could be made in *C. albicans*, additional phenotypic growth assays were performed. Systematic

phenotypic screening tests on different carbon sources had been successfully applied in *S. cerevisiae* transcription factor mutants to reveal their functional categorization (Akache *et al.*, 2001). Even though *C. albicans* transcription factors can share similar functions in *S. cerevisiae* that have been attributed by functional assays or sequence comparisons, their function in *C. albicans* can be considerably divergent, a notion described as 'transcriptional rewiring' (Martchenko *et al.*, 2007). We addressed this possibility by performing a restricted number of phenotypic tests, which revealed growth defects on acetate- and ethanol-containing media when *ASG1* was inactivated.

In *C. albicans*, acetate or ethanol metabolism involves gluconeogenesis via the glyoxylate cycle, thus raising the possibility that *ASG1* controls specific metabolic steps of this pathway. However, because the *ASG1* mutant was not compromised in oleate assimilation (Fig. 3D), this possibility remains unlikely. Toxicity of sodium acetate or acetic acid was revealed only in the *ASG1* mutant (Fig. 3C). This toxicity was not pH-dependent, since independent experiments performed with this mutant on media buffered from pH 3 to pH 9 did not result in growth alteration as compared to the wild-type (data not shown). The exposure of *C. albicans* to acetic acid (40–60 mM, corresponding to a content of 0.3%) triggers apoptotic events (Phillips *et al.*, 2003) that are dependent on the RAS signalling pathway (Phillips *et al.*, 2006). We have not yet investigated the involvement of *ASG1* in acetic acid-dependent apoptosis of *C. albicans*; however, it is possible that this transcription factor acts as a downstream target of the RAS signalling pathway. Transcript profiling experiments using the *ASG1* mutant in the presence/absence of sodium acetate and acetic acid are currently under way in our laboratory to identify *ASG1*-dependent genes in *C. albicans* and thus we hope to elucidate more precisely the role of this gene in carbon source assimilation and its hypothetical relationship to the RAS pathway.

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REFERENCES

- Akache, B., Wu, K. & Turcotte, B. (2001). Phenotypic analysis of genes encoding yeast zinc cluster proteins. *Nucleic Acids Res* **29**, 2181–2190.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1987). *Current Protocols in Molecular Biology*. Greene Publishing Associates and Wiley-Interscience.
- Balzi, E., Wang, M., Leterme, S., Van Dyck, L. & Goffeau, A. (1994). *PDR5*, a novel yeast multidrug resistance conferring transporter controlled by the transcription regulator *PDR1*. *J Biol Chem* **269**, 2206–2214.
- Brand, A., MacCallum, D. M., Brown, A. J., Gow, N. A. & Odds, F. C. (2004). Ectopic expression of *URA3* can influence the virulence phenotypes and proteome of *Candida albicans* but can be overcome by targeted reintegration of *URA3* at the *RPS10* locus. *Eukaryot Cell* **3**, 900–909.
- Carvajal, E., van den Hazel, H. B., Cybularz-Kolaczowska, A., Balzi, E. & Goffeau, A. (1997). Molecular and phenotypic characterization of yeast *PDR1* mutants that show hyperactive transcription of various ABC multidrug transporter genes. *Mol Gen Genet* **256**, 406–415.
- Chiranand, W., McLeod, I., Zhou, H., Lynn, J. J., Vega, L. A., Myers, H., Yates, J. R., III, Lorenz, M. C. & Gustin, M. C. (2008). *CTA4* transcription factor mediates induction of nitrosative stress response in *Candida albicans*. *Eukaryot Cell* **7**, 268–278.
- Coste, A. T., Karababa, M., Ischer, F., Bille, J. & Sanglard, D. (2004). *TAC1*, transcriptional activator of *CDR* genes, is a new transcription factor involved in the regulation of *Candida albicans* ABC transporters *CDR1* and *CDR2*. *Eukaryot Cell* **3**, 1639–1652.
- Coste, A., Turner, V., Ischer, F., Morschhäuser, J., Forche, A., Semelcki, A., Berman, J., Bille, J. & Sanglard, D. (2006). A mutation in *Tac1p*, a transcription factor regulating *CDR1* and *CDR2*, is coupled with loss of heterozygosity at chromosome 5 to mediate antifungal resistance in *Candida albicans*. *Genetics* **172**, 2139–2156.
- Coste, A., Selmecki, A., Forche, A., Diogo, D., Bounoux, M. E., d'Enfert, C., Berman, J. & Sanglard, D. (2007). Genotypic evolution of azole resistance mechanisms in sequential *Candida albicans* isolates. *Eukaryot Cell* **6**, 1889–1904.
- De Micheli, M., Bille, J., Schueller, C. & Sanglard, D. (2002). A common drug-responsive element mediates the upregulation of the *Candida albicans* ABC transporters *CDR1* and *CDR2*, two genes involved in antifungal drug resistance. *Mol Microbiol* **43**, 1197–1214.
- Decottignies, A., Grant, A. M., Nichols, J. W., de Wet, H., McIntosh, D. B. & Goffeau, A. (1998). ATPase and multidrug transport activities of the overexpressed yeast ABC protein Yor1p. *J Biol Chem* **273**, 12612–12622.
- Delaveau, T., Delahodde, A., Carvajal, E., Subik, J. & Jacq, C. (1994). *PDR3*, a new yeast regulatory gene, is homologous to *PDR1* and controls the multidrug resistance phenomenon. *Mol Gen Genet* **244**, 501–511.
- DeRisi, J., van den Hazel, B., Marc, P., Balzi, E., Brown, P., Jacq, C. & Goffeau, A. (2000). Genome microarray analysis of transcriptional activation in multidrug resistance yeast mutants. *FEBS Lett* **470**, 156–160.
- Devaux, F., Marc, P., Bouchoux, C., Delaveau, T., Hikkel, I., Potier, M. C. & Jacq, C. (2001). An artificial transcription activator mimics the genome-wide properties of the yeast *Pdr1* transcription factor. *EMBO Rep* **2**, 493–498.
- Feinberg, A. P. & Vogelstein, B. (1984). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* **137**, 266–267.
- Fonzi, W. A. & Irwin, M. Y. (1993). Isogenic strain construction and gene mapping in *Candida albicans*. *Genetics* **134**, 717–728.
- Gietz, D., St. Jean, A., Woods, R. A. & Schiestl, R. H. (1992). Improved method for high efficiency transformation of intact yeast cells. *Nucleic Acids Res* **20**, 1425.
- Gupta, V., Kohli, A., Krishnamurthy, S., Puri, N., Aalamgeer, S. A., Panwar, S. & Prasad, R. (1998). Identification of polymorphic mutant alleles of *CaMDR1*, a major facilitator of *Candida albicans* which confers multidrug resistance, and its in vitro transcriptional activation. *Curr Genet* **34**, 192–199.
- Hanahan, D. (1985). Techniques for transformation of *E. coli*. In *DNA Cloning: a Practical Approach*. Edited by D. M. Glover. Oxford: IRL Press.

- Harry, J. B., Oliver, B. G., Song, J. L., Silver, P. M., Little, J. T., Choiniere, J. & White, T. C. (2005). Drug-induced regulation of the *MDR1* promoter in *Candida albicans*. *Antimicrob Agents Chemother* **49**, 2785–2792.
- Hiller, D., Sanglard, D. & Morschhauser, J. (2006a). Overexpression of the *MDR1* gene is sufficient to confer increased resistance to toxic compounds in *Candida albicans*. *Antimicrob Agents Chemother* **50**, 1365–1371.
- Hiller, D., Stahl, S. & Morschhauser, J. (2006b). Multiple *cis*-acting sequences mediate upregulation of the *MDR1* efflux pump in a fluconazole-resistant clinical *Candida albicans* isolate. *Antimicrob Agents Chemother* **50**, 2300–2308.
- Kaiser, B., Munder, T., Saluz, H. P., Kunkel, W. & Eck, R. (1999). Identification of a gene encoding the pyruvate decarboxylase gene regulator CaPdc2p from *Candida albicans*. *Yeast* **15**, 585–591.
- Karababa, M., Coste, A. T., Rognon, B., Bille, J. & Sanglard, D. (2004). Comparison of gene expression profiles of *Candida albicans* azole-resistant clinical isolates and laboratory strains exposed to drugs inducing multidrug transporters. *Antimicrob Agents Chemother* **48**, 3064–3079.
- Karpichev, I. V. & Small, G. M. (1998). Global regulatory functions of Oaf1p and Pip2p (Oaf2p), transcription factors that regulate genes encoding peroxisomal proteins in *Saccharomyces cerevisiae*. *Mol Cell Biol* **18**, 6560–6570.
- Katzmann, D. J., Hallstrom, T. C., Mahe, Y. & Moye-Rowley, W. S. (1996). Multiple Pdr1p/Pdr3p binding sites are essential for normal expression of the ATP binding cassette transporter protein-encoding gene *PDR5*. *J Biol Chem* **271**, 23049–23054.
- Leberer, E., Harcus, D., Broadbent, I. D., Clark, K. L., Dignard, D., Ziegelbauer, K., Schmidt, A., Gow, N. A., Brown, A. J. & Thomas, D. Y. (1996). Signal transduction through homologs of the Ste20p and Ste7p protein kinases can trigger hyphal formation in the pathogenic fungus *Candida albicans*. *Proc Natl Acad Sci U S A* **93**, 13217–13222.
- Longtine, M. S., McKenzie, A., III, Demarini, D. J., Shah, N. G., Wach, A., Brachat, A., Philippsen, P. & Pringle, J. R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast* **14**, 953–961.
- MacPherson, S., Laroche, M. & Turcotte, B. (2006). A fungal family of transcriptional regulators: the zinc cluster proteins. *Microbiol Mol Biol Rev* **70**, 583–604.
- Martchenko, M., Levitin, A., Hogues, H., Nantel, A. & Whiteway, M. (2007). Transcriptional rewiring of fungal galactose-metabolism circuitry. *Curr Biol* **17**, 1007–1013.
- Morschhauser, J., Barker, K. S., Liu, T. T., Bla, B. W. J., Homayouni, R. & Rogers, P. D. (2007). The transcription factor Mrr1p controls expression of the *MDR1* efflux pump and mediates multidrug resistance in *Candida albicans*. *PLoS Pathog* **3**, e164.
- Nourani, A., Papajova, D., Delahodde, A., Jacq, C. & Subik, J. (1997). Clustered amino acid substitutions in the yeast transcription regulator Pdr3p increase pleiotropic drug resistance and identify a new central regulatory domain. *Mol Gen Genet* **256**, 397–405.
- Phillips, A. J., Sudbery, I. & Ramsdale, M. (2003). Apoptosis induced by environmental stresses and amphotericin B in *Candida albicans*. *Proc Natl Acad Sci U S A* **100**, 14327–14332.
- Phillips, A. J., Crowe, J. D. & Ramsdale, M. (2006). Ras pathway signaling accelerates programmed cell death in the pathogenic fungus *Candida albicans*. *Proc Natl Acad Sci U S A* **103**, 726–731.
- Riggle, P. J. & Kumamoto, C. A. (2006). Transcriptional regulation of *MDR1*, encoding a drug efflux determinant, in fluconazole-resistant *Candida albicans* strains through an Mcm1p binding site. *Eukaryot Cell* **5**, 1957–1968.
- Rognon, B., Kozovska, Z., Coste, A. T., Pardini, G. & Sanglard, D. (2006). Identification of promoter elements responsible for the regulation of *MDR1* from *Candida albicans*, a major facilitator transporter involved in azole resistance. *Microbiology* **152**, 3701–3722.
- Sanglard, D., Kuchler, K., Ischer, F., Pagani, J. L., Monod, M. & Bille, J. (1995). Mechanisms of resistance to azole antifungal agents in *Candida albicans* isolates from AIDS patients involve specific multidrug transporters. *Antimicrob Agents Chemother* **39**, 2378–2386.
- Sanglard, D., Ischer, F., Monod, M. & Bille, J. (1996). Susceptibilities of *Candida albicans* multidrug transporter mutants to various antifungal agents and other metabolic inhibitors. *Antimicrob Agents Chemother* **40**, 2300–2305.
- 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**, 405–416.
- Shen, H., An, M. M., Wang de, J., Xu, Z., Zhang, J. D., Gao, P. H., Cao, Y. Y., Cao, Y. B. & Jiang, Y. Y. (2007). Fcr1p inhibits development of fluconazole resistance in *Candida albicans* by abolishing *CDR1* induction. *Biol Pharm Bull* **30**, 68–73.
- 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.
- Wolfger, H., Mahe, Y., Parle-McDermott, A., Delahodde, A. & Kuchler, K. (1997). The yeast ATP binding cassette (ABC) protein genes *PDR10* and *PDR15* are novel targets for the Pdr1 and Pdr3 transcriptional regulators. *FEBS Lett* **418**, 269–274.
- 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.

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