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Role of the PDR Gene Network in Yeast Susceptibility to the Antifungal Antibiotic Mucidin

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Yeast strains disrupted in the *PDR1*, *PDR3*, or *PDR5* gene, but not in *SNQ2*, exhibited higher sensitivity to mucidin (strobilurin A) than did the isogenic wild-type strains. Different gain-of-function mutations in the *PDR1* and *PDR3* genes rendered yeast mutants resistant to this antibiotic. Mucidin induced *PDR5* expression, but the changes in the expression of *SNQ2* were only barely detectable. The results indicate that *PDR5* provides the link between transcriptional regulation by *PDR1* and *PDR3* and mucidin resistance of yeast.

The antifungal antibiotic mucidin (strobilurin A) has been successfully used in clinical treatment of dermatomycoses (7) and has become a valuable tool in biochemical and molecular genetic studies with yeasts (10). Mucidin specifically inhibits electron transport in the cytochrome bc_1 complex of the mitochondrial respiratory chain (16). Resistance to mucidin in the yeast *Saccharomyces cerevisiae* results from mutations mapping in either mitochondrial or nuclear genes (17). The nuclear mucidin-resistant mutants displayed a pleiotropic drug resistance phenotype due to the mutations in the *PDR3* gene (17, 18). This gene has been cloned, sequenced (4), and subjected to mutational analysis (13). It specifies a transcriptional activator homologous to that encoded by the *PDR1* gene (4). *PDR1* and *PDR3* together with the drug efflux pump-encoding genes like *PDR5* and *SNQ2* create the PDR network of genes (8) which, together with another network of stress response genes activated by the transcription factor *YAP1* (11), are involved in multiple drug resistance in *S. cerevisiae*. Their homologues were also identified in the yeast *Candida albicans* and contribute significantly to the fungicide resistance of this most prominent fungal pathogen of humans (15).

The aim of the present study was to analyze the molecular mechanisms of mucidin susceptibility in *S. cerevisiae* using different mutants of the PDR network of genes involved in yeast multidrug resistance.

The strains of *S. cerevisiae* used in this study were derived from the wild-type strains FY1679-28C (*MAT***a** *ura3-52 trp1*Δ*63 leu2*Δ*1 his3*Δ*200*) (1, 4) and YPH500 (*MAT*α *ura3-52 trp1*Δ*63 leu2*Δ*1 his3*Δ*200 lys2-801^{amb} ade2-101^{ochr}*) (9). The *PDR3* gene and its mutant alleles were cloned on centromeric plasmid pFL38-PP3 (*ARS1 CEN4 URA3 PDR3*) under the control of its promoter (13). Six mutant alleles (*pdr3-4* to *pdr3-11*) contained gain-of-function mutations in the N terminus (13), and another six mutant alleles (*pdr3-15* to *pdr3-20*) contained mutations in the C-terminal moiety of *PDR3* (J. Subik, A. Nourani, A. Delahodde, T. Simonic, V. Subikova, and C. Jacq, Abstr. Sixth Int. Mycol. Congr., p. 19, 1998). Yeast strains were grown in YPGE medium (1% Bacto Peptone, 1% yeast extract, 2% glycerol, and 2% ethanol) or in a minimal medium containing 0.67% yeast nitrogen base without amino acids and 2% glucose or 2% glycerol plus 2% ethanol. The appropriate nutritional requirements and drugs were added at the indicated concentrations. Solid media were prepared with 2% agar. Bacterial strains were grown in Luria-Bertani medium supplemented with ampicillin at 50 μg/ml.

Plasmid DNA from *Escherichia coli* TG1 cells was prepared by the alkaline lysis method and used to transform *S. cerevisiae* as described previously (13). The sensitivity of yeast cells to mucidin was assayed by measuring the MICs (13) and by determination of growth inhibition zones on the solid YPGE medium (17). Mucidin resistance of a set of independent transformants was scored for each of the DNA constructs after 5 to 12 days of growth at 30°C.

The abundance of Pdr5p and Snq2p in total yeast extracts was determined by Western blot analysis as described previously (5). Equivalent protein loading in each lane was verified by probing the immunoblots with polyclonal antibodies against Pgk1p. The level of Pdr5p after mucidin response was quantitated by densitometric scanning of the developed film using Pharmacia Biotech ImageMaster 1D software.

The susceptibility of the yeast *S. cerevisiae* to mucidin was studied using the isogenic sets of mutants derived from two wild-type strains, FY1679-28C and YPH500. It was found that the strains with the disrupted *PDR1* or *PDR3* gene were significantly more sensitive to mucidin than a corresponding wild-type strain (Fig. 1A). The disruption of both *PDR1* and *PDR3* led to the highest level of mucidin sensitivity, indicating that the two transcriptional activators play a significant role in the control of mucidin susceptibility of yeast cells.

Whereas the disruption of the *PDR1* and/or the *PDR3* gene led to an increased sensitivity of cells to mucidin, all independently isolated gain-of-function mutations in the *PDR1* gene or in the *PDR3* gene resulted in mucidin resistance. In a genetic background of Δ*pdr3* strains (1), the most resistant was the strain containing the chromosomal *pdr1-3* allele and less resistant was the strain with the *pdr1-6* allele (Fig. 2A). Different levels of mucidin resistance were also observed in the isogenic transformants of the Δ*pdr1*Δ*pdr3* host strain FY1679-28C/TDEC bearing on a centromeric plasmid the wild-type *PDR3* gene or the *pdr3* mutant alleles containing mutations either in the central regulatory domain (Fig. 2B) or in the C-terminal activation domain (Fig. 2C).

Mucidin added to the wild-type strain FY1979-28C grown in minimal glucose medium at a concentration (0.4 μg/ml) that inhibits cell growth on nonfermentable carbon sources but does not affect the growth on glucose induced an increased expression of *PDR5*. The abundance of Pdr5p in cells grown in

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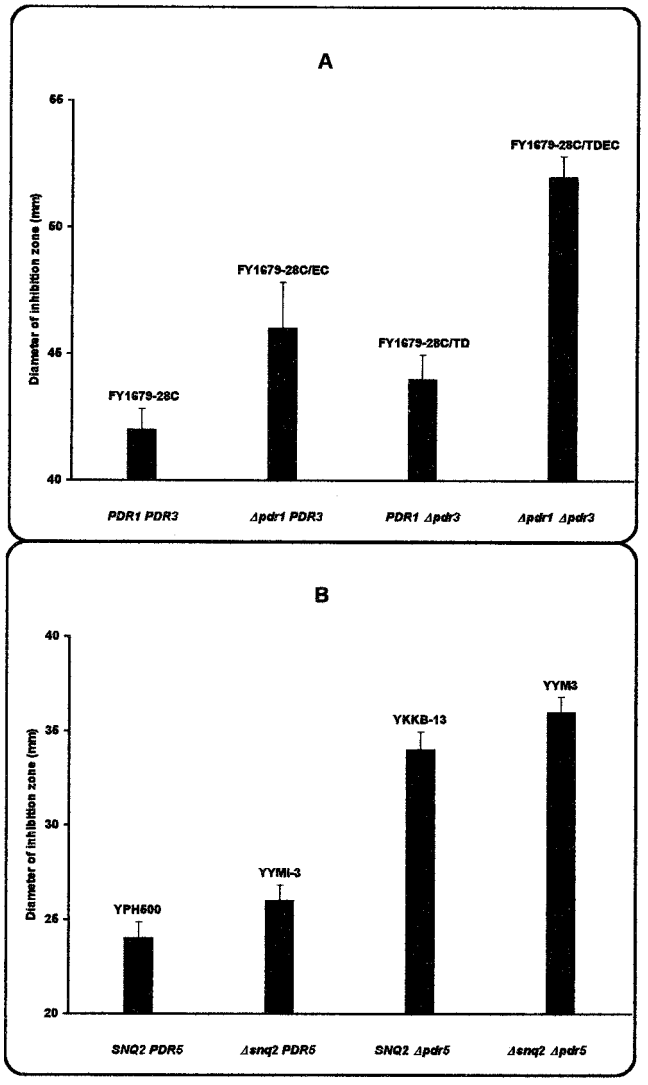

FIG. 1. Sensitivity to mucidin of yeast strains with deletions of *PDR1* and *PDR3* (A) or of *PDR5* and *SNQ2* (B). The amount of mucidin applied to the paper disk was 1 μg (A) and 0.5 μg (B). The bars represent the standard deviations.

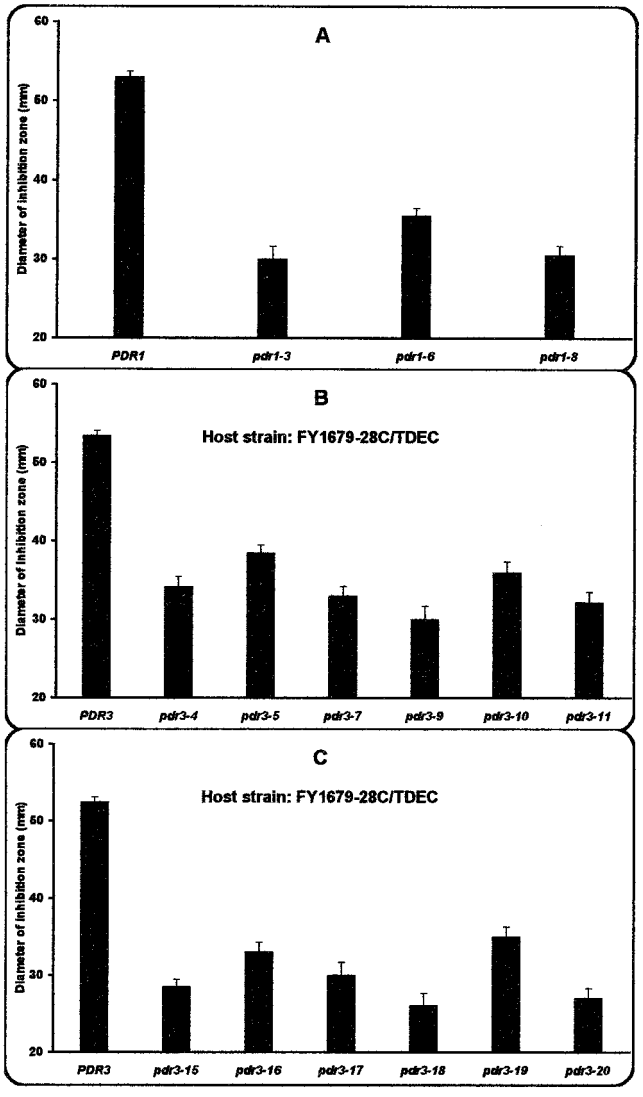

FIG. 2. Resistance to mucidin of the isogenic strains bearing different *pdr1* (A) and *pdr3* (B and C) mutant alleles. The amount of mucidin applied to the disk was 5 μg. The bars represent the standard deviations.

the presence of mucidin for 1 and 2 h was 2.0 and 2.7 times higher, respectively, than that in the control cells (Fig. 3). Under similar conditions, no significant changes were observed in the level of Snq2p, indicating that mucidin is an inducer and apparently also a substrate of Pdr5p in *S. cerevisiae*. A similar induction of expression of the drug transporter genes like *PDR5* (12), *SNQ2* (12), *YOR1* (2), and *PDR12* (14) was already demonstrated by their respective substrates—cycloheximide, cations, 4-nitroquinoline-*N*-oxide, reveromycin, and sorbate.

To assess whether mucidin is a substrate for Pdr5p, the susceptibility to mucidin was determined in the strains with deletion either of single *PDR5* and *SNQ2* or simultaneously of both genes (9). As shown in Fig. 1B, in zone-of-inhibition assays the mucidin sensitivity of Δ*snq2* disruptant YYMI-3 was only slightly higher than that of the wild-type strain YPH500. Such a small change in mucidin sensitivity was not observed in the spot test experiments (Fig. 4). On the other hand, in both experiments the Δ*pdr5* strain YKKB-13 exhibited clear hypersensitivity to mucidin which was faintly elevated when the strain YYM3 possessed the disruptions in both genes (Δ*pdr5* and Δ*snq2*) (Fig. 1B). However, the transformants of this strain containing the *pdr3-4* or the *pdr3-7* allele on the centromeric plasmid were still able to grow in the presence of 0.05 μg of mucidin per ml, which already prevented the growth of the host strain with deletion simultaneously of both *PDR5* and *SNQ2* (Fig. 4). Under the same conditions, no growth of the wild-type strain YPH500, Δ*pdr5*Δ*snq2* double mutant YYM3,

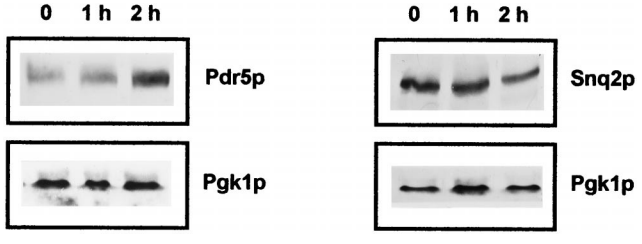

FIG. 3. Immunological detection of Pdr5p and Snq2p in the wild-type strain FY1679-28C exposed to mucidin.

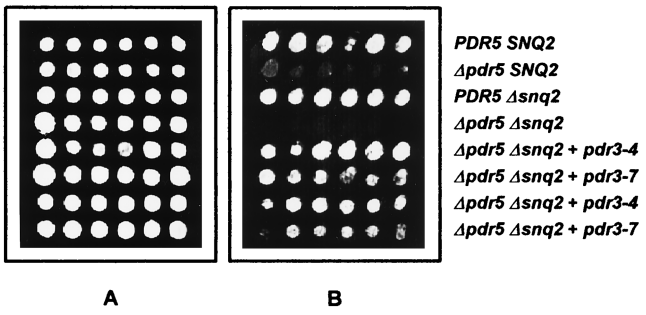

FIG. 4. Mucidin susceptibility of the strain YYM3 with deletions of *PDR5* and *SNQ2* and transformed with the *pdr3* mutant alleles. Cells were grown for 5 days at 30°C on YPGE medium containing mucidin at concentrations of 0.025 (A) and 0.05 (B) μg/ml.

and its transformants was observed at a mucidin concentration of 0.1 μg per ml (data not shown).

These results indicate that mucidin-inducible Pdr5p is a primary membrane transporter responsible for mucidin resistance. Nevertheless, another as-yet-unidentified drug transporter protein(s) different from that of Pdr5p and Snq2p but overexpressible by the mutated Pdr3p can also modulate mucidin sensitivity. In fact, 29 members of the superfamily of ATP binding cassette transporters (3) and 28 members of the major facilitator superfamily (6) were identified in the complete *S. cerevisiae* genome. However, in comparison with Pdr5p their contribution to mucidin efflux seems to be only marginal.

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