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Role of ABC Transporters in Aureobasidin A Resistance

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Aureobasidin A (AbA) has strong antifungal effects arising from an unusual mechanism. We show that AbA interacts with ATP-binding cassette (ABC) transporters in yeast and mammalian cells. We isolated a gene of *Saccharomyces cerevisiae* that conferred resistance to AbA when the gene was present in multiple copies. The gene was identical to *YOR1*/*YRS1*, which confers resistance to oligomycin, reveromycin, and organic anions, none of which have structures similar to that of AbA. We also isolated an *aur3*^{R} recessive mutant of *S. cerevisiae* with increased resistance to AbA. Northern hybridization showed that the *aur3*^{R} mutant expressed not only *YOR1* but also the ABC transporter-encoding gene *PDR5* at high levels. Genetic studies showed that the *aur3*^{R} mutant had a mutation in the *PDR1* gene, which encodes a transcriptional regulator of *PDR5* and *YOR1*. Analysis of a *yor1* disruptant of the *aur3*/*pdr1* mutant showed that both the functional *YOR1* gene and the mutation in *PDR1* were necessary for AbA resistance. These results suggest that *YOR1* is more important than *PDR5* for AbA resistance. We found in *Candida albicans* a novel gene whose sequence was similar to the sequence of *YOR1* in *S. cerevisiae*. The amino acid sequence of the *C. albicans YOR1* homolog showed no significant similarity to the sequences of *CDR1* and *CDR2*, which are ABC transporters of *C. albicans*. Furthermore, AbA inhibited the efflux of the anticancer agent vincristine through P glycoproteins in cancer cells with multidrug resistance.

Aureobasidin A (AbA) is an antifungal antibiotic produced by *Aureobasidium pullulans* R106. It is a cyclic depsipeptide with a molecular weight of 1,100, and it contains eight amino acids and a hydroxy acid (15, 33). AbA is active against a variety of fungi including the budding yeast *Saccharomyces cerevisiae*, killing it by inhibiting bud growth due to abnormal deposition of actin and chitin (9). To identify the mechanism of action of AbA against yeasts, we and another group isolated an AbA resistance gene from the budding yeast (12, 13). The dominant resistance gene isolated from *S. cerevisiae*, *AUR1*, encodes a putative integral membrane protein and is essential for growth. The protein encoded by the *AUR1* gene is associated with the activity of inositol phosphatidylceramide synthase, which is involved in sphingolipid synthesis (20), and seems to be an intracellular target in the resistance (12).

The resistance of tumor cells and pathogenic fungi to some chemotherapeutic drugs is a problem in the treatment of cancer and fungal infections. The multidrug resistance of tumors is caused by overexpression of a 170-kDa plasma membrane protein, the P glycoprotein belonging to the superfamily of ATP-binding cassette (ABC) transporters (8, 11, 14). In recent years, the frequent use of the antifungal agent fluconazole for the treatment of oropharyngeal candidiasis in AIDS patients has caused the appearance of *C. albicans* strains resistant to this and other azoles. Resistance to antifungal agents in *C. albicans* can be mediated by multidrug efflux transporters (26). Multidrug transporters are divided into two classes: the ABC multidrug transporters (22, 27) and the major facilitated transporter (10). Furthermore, ABC transporter proteins are classified into two subgroups according to their structures (14). One is the MDR subgroup represented by the mammalian multidrug resistance P glycoprotein (encoded by the *MDR1* gene), and the other is the CFTR subgroup represented by human cystic fibrosis transmembrane conductance regulator (CFTR) (23) and multidrug resistance-associated protein (MRP1) (5). In human tumor cells, two ABC transporter genes, *MDR1* and *MRP1*, elicit multidrug resistance when the genes are overexpressed. The budding yeast *S. cerevisiae* also has several ABC transporters from each subgroup. The mating factor transporter gene *STE6* (18), the pleiotropic drug resistance gene *PDR5*/*STS1* (1, 4), and the 4-nitroquinoline-*N*-oxide resistance gene *SNQ2* (29) have sequences similar to the sequence of MDR1. The *PDR5* and *SNQ2* genes are involved in the cross-resistance of *S. cerevisiae* to antifungal azole drugs (19, 26). Pdr5p contributes to the resistance by pumping azoles out of the cell. A gene with a sequence similar to that of *PDR5* in *C. albicans*, *CDR1* (22), is overexpressed in azole-resistant clinical isolates (26). In contrast, a cadmium resistance gene (*YCF1*) and a gene involved in resistance to oligomycin and organic anions, *YOR1*/*YRS1*, encode proteins of the CFTR subgroup in *S. cerevisiae* (6, 16, 32).

In this paper, we report the role of ABC transporters in the AbA resistance of *S. cerevisiae*. AbA was a substrate of ABC transporters in both *S. cerevisiae* and human tumor cells.

MATERIALS AND METHODS

Yeast strains, media, and genetic methods. The *S. cerevisiae* strains used in this study are listed in Table 1. *Candida albicans* TIMM 0136 was also used. Yeast cells were grown aerobically in YPD (1% yeast extract, 2% Bacto Peptone, 2% dextrose) at 30°C. Synthetic minimal medium (SD; 2% glucose, 0.7% yeast nitrogen base without amino acids, appropriate amino acid supplements) and sporulation medium (1% potassium acetate) were used. Standard genetic techniques of crossing, sporulation, and tetrad analysis were performed as described by Sherman et al. (30).

Bioassay of drug sensitivity. The sensitivities of yeast cells to various drugs and toxic compounds were assayed by measuring the MIC as follows. Yeast cells suspended in water were streaked with sterile toothpicks onto YPD agar plates containing various concentrations of drugs or other compounds. The plates were incubated at 30°C for 2 days.

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TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype	Reference or source
SH3328	*MAT*α *his4 leu2-3,112 thr4 ura3-52*	S. Harashima
DKD-5D	*MAT***a** *his3 leu2-3,112 trp1*	S. Harashima
AL22-4A	*MAT***a** *his3 leu2-3,112 trp1 pdr1*^{R}-A1	This study
AL49-3A	*MAT***a** *his3 leu2-3,112 trp1 pdr1*^{R}-A3	This study
AL33-9C	*MAT*α *his4 leu2-3,112 thr4 pdr1*^{R}-A2	This study
AL33-18C	*MAT***a** *his4 leu2-3,112 trp1 ura3-52 pdr1*^{R}-A2	This study
A141-9A	*MAT*α *his4 leu2-3,112 thr4 ura3-52 yor1*::*URA3*	This study
AR9-4A	*MAT***a** *his3 leu2-3,112 trp1 AUR*^{R} *1-1*	Hashida-Okado et al. (12)
AOD1	*MAT***a**/*MAT*α *trp1*/+ +/*thr4* +/*his4 his3*/+ *ura3-52*/*ura3-52 leu2-3,112*/*leu2-3,112*	Hashida-Okado et al. (12)
AOD3	*MAT***a**/*MAT*α *trp1*/+ +/*thr4* +/*his4 his3*/+ *ura3-52*/*ura3-52 leu2-3,112*/*leu2-3,112* *yor1*::*URA3*/+	This study
A07	*MAT*α *his4 leu2-3,112 thr4 ura3-52* (pWL7)	This study
T73-1	*MAT***a** *his3 leu2-3,112 trp1 pdr1*^{R}::*LEU2*	This study

Isolation and sequencing of *YOR1*. Standard molecular cloning techniques were performed as described by Sambrook et al. (25). For construction of a genomic DNA library, chromosomal DNA was isolated from AbA-resistant mutant AR9-4A and wild-type strain DKD-5D of *S. cerevisiae*, as reported by Philipsen et al. (21). Each DNA was partially digested with *Hin*dIII, and fragments of 3 to 15 kb were obtained by agarose gel electrophoresis. The fragments were ligated to a *Hin*dIII-digested pWH5 vector and were then introduced into *Escherichia coli* HB101. The mutant genomic library was introduced by the modified lithium acetate procedure (28) into the wild-type strain SH3328, for which the MIC of AbA was 0.4 μg/ml. Colonies of Leu^{+} transformants were replicated on YPD agar plates with AbA at 1.5 μg/ml. From one transformant for which the MIC was 5 μg/ml, plasmid DNA was recovered and was designated pWL7. The ability of pWL7 to confer AbA resistance was checked by reintroduction of pWL7 into the wild-type strain.

Isolation of *YOR1* homolog of *C. albicans*. Chromosomal DNA was isolated from *C. albicans* TIMM 0136 as described by Philippsen et al. (21). The DNA was partially digested with either *Hin*dIII or *Bam*HI and was ligated to pTV119 for construction of genomic DNA libraries. Plasmid pA8.3 containing the *YOR1* homolog of *C. albicans* was isolated from the library by colony hybridization with a 1.2-kb *Hin*dIII-*Pst*I fragment of *S. cerevisiae YOR1* (see Fig. 1) as the probe. Plasmid pA6.5 was isolated by colony hybridization with a PCR product containing the carboxyl-terminal region of pA8.3 as the probe.

Isolation of AbA-resistant mutants. The *S. cerevisiae* wild-type strain DKD-5D formed no colonies on YPD agar plates containing AbA at 0.4 μg/ml. Cells grown in YPD liquid medium were suspended in 0.2 M phosphate buffer (pH 8.0) containing 0.2% glucose and were treated with 3% ethyl methanesulfonate for 90 min, leaving about 40% of the cells viable. Mutagenized cells were cultured overnight in YPD medium containing 1 μg of AbA per ml and were plated onto YPD agar plates containing 1 μg of AbA per ml. The MICs of the antifungal agents cycloheximide, miconazole, amphotericin B, and AbA for the mutants were tested.

Gene disruption. Gene disruption was done by a one-step method (24). For disruption of the *YOR1* gene, an 8.5-kb *Hin*dIII fragment of pWL7 was subcloned into pUC119, and the plasmid obtained was cleaved with *Bst*XI and blunted with T4 DNA polymerase. A 1.1-kb *Hin*dIII-*Eco*RI fragment of *URA3* was blunted and inserted into the blunted *Bst*XI sites of pWL7. By ligation of the blunted *Bst*XI and *Hin*dIII, a *Hin*dIII recognition sequence was created. A linear 4-kb fragment containing *yor1*::*URA3* was obtained by *Eco*RI digestion. This linear fragment was introduced into diploid strain AOD1, which was spread onto SD plates without uracil. The stable Ura^{+} transformants obtained were sporulated, and the tetrads that were obtained were dissected on YPD agar plates. All four spores from the tetrads formed colonies, indicating that *YOR1* is not essential for growth. The segregation of Ura^{+}:Ura^{-} strains was 2:2. Disruption of *YOR1* was confirmed by Southern hybridization of genomic DNAs from each of the four segregants. The pattern of hybridizing bands was identical to that expected from the restriction map, with the results showing disruption of the *YOR1* gene of the Ura^{+} spores.

For the disruption of *PDR1*, a DNA fragment containing this gene was obtained by PCR. The primers used for PCR were 5′-ATCTTCGATATCATCTG CAGGG-3′ (positions +1032 to +1053) as the 5′ primer and 5′-TGCTGAGC GACCATTGAATGGC-3′ (positions +2820 to +2799) as the 3′ primer; the primers were based on the DNA sequence of *PDR1* (1). Amplification by PCR was done with *S. cerevisiae* genomic DNA as the template. An amplified fragment was cloned into the *Hin*cII site of modified pUC19 lacking the *Hin*dIII site, generating pUCPDR1. The 0.8-kb *Hin*dIII fragment in *PDR1* was replaced with a 2.2-kb *Hin*dIII fragment containing *LEU2*. The resulting plasmid, pUCPDR1::LEU2, was digested with *Sph*I and *Bam*HI to generate a linear 3.1-kb fragment, which was introduced into the *aur3* mutant AL22-4A.

DNA and RNA analysis. Southern hybridization was done as described by Sambrook et al. (25). *S. cerevisiae* RNA for Northern hybridization was prepared as described previously (12). The quantitation of the RNA was done by measuring the autoradiograph by densitometry. The *PDR5* probe was obtained by PCR with oligonucleotides 5′-ACGTTACTAGCTACTCCTCCG-3′ (positions +35 to +55 of *PDR5*) as the 5′ primer, 5′-TTATTGAACAAGTCGTACGC-3′ (positions +1136 to +1117) as the 3′ primer, and *S. cerevisiae* genomic DNA as the template. The resulting 1.1-kb fragment was labeled and used as the probe.

Intracellular accumulation of [^{3}H]vincristine. AbA and verapamil were dissolved in dimethyl sulfoxide and diluted with phosphate-buffered saline (pH 7.2). An adriamycin-resistant cell line, A2780AD, of a human ovarian tumor was used as described previously (34). In brief, A2780AD cells (10^{6}/ml) in RPMI 1640 medium containing 5% fetal calf serum and 100 μg of kanamycin per ml were plated in wells of 24-well tissue culture dishes. After incubation of the cells at 37°C for 24 h, [^{3}H]vincristine (222 GBq/mmol; Amersham) was added to a final concentration of 20 nM. Then various concentrations of drugs in a volume of 5 μl or the same volume of saline was added. After incubation of the cells at 37°C for 2 h, the intracellular concentration of vincristine was assayed. Means for triplicate samples were calculated.

Nucleotide sequence accession number. The *C. albicans YOR1* gene sequence has been deposited in the GenBank database under accession no. AF034608.

RESULTS

Cloning of a gene conferring AbA resistance. While isolating an *AUR1*^{R} mutant gene from a genomic library of resistant mutants constructed with the multicopy vector pWH5, we obtained a transformant with somewhat more AbA resistance than wild-type cells; the transformant grew on YPD agar plates containing 1.5 μg of AbA per ml but not on plates containing 5 μg of AbA per ml. Most of the other transformants obtained had higher levels of resistance, even growing in the presence of 25 μg of AbA per ml, so resistance was high; the *AUR1*^{R} gene has been recovered from such transformants (12). Plasmid pWL7, which contained an 8.5-kb DNA fragment, was recovered from the transformant with the lowest level of resistance. Digested DNA fragments derived from the 8.5-kb insert shown in Fig. 1 did not confer AbA resistance on wild-type cells, so the insert contained an AbA resistance gene other than *AUR1*^{R}. The ability of pWL7 to confer AbA resistance was checked by reintroduction of pWL7 into wild-type strain SH3328. Nucleotide sequencing showed that the insert DNA had a large open reading frame (ORF) of 4,431 bp encoding a protein of 1,477 amino acid residues. Searches for sequences similar to the predicted polypeptide sequence have shown that the sequence of the ORF is identical to that of *YOR1*/*YRS1*, which confers resistance to oligomycin, reveromycin, and organic anions (6, 16). Yor1p/Yrs1p is a member of the ABC transporter superfamily, like MDR1 and CFTR, and is most closely related to human MRP1 (5) and the cadmium resistance factor Ycf1p in *S. cerevisiae* (32). We constructed by one-step gene disruption a strain depleted of the *YOR1* gene. The disrupted cells (Ura^{+}; clones b and c, Fig. 2) did not grow on YPD agar plates containing 0.2 μg of AbA per ml, but the

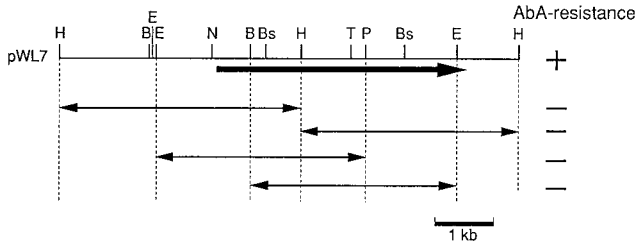


FIG. 1. Restriction map and subcloning of the *YOR1* locus. The restriction map of an 8.5-kb genomic insert of pWL7 is shown at the top. The thick arrow indicates the location and direction of an ORF. DNAs subcloned on the pWH5 vector were examined for their ability to confer AbA resistance on wild-type cells. B, *Bam*HI; Bs, *Bst*XI; E, *Eco*RI; H, *Hin*dIII; N, *Nhe*I; P, *Pst*I; T, *Tth*111I. +, resistance conferred; −, resistance not conferred.

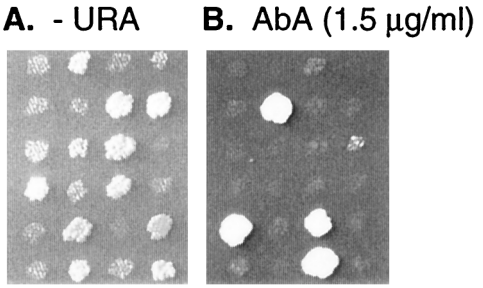


FIG. 3. Correlation between the *YOR1* gene and the *aur3*^{R} mutation in AbA resistance. *yor1*-disrupted haploid A141-9A cells were crossed with *aur3*^{R} mutant AL33-18C, and the diploids obtained were allowed to sporulate. Tetrad segregants from the diploids were streaked onto YPD agar plates, the plates were incubated at 30°C for 2 days, and the segregants were replicated on a YPD agar plate with 1.5 μg of AbA per ml (B) or an SD plate without uracil (−URA) (A).

YOR1$^+$ cells (clones a and b, Fig. 2) grew well. This result showed that the *yor1*-null cells were hypersensitive to AbA. Next, we examined the sensitivities of wild-type (DKD-5D, SH3328), *yor1*-null (A141-9A), multicopy *YOR1*-containing (A07), and *aur3*^{R} (AL22-3A; see below) cells to various drugs and compounds on YPD agar plates (Table 2). The MICs of these drugs and compounds on YPD agar plates were the same for these strains and the wild-type strain. This result indicated that the *YOR1* gene is specifically involved in resistance to AbA.

Overexpression of the *YOR1* gene by *aur3* mutation. We searched for mutants that were specifically resistant to AbA and that grew in the presence of 1 μg of AbA per ml after mutagenization of wild-type DKD-5D cells. Several haploid AbA-resistant mutants were crossed with wild-type strain SH3328, and heterozygous diploids were obtained. The diploids derived from three mutants, AL22-4A, AL33-9C, and AL49-3A, were as sensitive to AbA as the wild-type strain, indicating that in these mutants the mutations are recessive. In contrast, other mutants had higher levels of resistance (to more than 25 μg of AbA per ml) than those of the three AL mutants, indicating that the mutations in these mutants are dominant, and the mutations were in the *aur1*$^+$ gene. Analysis of tetrads from the diploids of the AL mutants indicated that they all carried a single chromosomal mutation, and complementation tests showed that the mutations were all in one locus, designated *aur3*. Analysis of tetrads from the diploids obtained by crosses between *aur3*^{R} mutant AL33-9C and *AUR1*^{R} mutant AR9-4A indicated that *aur3* is not an allele of *AUR1* (data not shown). Tetrads from diploids obtained by crosses between the *yor1*::*URA3* mutant A141-9A and *aur3*^{R} mutant AL33-18C were tested for resistance to AbA at 1.5 μg/ml and for the Ura$^+$ phenotype. Of the 22 four-spored tetrads analyzed, 4 tetrads had two resistant spores, 15 tetrads had one resistant spore, and 3 tetrads had no resistant spores. This ratio, 4:15:3, is close to 1:4:1, showing that *aur3* is not linked genetically with *YOR1*. All Ura$^+$ spores having the *yor1*-null allele, some of which had the *aur3*^{R} mutation, were sensitive to AbA (Fig. 3), suggesting that a functional *YOR1* gene is needed for the *aur3*^{R} mutant to be resistant to AbA. These results also suggest the possibility that the *aur3*^{R} mutation causes overexpression of the *YOR1* gene, leading to resistance to AbA, although the possibility that the function of *AUR3* may be downstream of the *YOR1* gene function remains.

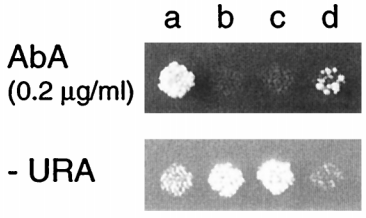


FIG. 2. Sensitivity of *yor1*-disrupted cells to AbA. Tetrads derived from heterozygous diploid strain AOD3 (*YOR1*/*yor1*::*URA3*) were incubated on YPD agar plates and replicated onto YPD agar plates with 0.2 μg of AbA per ml or SD plates without uracil (−URA). Drug supersensitivity and Ura$^+$ also segregated together in other tetrads (data not shown).

To find whether expression of the *YOR1* gene was controlled by *AUR3*, the *YOR1* mRNA in *aur3*^{R} mutants was examined by Northern hybridization (Fig. 4A). The AL22-4A mutant contained 20-fold as much *YOR1* mRNA as the parental strain. The mutant also overexpressed mRNA of another ABC transporter gene, *PDR5*. Southern blot analysis (Fig. 4B) showed that mutant AL22-4A had the same number of copies of *YOR1* as the parental strain. These results suggest that the transcription of both *YOR1* and *PDR5* is regulated by the *AUR3* gene product and that overexpression of the *YOR1* gene occurs because its regulation is abnormal as a result of a mutation in the *AUR3*$^+$ gene. Genetic mapping by tetrad analysis showed that the *aur3* locus is linked loosely to the centromere (for *aur3-trp1*, parental ditype:nonparental ditype:tetrad [PD:NPD:T] = 18:17:5; for *aur3-met14*, PD:NPD:T = 7:6:0) and is linked tightly to *LEU1* (PD:NPD:T = 12:0:0) on chromosome VII. *LEU1* has been mapped to a position near *PDR1* (1), a transcriptional regulator gene of *PDR5* and *YOR1*, suggesting that the mutation in the *aur3*^{R} mutants was in the *PDR1* locus. To examine this suggestion, we introduced *pdr1*::*LEU2* DNA into *aur3* mutant AL22-4A to disrupt the *PDR1* gene and selected Leu$^+$ transformants. All transformants had lost their resistance to AbA and had the same sensitivity to AbA as wild-type cells (Fig. 5). Furthermore, one of these transformants was crossed with wild-type strain SH3328, and the diploid that was obtained was sporulated. Segregation of the Leu$^+$ and AbA resistance phenotype among the resulting spores was examined by random spore analysis (Fig. 5). No AbA-resistant clone (*aur3*^{R} *PDR1*) appeared among ~10^4 viable segregants. These results indicate that *AUR3* is identical to *PDR1*. Therefore, the *aur3*^{R} mutations in strains AL22-4A, AL33-9C, and AL49-3A were designated *pdr1*^{R}-A1, *pdr1*^{R}-A2, and *pdr1*^{R}-A3, respectively.

Isolation of *YOR1* homolog from *C. albicans*. To search for a *C. albicans* gene with a sequence similar to that of the *S. cerevisiae YOR1* gene, Southern hybridization of genomic DNA

TABLE 2. Sensitivities of the *yor1* gene mutants

Compound (MIC units)	MIC[a]				
	DKD-5D (wild type)	SH3328 (wild type)	AL22-4A (*aur3*^{R})	A141-9A (*yor1*::*URA3*)	A07 (pWL7)
AbA (μg/ml)	0.4	0.4	>25	0.2	5.0
Cycloheximide (μg/ml)	0.2	0.4	0.2	0.2	0.2
Miconazole (μg/ml)	1.0	1.0	1.0	1.0	1.0
Amphotericin B (μg/ml)	12.5	6.3	6.3	6.3	3.2
$CdCl_2$ (μg/ml)	50	50	50	50	50
LiCl (μg/ml)	8.4	4.2	8.4	4.2	4.2
$CaCl_2$ (mg/ml)	55.5	11.1	11.1	27.8	27.8
NaCl (mg/ml)	87.6	87.6	87.6	87.6	87.6

[a] MICs were determined on YPD (pH 6.4) agar plates containing each compound. The plates were incubated at 30°C for 2 days.

of *C. albicans* was performed with a 1.2-kb *Hin*dIII-*Pst*I fragment of *S. cerevisiae YOR1* (Fig. 1) as a probe. A single band hybridized with the probe (data not shown), indicating that *C. albicans* has a gene hybridizable to the *YOR1* gene and, therefore, a gene with a high degree of sequence similarity to it. The *YOR1* homolog was isolated from the genomic DNA library of *C. albicans* by hybridization with the same probe described above. A plasmid, pA8.3, that contained an 8.3-kb *Bam*HI fragment was selected. Nucleotide sequencing of the fragment showed that it lacked the region coding for the carboxyl terminus of the protein. A residual coding region, plasmid pA6.5, was isolated by screening of another genomic library with a part of the 8.3-kb fragment as a probe. Figure 6A shows a restriction map of these DNA fragments. Parts of the 8.3-kb *Bam*HI and 6.5-kb *Hin*dIII fragments were sequenced. The results indicated that the predicted amino acid sequence had a high degree of similarity (61%) to the sequence at residues 1107 to 1352 of *S. cerevisiae* Yor1p (Fig. 6B and C), but no similarity to the sequences of *CDR1* (22) and *CDR2* (27), which have been identified as multidrug resistance genes in *C. albicans*. Therefore, this gene was designated *YOR1* of *C. albicans.*

Inhibition of ABC transporter by AbA. To identify the direct effect of AbA on the ABC transporter, we used multidrug-resistant tumor cells and tested AbA for its ability to inhibit the efflux of vincristine out of the cells. This efflux is caused by the P glycoprotein. AbA had no cytotoxic effects on the cells at the concentrations tested. AbA caused as much of an increase in the amount of vincristine that accumulated in A2780AD cells (Table 3) as verapamil did. This result indicates that AbA inhibits the drug efflux caused by mammalian ABC transporters.

DISCUSSION

We showed that overexpression of *YOR1*/*YRS1* confers resistance to AbA. Katzmann et al. (16) have shown that the

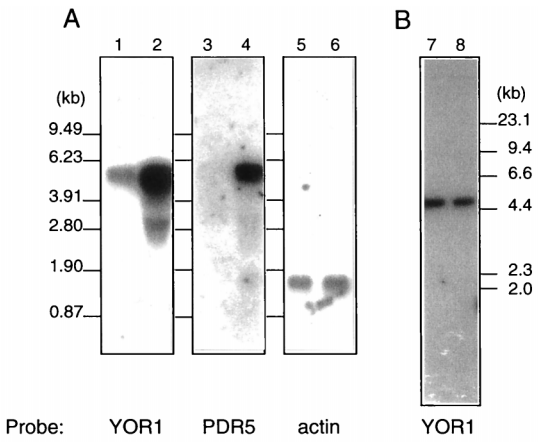


FIG. 4. Northern blot analysis of the *YOR1* gene in *aur3*^{R} mutants. (A) Total RNA from wild-type DKD-5D cells (lanes 1, 3, and 5) or *aur3*^{R} mutant AL22-4A cells (lanes 2, 4, and 6) was studied by Northern hybridization. The probes used were a 1.2-kb *Hin*dIII-*Pst*I fragment of *YOR1* (lanes 1 and 2), a 1.1-kb *PDR5* fragment (lanes 3 and 4), and a 1.5-kb actin DNA fragment (lanes 5 and 6). (B) Genomic DNAs from DKD-5D cells (lane 7) and AL22-4A cells (lane 8) were digested with *Hin*dIII and studied by Southern hybridization with the 1.0-kb *Nhe*I-*Bst*XI fragment of *YOR1* as the probe (see Fig. 1).

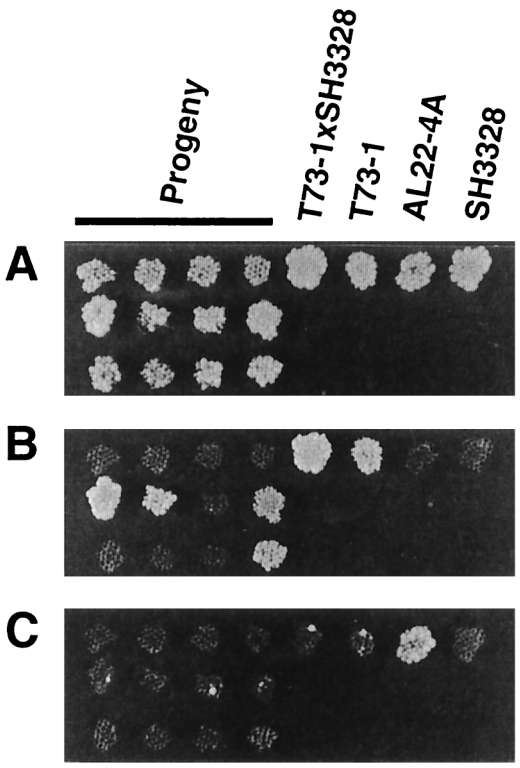
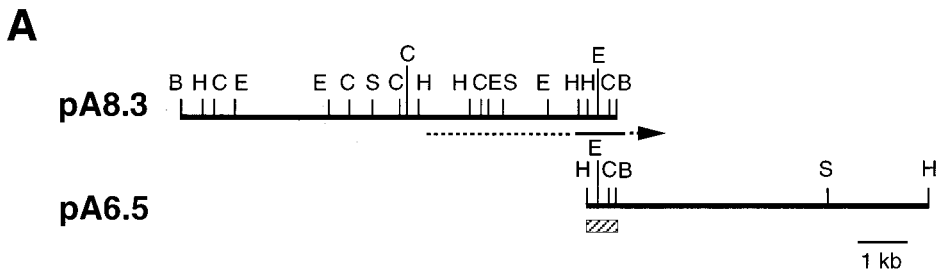


FIG. 5. Genetic analysis of a linkage between *aur3* and *PDR1*. The wild-type strain (SH3328), *aur3*^{R} mutant AL22-4A, and *aur3*^{R} mutant with a disrupted *PDR1* gene T73-1, a diploid strain (T73-1 × SH3328), and random spore isolates derived from the diploid cells were streaked onto a YPD agar plate. The plate was incubated at 30°C for 2 days and then replicated onto YPD agar plates (A), an SD plate lacking leucine (B), and a YPD agar plate containing 2 μg of AbA per ml (C). Incubation was carried out for 2 days at 30°C. No progeny showing AbA resistance appeared. Only 12 of the progenies examined are shown.



B

```
   1 AAGCTTATTATATCACAATTGCTAATCAACGTTGGTTAGCCATTCATATGGATTTTGTGG
     A  Y  Y  I  T  I  A  N  Q  R  W  L  A  I  H  M  D  F  V

  61 CTGCACTTTTCGCATTATTAATTGCCTTACTTTGTGTTAATCGAGTTTTCAATATTAGTG
     A  A  L  F  A  L  L  I  A  L  L  C  V  N  R  V  F  N  I  S

 121 CTTCATCAGTTGGGTTAATTGTATCATATGTTTTCCAAATTGCTGGTCAATTATCCATGC
     A  S  S  V  G  L  I  V  S  Y  V  F  Q  I  A  G  Q  L  S  M

 181 TTATTAGAACTTTCACTCAAGTGGAAAATGAAATGAATTCTGCCGAAAGATTACATACTT
     L  I  R  T  F  T  Q  V  E  N  E  M  N  S  A  E  R  L  H  T

 241 ATGCACAAAATTTACCTAAAGAAGCTCCTTATGTCATTACCGAAAATACTCCACCACCAA
     Y  A  Q  N  L  P  K  E  A  P  Y  V  I  T  E  N  T  P  P  P

 301 ATTGGCCCCACAGGGGAGCTATTGAATTTGATAATGCTTCATTAGCTTATAGACCAGGAT
     N  W  P  H  R  G  A  I  E  F  D  N  A  S  L  A  Y  R  P  G

 361 TACCATTAGTTTTGAAAAATTTGAATTTTAAAATTAATCCAATGGAAAAAATTGGTATTT
     L  P  L  V  L  K  N  L  N  F  K  I  N  P  M  E  K  I  G  I

 421 GTGGAAGAACCGGTGCTGGTAAATCATCAATTATGACAGCATTATATCGATTATCAGAAT
     C  G  R  T  G  A  G  K  S  S  I  M  T  A  L  Y  R  L  S  E

 481 TAGAACTGGGGAAAATTATTATTGATGATATTGATATTTCAACTTTGGGTTTAAAAGATC
     L  E  L  G  K  I  I  I  D  D  I  D  I  S  T  L  G  L  K  D

 541 TTCGATCAAAATTATCAATTATTCCTCAAGATCCAGTATTATTCCGAGGTTCAATTCGGA
     L  R  S  K  L  S  I  I  P  Q  D  P  V  L  F  R  G  S  I  R

 601 AAAACTTGGATCCATTCAATGAACATTCCGATGATAAACTTTGGGATGCATTAAGACGTA
     K  N  L  D  P  F  N  E  H  S  D  D  K  L  W  D  A  L  R  R

 661 CTGGATTAATTGAAGAATCAAGATTAGATATCGTTAAGAAACAAATCAAACCAACCATTG
     T  G  L  I  E  E  S  R  L  D  I  V  K  K  Q  I  K  P  T  I

 721 GACACAAGAACCAGGACACC
     G  H  K  N  Q  D  T
```

C

```
                    10        20        30        40        50        60
CaYOR1      AYYITIANQRWLAIHMDFVAALFALLIALLCVNRVFNISASSVGLIVSYVFQIAGQLSML
            : :. .  :::. : .: ::  :::.: :::: : : :::.:::.....:: :. : :. .
ScYOR1      AGYLVVVLQRWVGIFLDMVAIAFALIITLLCVTRAFPISAASVGVLLTYVLQLPGLLNTI
            1110      1120      1130      1140      1150      1160
                   70        80        90       100       110       120
CaYOR1      IRTFTQVENEMNSAERLHTYAQNLPKEAPYVITENTPPPNWPHRGAIEFDNASLAYRPGL
            .:  :: ::.:::::: :::  :: :: :   : ::: .::  : : :.:   ::::::
ScYOR1      LRAMTQTENDMNSAERLVTYATELPLEASYRKPEMTPPESWPSMGEIIFENVDFAYRPGL
            1170      1180      1190      1200      1210      1220
                  130       140       150       160       170       180
CaYOR1      PLVLKNLNFKINPMEKIGICGRTGAGKSSIMTALYRLSELELGKIIIDDIDISTLGLKDL
            :.:::::  :   :::::::::::::::.::.::::::.::  :::.:::..::: ::: ::
ScYOR1      PIVLKNLNLNIKSGEKIGICGRTGAGKSTIMSALYRLNELTAGKILIDNVDISQLGLFDL
            1230      1240      1250      1260      1270      1280
                  190       200       210       220       230       240
CaYOR1      RSKLSIIPQDPVLFRGSIRKNLDPFNEHSDDKLWDALRRTGLIEESRLDIVKKQIKPTIG
            : ::.:::::::::::.:::::::::: .::.::::: : : : .  :  :: :     :
ScYOR1      RRKLAIIPQDPVLFRGTIRKNLDPFNERTDDELWDALVRGGAIAKDDLPEVKLQKPDENG
            1290      1300      1310      1320      1330      1340

CaYOR1      HKNQDT

ScYOR1      THGKMH
            1350
```

FIG. 6. *YOR1* homolog of *C. albicans*. (A) Restriction map of the cloned DNA. The hatched box indicates the region sequenced. The arrow indicates the direction of transcription. Restriction sites are as follows: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; S, *Spe*I. (B) Nucleotide sequence and predicted amino acid sequence of the partially sequenced region of the *YOR1* homolog. (C) Alignment of the *C. albicans YOR1* homolog (CaYOR1) and the *S. cerevisiae YOR1* gene (ScYOR1). The Walker A motif in the nucleotide-binding domain and the membrane-spanning domains of *S. cerevisiae* YOR1p are indicated by double underlines and underlines, respectively. Identical and similar amino acid residues are marked by colons and dots, respectively.

TABLE 3. Change in vincristine accumulation caused by AbA in tumor cells with multidrug resistance

Drug	Intracellular concn (pmol/10^6 cells) (% change[a] in concn of [^{3}H]vincristine at the following different doses [μg/ml] of AbA or verapamil):			
	0	0.1	1	10
AbA	0.052 (100)	0.042 (82)	0.077 (149)	0.247 (478)
Verapamil		0.060 (116)	0.162 (312)	0.366 (708)

[a] The change in concentration is given as a percentage of the concentration without AbA or verapamil.

expression of *YOR1* is regulated by *PDR1* and *PDR3*, both of which also regulate the expression of the *PDR5* gene. We showed by genetic analysis that the *aur3*^{R} mutation causes overexpression of *YOR1* as well as *PDR5* and that *AUR3* is identical to *PDR1*.

The fact that the amount of the *YOR1* gene had more of an effect than *PDR5* on resistance to AbA suggests that *YOR1* is more important for AbA resistance in *S. cerevisiae*. The Yor1 protein that confers resistance was more similar to the CFTR subgroup (including MRP1) than the MDR1 subgroup. Therefore, AbA may be a better substrate for members of the CFTR subgroup than the MDR1 subgroup in human cells as well. In small-cell lung cancer cells, overexpression of MRP1 causes resistance to several anticancer agents (5). AbA may overcome the multidrug resistance of such cancer cells overexpressing MRP1 better than it overcomes the resistance of the A2780AD cancer cells overexpressing MDR1 examined in this study.

We identified a gene of *C. albicans* whose sequence was similar to that of *YOR1* of *S. cerevisiae*. A search for a sequence homologous to that of *C. albicans* Yor1p showed that it is more similar to the CFTR subgroup than to the MDR1 subgroup, to which *C. albicans* Cdr1p and Cdr2p belong (22, 27). Of the fungal pathogens, clinical isolates of *C. albicans* resistant to fluconazole overexpress the *PDR5* homolog *CDR1* gene (22) or the gene for another efflux pump, *BEN*^{R} (10). Whether these strains have proteins in the CFTR subgroup including Yor1p is not known. In *S. cerevisiae*, several loci are involved in pleiotropic drug resistance (PDR) (2), and they seem to interact, causing the expression of resistance (7, 19). At times, the mutation of a PDR locus such as *AUR3*/*PDR1* or *PDR3* causes overexpression of other PDR genes encoding ABC transporters (3). This fact suggests the possible emergence of multidrug resistant strains of pathogenic *Candida* isolates which have mutations in yet unidentified genes similar to *PDR1* and *PDR3* of *S. cerevisiae*, and the resistance may develop particularly readily in haploid species such as *Candida glabrata.*

It is important that the intrinsic roles and substrates of ABC transporters in cell metabolism be known. Some are already known; for example, mouse *mdr2* is essential in the liver for the export of phospholipids from the apical surface of the canalicular membrane into the bile (31), CFTR acts as a chloride ion channel in the lungs (23), and yeast Ste6p is a transporter of the **a** factor (18). Cells overexpressing *YOR1*/*YRS1* are resistant to oligomycin when the cells are grown with unfermentable carbon sources such as glycerol and ethanol (6). Such cells are also resistant to reveromycin and organic anions when they are grown in medium with a low pH (pH 4.5) (16). However, resistance to AbA is seen at pH 6.4 in ordinary YPD medium, which contains glucose as the carbon source. It seems likely that *YOR1* contributes to cellular resistance to AbA and structurally related compounds rather than to resistance to other toxic compounds.

A variety of hydrophobic and ionic compounds can be transported through membranes by ABC transporters, and some also inhibit the transporters. Thus, the inhibitory action of AbA in tumor cells may result from competition because of structural or ionic similarity to the usual substrate of ABC transporters. The antifungal activity of AbA against yeasts could be used to investigate the effects of inhibitors on various ABC transporters, including mammalian ones, as described by Kino et al. (17).

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