JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 400–404 Vol. 182, No. 2
0021-9193/00/$04.00+0

Activation of the Multiple Drug Resistance Gene *MDR1* in Fluconazole-Resistant, Clinical *Candida albicans* Strains Is Caused by Mutations in a *trans*-Regulatory Factor

STEPHANIE WIRSCHING, SONJA MICHEL, GERWALD KÖHLER, AND JOACHIM MORSCHHÄUSER*

Zentrum für Infektionsforschung, Universität Würzburg, Röntgenring 11, D-97070 Würzburg, Germany

Received 26 July 1999/Accepted 20 October 1999

Resistance of *Candida albicans* against the widely used antifungal agent fluconazole is often due to active drug efflux from the cells. In many fluconazole-resistant *C. albicans* isolates the reduced intracellular drug accumulation correlates with constitutive strong expression of the *MDR1* gene, encoding a membrane transport protein of the major facilitator superfamily that is not detectably expressed in vitro in fluconazole-susceptible isolates. To elucidate the molecular changes responsible for *MDR1* activation, two pairs of matched fluconazole-susceptible and resistant isolates in which drug resistance coincided with stable *MDR1* activation were analyzed. Sequence analysis of the *MDR1* regulatory region did not reveal any promoter mutations in the resistant isolates that might account for the altered expression of the gene. To test for a possible involvement of *trans*-regulatory factors, a *GFP* reporter gene was placed under the control of the *MDR1* promoter from the fluconazole-susceptible *C. albicans* strain CAI4, which does not express the *MDR1* gene in vitro. This *MDR1P-GFP* fusion was integrated into the genome of the clinical *C. albicans* isolates with the help of the dominant selection marker *MPA*^{R} developed for the transformation of *C. albicans* wild-type strains. Integration was targeted to an ectopic locus such that no recombination between the heterologous and resident *MDR1* promoters occurred. The transformants of the two resistant isolates exhibited a fluorescent phenotype, whereas transformants of the corresponding susceptible isolates did not express the *GFP* gene. These results demonstrate that the *MDR1* promoter was activated by a *trans*-regulatory factor that was mutated in fluconazole-resistant isolates, resulting in deregulated, constitutive *MDR1* expression.

Candida albicans is an important opportunistic fungal pathogen of humans and is the major cause of oropharyngeal candidiasis (OPC) in patients with AIDS (21). The azole antifungal agent fluconazole is a widely used compound to treat OPC. In recent years, however, the incidence of treatment failures has been rising. Especially in patients with AIDS who have recurrent OPC and who are receiving prolonged fluconazole therapy, treatment failures are due to the emergence of fluconazole-resistant strains (10, 22). Resistant *C. albicans* isolates frequently exhibit reduced intracellular drug accumulation that correlates with enhanced expression of certain multiple drug resistance genes, the ATP-binding cassette (ABC) transporters *CDR1* and *CDR2*, and the major facilitator *MDR1* (8, 14, 24, 25, 29). Fluconazole resistance is usually a stable phenotype that is maintained in the absence of selection pressure by the drug. This implies that genetic alterations have occurred in the resistant isolates that result in a constitutive overexpression of the drug efflux pumps. The *MDR1* gene is not detectably expressed in vitro in fluconazole-susceptible *C. albicans* isolates but is strongly activated in many strains after the development of fluconazole resistance. The molecular changes responsible for the constitutive activation of the *MDR1* gene in fluconazole-resistant, clinical *C. albicans* isolates have not been identified. Possible mechanisms include mutations in the *MDR1* promoter region that might result in deregulated *MDR1* expression or mutations in a regulatory factor controlling expression of the *MDR1* gene.

In a previous report (8) we have described two series of *C. albicans* isolates from patients with AIDS who had recurrent episodes of OPC and developed fluconazole resistance during therapy. It was shown by DNA fingerprinting that in both cases fluconazole resistance had developed in a previously susceptible strain and that multiple mechanisms had contributed to a stepwise development of drug resistance. In both series of isolates the observed reduced intracellular drug accumulation correlated with high *MDR1* mRNA levels. These two series of matched isolates gave us an opportunity to investigate which molecular changes were responsible for activation of the *MDR1* gene in fluconazole-resistant, clinical *C. albicans* strains.

* Corresponding author. Mailing address: Zentrum für Infektionsforschung, Universität Würzburg, Röntgenring 11, D-97070 Würzburg, Germany. Phone: 49-931-31 21 52. Fax: 49-931-31 25 78. E-mail: joachim.morschhaeuser@mail.uni-wuerzburg.de.

MATERIALS AND METHODS

***C. albicans* strains.** The clinical *C. albicans* isolates used in this study have been described previously (8). The two isolate pairs F2 and F5 and G2 and G5 represent fluconazole-susceptible and resistant isolates of the same *C. albicans* strains. The isolates were kept as frozen stocks at −80°C and were subcultured on YPD agar plates (10 g of yeast extract, 20 g of peptone, 20 g of glucose, 15 g of agar per liter) at 30°C. Strains F2G54, F5G54, G2G54, and G5G54 are derivates of these clinical isolates that contain a transcriptional fusion of the *MDR1* promoter (*MDR1P*) with the *GFP* gene, integrated at the *CDR4* locus (see below). The fluconazole-susceptible *C. albicans* strain CAI4 (7) was used as a source of the *MDR1* promoter for construction of the *MDR1P-GFP* fusion.

DNA sequencing. The *MDR1* promoter regions from the clinical *C. albicans* isolates were amplified with the primers MDR1p1, 5′-CGATAAATGATAAG TCACTCTACC-3′ (positions 57 to 34 within the coding region), and MDR1p2, 5′-CAACTCTACTGGTAACTATTGGCG-3′ (positions −561 to −538 with respect to the start codon), deduced from the published sequence of the *MDR1* gene (6). The PCR products were phosphorylated and cloned into the *Sma*I site of the vector pUC18. Using the universal and reverse primers, the sequences of both strands of the cloned PCR products were determined from several independent clones of each isolate until the sequences of both *MDR1* alleles had been obtained. Direct sequencing of the PCR products from each isolate was also performed with 200 ng of the phenol-extracted, ethanol-precipitated amplicons as a template and the primers Mdr1p1 and Mdr1pseq1, 5′-CTGAAAAGGAT ATCCCATCCC-3′. Sequencing was performed with the Thermo Sequenase fluorescence-labeled-primer cycle sequencing kit with deaza dGTP (Amersham, Braunschweig, Germany) and IRD 800 dye-labeled primers (MWG Biotech,

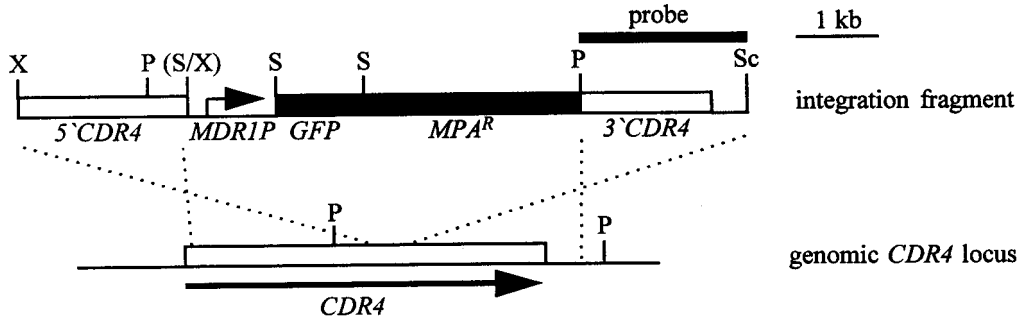

FIG. 1. Integration of the *MDR1P-GFP* fusion into the *CDR4* locus of *C. albicans*. The genetic structure of the linear DNA fragment from pGFP54 used for transformation and the genomic structure at the *CDR4* locus of the parent strains are delineated. The *CDR4* coding region is represented by an open bar. The straight arrow indicates the direction of transcription. The *MDR1* promoter is represented by the angled arrow. The probe used to verify the correct integration is indicated by a thick line. Only relevant restriction sites are shown. P, *Pst*I, S, *Sal*I, Sc, *Sac*I X, *Xho*I. The *Sal*I and *Xho*I sites shown in parentheses were destroyed by the cloning procedure.

Ebersberg, Germany). Sequences were analyzed on a LI-COR model 4000 automated sequencer (MWG Biotech). The sequences obtained by direct sequencing of PCR products were analyzed visually to detect positions of heterozygosity.

Plasmid construction. Plasmid pGFP41 has been described previously (18). It contains a *GFP* gene, genetically modified for expression in *C. albicans* under control of the *SAP2* promoter, and the *URA3* selection marker in the vector pBluescript. After removal of the *Pst*I site in the polylinker by digestion with *Cla*I and *Xba*I, filling in the ends, and religation, the *Sal*I-*Pst*I fragment with the *URA3* gene was replaced by a *Sal*I-*Pst*I fragment containing the *MPA*^{R} marker from plasmid pAFI3 (26), resulting in plasmid pGFP49. The *MDR1* promoter (*MDR1P*) from strain CAI4 was obtained by PCR amplification with the primers MDR1p5, 5′-GCATTGTCGACGTTCTATGTAAGTAGATGTATTGC-3′ (positions +4 to −30 of the *MDR1* gene), and MDR1p7, 5′-CGTAAATCTCGAG AAACGGACTCCG-3′ (positions −1109 to −1085), thereby introducing an upstream *Xho*I site and a *Sal*I site in front of the start codon (underlined). The *MDR1P* fragment was substituted for the *Xho*I-*Sal*I fragment containing the *SAP2* promoter in pGFP49, resulting in pGFP50. Subsequently, the 3′ *SAP2* fragment was replaced by a *Pst*I-*Sac*I fragment comprising the 3′ region of the *CDR4* gene (positions 2818 to 4901 with respect to the start codon [9]) to yield pGFP51. Finally, an *Xho*I-*Sal*I fragment containing the 5′ *CDR4* region (positions 103 to 2217) was inserted into the *Xho*I site of pGFP51, resulting in pGFP54. The insert of pGFP54 was excised by digestion with *Xho*I and *Sac*I and used for integration of the *MDR1P-GFP* fusion at the *CDR4* locus of the clinical *C. albicans* strains (Fig. 1).

***C. albicans* transformation.** *C. albicans* strains were transformed with the gel-purified linear DNA fragment from pGFP54 described above by electroporation (13). Mycophenolic acid (MPA)-resistant transformants were selected on minimal agar plates (6.7 g of yeast nitrogen base without amino acids [YNB; BIO 101, Vista, Calif.], 2 g of glucose, and 0.77 g of complete supplement medium [CSM-URA; BIO 101] per liter) containing 10 μg of MPA ml^{-1}. Single colonies were picked after 5 to 7 days of growth and restreaked on plates containing 10 μg of MPA ml^{-1}. Clones containing the correct insertion of the *MDR1P-GFP* fusion at the *CDR4* locus were then further propagated on YPD agar plates.

Isolation of chromosomal DNA and Southern hybridization. Chromosomal DNA from *C. albicans* strains was isolated as described by Millon et al. (17). DNA (10 μg) was digested with *Pst*I, separated on a 1% (wt/vol) agarose gel and, after ethidium bromide staining, transferred by vacuum blotting onto a nylon membrane and fixed by UV cross-linking. Probe labeling, hybridization, washing, and signal detection were performed with the ECL labeling and detection kit provided by Amersham according to the instructions of the manufacturer.

Fluorescence microscopy. The strains were grown overnight in YPD liquid medium, and aliquots were spotted on microscope slides. Fluorescence was detected with a Zeiss Axiolab microscope equipped for epifluorescence microscopy with a 50-W mercury high-pressure bulb and the Zeiss fluorescein-specific filter set 09.

RESULTS

Sequence analysis of the *MDR1* promoter region of fluconazole-susceptible and -resistant *C. albicans* isolates. From each of the two series of clinical *C. albicans* isolates described in a previous report (8), one fluconazole-susceptible and one resistant isolate were selected for the present study. Isolates F2 (MIC of fluconazole, 6.25 μg ml^{-1}) and G2 (MIC, 0.39 μg ml^{-1}) were the last isolates in each series that did not detectably express the *MDR1* gene. Isolates F5 and G5, both with an MIC of ≥50 μg ml^{-1}, were the most resistant isolates in each series and exhibited high *MDR1* mRNA levels. To investigate if the activation of the *MDR1* gene in the fluconazole-resistant isolates was caused by mutations in the *MDR1* regulatory region, the sequence of more than 500 bp upstream of the start codon was determined. This region was chosen because there was a good chance of possible promoter mutations occurring within this distance from the *MDR1* coding region and because the sequences of both DNA strands of the cloned PCR products could conveniently be determined with single sequencing reactions. For each isolate, several independent plasmid clones containing the PCR-amplified *MDR1* upstream region were analyzed to obtain the sequences of both *MDR1* alleles and to exclude PCR artifacts (point mutations and hybrids between the two alleles that were also obtained). Nucleotide polymorphisms between the two *MDR1* alleles were detected in all four isolates, but the same two alleles found in the susceptible isolates F2 and G2 were also present in the corresponding resistant isolates F5 and G5 without any sequence alterations (Table 1). Direct sequencing of the PCR products confirmed the observed allelic differences occurring within each isolate and verified that no other nucleotide differences within the sequenced region were present in any of the four isolates. Several additional sequence differences with respect to the published *MDR1* sequence (6) were also found, but all eight *MDR1* alleles from the four isolates were identical at these positions (data not shown). These results demonstrate that, within the sequenced *MDR1* upstream region, no promoter mutations had occurred in the fluconazole-resistant isolates that could account for the constitutive activation of the *MDR1* gene.

Expression of an *MDR1-GFP* fusion in fluconazole-susceptible and -resistant *C. albicans* isolates. The sequence analysis of the *MDR1* upstream region suggested that mutations in a regulatory factor might be responsible for the activation of the *MDR1* gene in the two fluconazole-resistant *C. albicans* isolates. However, we could not exclude the possibility that *cis*-acting mutations might have occurred still further upstream at sites located considerably distant from the *MDR1* coding region, even if we had sequenced a larger region (see, for example, reference 23). To obtain direct evidence that the molecular changes involved a regulatory factor, we tested whether the *MDR1* promoter from a fluconazole-susceptible *C. albicans* strain would be activated in the fluconazole-resistant isolates. For this purpose, the *MDR1* promoter from strain CAI4, which does not detectably express the *MDR1* gene in vitro (8), was fused to the *GFP* gene and the reporter gene fusion was inte-

TABLE 1. Allelic differences in the *MDR1* promoter regions of the *C. albicans* isolates F2, F5, G2, and G5

Strain	Allele	Nucleotide at position[a]:								
		−473	−448	−407	−387	−343	−341	−301	−226	−199
F2	1	−[b]	A	T	G	T	G	T	C	T
	2	T	T	C	C	A	C	C	T	C
F5	1	−	A	T	G	T	G	T	C	T
	2	T	T	C	C	A	C	C	T	C
G2	1	−	A	C	G	T	G	T	C	T
	2	T	T	C	C	A	C	C	T	C
G5	1	−	A	C	G	T	G	T	C	T
	2	T	T	C	C	A	C	C	T	C

[a] Nucleotide positions are with respect to the *MDR1* start codon (+1).
[b] The thymidines occurring at this position in the published sequence (6) and in the second allele were deleted.

grated into the genome of the two fluconazole-resistant isolates, F5 and G5. To avoid recombination between the *MDR1* promoter from strain CAI4 and *MDR1* upstream sequences in the host strains, integration was targeted to an ectopic site in the genome. The *CDR4* locus was chosen for integration, as this region had been characterized previously by our group and it had been shown that inactivation of one of the *CDR4* alleles did not result in a detectable phenotype (18). In addition, *CDR4* expression levels did not differ between the fluconazole-susceptible and resistant isolates (9).

For integration of the reporter fusion into the *C. albicans* wild-type strains, a cassette was constructed that contained the *MDR1P-GFP* fusion and the dominant selection marker *MPA*^{R}, a mutated allele of the *C. albicans IMH3* gene conferring resistance to mycophenolic acid (13; Theiss et al., unpublished), flanked by 5′ and 3′ *CDR4* sequences (Fig. 1). The linear cassette was used for transformation of the two resistant *C. albicans* isolates and, for control purposes, also of the corresponding fluconazole-susceptible isolates, and the genomic structures of the transformants were analyzed by Southern hybridization. The majority of MPA-resistant transformants did not exhibit detectable genomic changes at the *CDR4* locus, probably because of integration of the *MPA*^{R} marker into one of the *IMH3* alleles, and these transformants were not further analyzed. For each parent strain, one transformant in which the *MDR1P-GFP* fusion had been correctly integrated at the *CDR4* locus is shown in Fig. 2.

Expression of the *MDR1P-GFP* fusion was analyzed by epifluorescence microscopy after growth of the transformants in YPD liquid medium, conditions under which the *MDR1* gene is activated in the fluconazole-resistant isolates, F5 and G5, but not detectably expressed in the corresponding susceptible isolates, F2 and G2 (8). One representative transformant of each parent strain is shown in Fig. 3. All transformants of strains F5 and G5 containing the *MDR1P-GFP* fusion showed a fluorescent phenotype. In contrast, strains F2G54 and G2G54 containing the identical *MDR1P-GFP* fusion integrated in the fluconazole-susceptible isolates F2 and G2, respectively, did not exhibit any fluorescence. None of the parent strains fluoresced under these experimental conditions (data not shown). The fact that an identical *MDR1* promoter was activated in the fluconazole-resistant isolates but not in the matched susceptible isolates demonstrates that *MDR1* activation in the resistant isolates was mediated by mutations in a *trans*-regulatory factor, resulting in *MDR1* expression under conditions under which the gene is normally repressed.

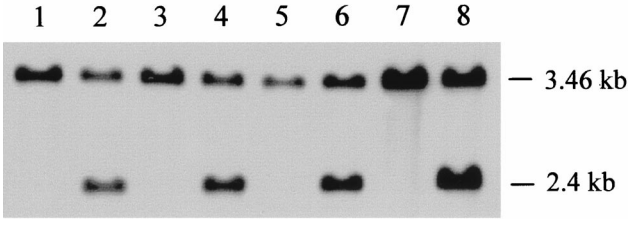

FIG. 2. Southern hybridization of *Pst*I-digested chromosomal DNA of *C. albicans* isolates F2, (lane 1), F5 (lane 3), G2 (lane 5), and G5 (lane 7) and the corresponding transformants F2G54 (lane 2), F5G54 (lane 4), G2G54 (lane 6), and G5G54 (lane 8). The sizes of the hybridizing fragments are indicated on the right-hand side of the blot. The correct integration of the *MDR1P-GFP* fusion at the *CDR4* locus reduces the size of one 3.46-kb *Pst*I fragment representing the intact *CDR4* gene (Fig. 1) to 2.4 kb.

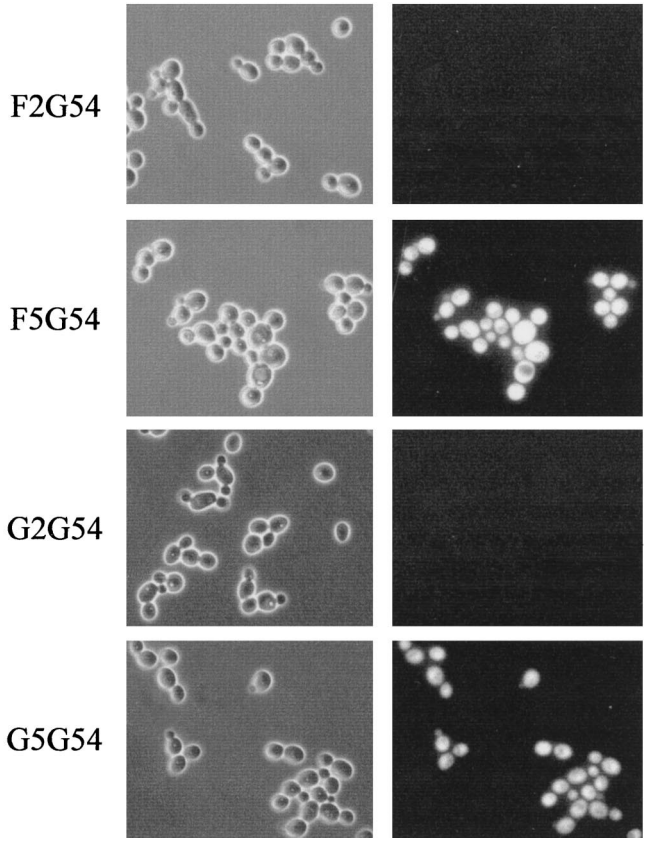

FIG. 3. Phase-contrast (left) and corresponding fluorescence (right) micrographs of transformants containing the chromosomally integrated *MDR1P-GFP* fusion.

DISCUSSION

For many clinical *C. albicans* strains that became fluconazole resistant during therapy, a correlation between drug resistance and activation of the *MDR1* gene has been found by several independent research groups (8, 14, 24, 29). In most cases, fluconazole resistance and *MDR1* expression are stable phenotypes that are maintained after in vitro passage of the clinical isolates in the absence of selection pressure by the drug. However, as with the stable overexpression of the ABC transporters *CDR1* and *CDR2*, the genetic basis for the constitutive activation of the *MDR1* gene in such strains has not been elucidated. In *Saccharomyces cerevisiae* several regulatory proteins (Pdr1p, Pdr3p, and Yap1p) controlling expression of multiple drug resistance genes of the ABC transporter and major-facilitator superfamilies are known (1, 3, 5, 11, 12, 15, 16, 20, 28, 30), and mutations in these regulators that result in upregulation of their respective target genes have been identified (4, 19). Recently, functional homologues of these regulators have also been found in *C. albicans* (1, 27); however, conflicting data about the roles of these transcriptional regulators have been obtained. Overexpression of the *YAP1* homologue *CAP1* in *S. cerevisiae* resulted in resistance of the transformants against fluconazole and other drugs that was mediated by the transcriptional activation of the *FLR1* gene encoding a major facilitator homologous to the *C. albicans* Mdr1 protein (1). Overexpression of a mutated form of *CAP1*, but not wild-type *CAP1*, in *C. albicans* CAI4 resulted in activation of the *MDR1* gene and, concomitantly, resistance against fluconazole and several other drugs (2), suggesting the possibility that similar mutations might also be responsible for *MDR1* activation in fluconazole-resistant clinical *C. albicans* isolates. On the other hand, disruption of the *CAP1* gene in the *MDR1*-overexpressing, fluconazole-resistant *C. albicans* strain FR2 did not suppress but further increased the level of *MDR1* expression, and it was concluded that *CAP1* was a negative regulator of *MDR1* that was not responsible for *MDR1* activation in this strain (2). Similarly, the *C. albicans* transcriptional regulator *FCR1* was identified by functional complementation of an *S. cerevisiae pdr1 pdr3* mutant (27). Overexpression of *FCR1* in this *S. cerevisiae* mutant resulted in fluconazole resistance that was mediated by the transcriptional activation of the ABC transporter *PDR5*. In contrast, disruption of *FCR1* in *C. albicans* resulted in hyperresistance against fluconazole, demonstrating that, similarly to *CAP1*, *FCR1* behaved as a transcriptional activator when overexpressed in *S. cerevisiae* but acted as a negative regulator of drug resistance in *C. albicans*. The transcriptional targets of *FCR1* in *C. albicans* have not been reported (27).

So far, none of the transcriptional regulators of drug resistance identified in *C. albicans* has been shown to be involved in the development of fluconazole resistance in clinical isolates, and it was suggested that mutations in the regulatory region of the multiple drug resistance genes themselves may be responsible for their overexpression in resistant isolates (27). This lack of knowledge about the molecular changes leading to activation of multiple drug resistance genes in clinical *C. albicans* strains is due to the fact that wild-type *C. albicans* is not easily accessible to genetic manipulation. The recent development of the dominant selection marker MPA^R (Theiss et al., unpublished) has eliminated this problem and allowed us to investigate the basis of *MDR1* activation in two different series of fluconazole-resistant, clinical *C. albicans* strains. Our results clearly demonstrate that in both cases *MDR1* activation was caused by mutations in a *trans*-regulatory factor, since the *MDR1* promoter from a fluconazole-susceptible *C. albicans* strain that did not detectably express the *MDR1* gene was activated in the two resistant isolates but not in the matched susceptible isolates. It is likely that a similar mechanism is responsible for *MDR1* activation in other fluconazole-resistant, clinical *C. albicans* strains and is, therefore, of general relevance. The mutations might directly affect a transcriptional activator or repressor binding to the *MDR1* regulatory region, but they may also involve regulatory proteins controlling the activity of transcription factors. To understand the mechanisms of drug resistance in more detail, it is necessary to elucidate the identity of the regulator(s), its mode of action, and the mutations occurring in drug-resistant, clinical *C. albicans* isolates that lead to constitutive expression of the *MDR1* gene.

ACKNOWLEDGMENTS

This study was supported by the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (BMBF grant O1 K1 8906-0). Gerwald Köhler was supported by the BMBF Stipendienprogramm Infektionsforschung.

REFERENCES

1. **Alarco, A.-M., I. Balan, D. Talibi, N. Mainville, and M. Raymond.** 1997. AP1-mediated multidrug resistance in *Saccharomyces cerevisiae* requires *FLR1* encoding a transporter of the major facilitator superfamily. J. Biol. Chem. **272:**19304–19313.
2. **Alarco, A.-M., and M. Raymond.** 1999. The bZip transcription factor Cap1p is involved in multidrug resistance and oxidative stress response in *Candida albicans*. J. Bacteriol. **181:**700–708.
3. **Balzi, E., M. Wang, S. Leterme, L. Van Dyck, and A. Goffeau.** 1994. *PDR5*, a novel yeast multidrug resistance conferring transporter controlled by the transcriptional regulator *PDR1*. J. Biol. Chem. **269:**2206–2214.
4. **Carvajal, E., H. B. van den Hasel, A. Cybularz-Kalaczkowska, E. Balzi, and A. Goffeau.** 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.
5. **Coleman, S. T., E. Tseng, and W. S. Moye-Rowley.** 1997. *Saccharomyces cerevisiae* basic region-leucine zipper protein regulatory networks converge at the *ATR1* structural gene. J. Biol. Chem. **272:**23224–23230.
6. **Fling, M. E., J. Kopf, A. Tamarkin, J. A. Gorman, H. A. Smith, and Y. Koltin.** 1991. Analysis of a *Candida albicans* gene that encodes a novel mechanism for resistance to benomyl and methothrexate. Mol. Gen. Genet. **227:**318–329.
7. **Fonzi, W. A., and M. Y. Irwin.** 1993. Isogenic strain construction and gene mapping in *Candida albicans*. Genetics **134:**717–728.
8. **Franz, R., S. L. Kelly, D. C. Lamb, D. E. Kelly, M. Ruhnke, and J. Morschhäuser.** 1998. Multiple molecular mechanisms contribute to a stepwise development of fluconazole resistance in clinical *Candida albicans* strains. Antimicrob. Agents Chemother. **42:**3065–3072.
9. **Franz, R., S. Michel, and J. Morschhäuser.** 1998. A fourth gene from the *Candida albicans CDR* family of ABC-transporters. Gene **220:**91–98.
10. **Ghannoum, M. A., J. H. Rex, and J. N. Galgiani.** 1996. Susceptibility testing of fungi: current status of correlation of in vitro data with clinical outcome. J. Clin. Microbiol. **34:**489–495.
11. **Katzmann, D. J., P. E. Burnett, J. Golin, Y. Mahé, and W. S. Moye-Rowley.** 1994. Transcriptional control of the yeast *PDR5* gene by the *PDR3* gene product. Mol. Cell. Biol. **14:**4653–4661.
12. **Katzmann, D. J., T. C. Hallstrom, M. Voet, W. Wysock, J. Golin, G. Volckaert, and W. S. Moye-Rowley.** 1995. Expression of an ATP-binding cassette transporter-encoding gene (*YOR1*) is required for oligomycin resistance in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **15:**6875–6883.
13. **Köhler, G. A., T. C. White, and N. Agabian.** 1997. Overexpression of a cloned IMP dehydrogenase gene of *Candida albicans* confers resistance to the specific inhibitor mycophenolic acid. J. Bacteriol. **179:**2331–2338.
14. **Lopez-Ribot, J. L., R. K. McAtee, L. N. Lee, W. R. Kirkpatrick, T. C. White, D. Sanglard, and T. F. Patterson.** 1998. Distinct patterns of gene expression associated with development of fluconazole resistance in serial *Candida albicans* isolates from human immunodeficiency virus-infected patients with oropharyngeal candidiasis. Antimicrob. Agents Chemother. **42:**2932–2937.
15. **Mahé, Y., A. Parle-McDermott, A. Nourani, A. Delahodde, A. Lamprecht, and K. Kuchler.** 1996. The ATP-binding cassette multidrug transporter Snq2 of *Saccharomyces cerevisiae*: a novel target for the transcription factors Pdr1 and Pdr3. Mol. Microbiol. **20:**109–117.
16. **Meyers, S., W. Schauer, E. Balzi, M. Wagner, A. Goffeau, and J. Golin.** 1992. Interaction of the yeast pleiotropic drug resistance genes *PDR1* and *PDR5*. Curr. Genet. **21:**431–436.
17. **Millon, L., A. Manteaux, G. Reboux, C. Drobacheff, M. Monod, T. Barale, and Y. Michel-Briand.** 1994. Fluconazole-resistant recurrent oral candidiasis

in human immunodeficiency virus-positive patients: persistence of *Candida albicans* strains with the same genotype. J. Clin. Microbiol. **32:**1115–1118.
18. **Morschhäuser, J., S. Michel, and J. Hacker.** 1998. Expression of a chromosomally integrated, single-copy *GFP* gene in *Candida albicans*, and its use as a reporter of gene regulation. Mol. Gen. Genet. **257:**412–420.
19. **Nourani, A., D. Papajova, A. Delahodde, C. Jacq, and J. Subik.** 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.
20. **Nourani, A., M. Wesolowski-Louvel, T. Delaveau, C. Jacq, and A. Delahodde.** 1997. Multiple-drug-resistance phenomenon in the yeast *Saccharomyces cerevisiae*: involvement of two hexose transporters. Mol. Cell. Biol. **17:**5453–5460.
21. **Odds, F. C.** 1988. *Candida* and candidosis: a review and bibliography. Bailliere Tindall, London, United Kingdom.
22. **Rex, J. H., M. G. Rinaldi, and M. A. Pfaller.** 1995. Resistance of *Candida* species to fluconazole. Antimicrob. Agents Chemother. **39:**1–8.
23. **Rupp, S., E. Summers, H.-J. Lo, H. Madhani, and G. Fink.** 1999. MAP kinase and cAMP filamentation signaling pathways converge on the unusually large promoter of the yeast *FLO11* gene. EMBO J. **18:**1257–1269.
24. **Sanglard, D., K. Kuchler, F. Ischer, J.-L. Pagani, M. Monod, and J. Bille.** 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.
25. **Sanglard, D., F. Ischer, M. Monod, and J. Bille.** 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.
26. **Staib, P., M. Kretschmar, T. Nichterlein, G. Köhler, S. Michel, H. Hof, J. Hacker, and J. Morschhäuser.** 1999. Host-induced, stage-specific virulence gene activation in *Candida albicans* during infection. Mol. Microbiol. **32:**533–546.
27. **Talibi, D., and M. Raymond.** 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.
28. **Wemmie, J. A., M. S. Szczypka, D. J. Thiele, and W. S. Moye-Rowley.** 1994. Cadmium tolerance mediated by the yeast AP-1 protein requires the presence of an ATP-binding cassette transporter-encoding gene, *YCF1*. J. Biol. Chem. **269:**32592–32597.
29. **White, T. C.** 1997. Increased mRNA levels of *ERG16*, *CDR*, and *MDR1* correlate with increases in azole resistance in *Candida albicans* isolates from a patient infected with human immunodeficiency virus. Antimicrob. Agents Chemother. **41:**1482–1487.
30. **Wolfger, H., Y. Mahé, A. Parle-McDermott, A. Delahodde, and K. Kuchler.** 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.