The involvement of the *Saccharomyces cerevisiae* multidrug resistance transporters Pdr5p and Snq2p in cation resistance

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Abstract The ATP-binding cassette superfamily proteins Pdr5p and Snq2p of *Saccharomyces cerevisiae* are implicated in multidrug resistance. Here, we show that these transporters are also involved in cation resistance. Null mutants of *PDR5* and *SNQ2* genes exhibit increased sensitivity to NaCl, LiCl and $MnCl_2$. The mutant cells grown in the presence of high concentrations of these metal salts contain higher levels of the metals than wild-type cells. The expression of *PDR5* and *SNQ2* is induced by the metal salts. These results provide evidence that the yeast drug transporters contribute to cation resistance by regulating cellular cation homeostasis under ionic stress conditions.

Key words: ABC transporter; Multidrug resistance; Sodium; Lithium; Manganese; *Saccharomyces cerevisiae*

1. Introduction

Multidrug resistance (MDR), which involves resistance to a wide variety of unrelated cytotoxic drugs, is important for drug resistance of cancer cells. The MDR protein is a member of the ATP-binding cassette (ABC) superfamily of transporters which consist of two transmembrane domains (each with six membrane-spanning α-helices) and two ATP-binding domains [1,2]. The members of the ABC superfamily so far characterized are suggested to transport a variety of molecules, such as drugs, metabolites and peptides across membranes. In the yeast *Saccharomyces cerevisiae*, an MDR-like phenomenon (referred to as pleiotropic drug resistance, PDR) is known and the genes encoding the ABC superfamily proteins functionally related to MDR1 (P-glycoprotein) have been identified (reviewed by Balzi and Goffeau [3,4]). The *PDR5* gene [5], also referred to as *STS1/YDR1* [6,7], encodes a putative transporter protein of the ABC superfamily, which is responsible for the resistance of growth to various drugs, such as cycloheximide, sulfomethuron methyl, sporidesmin, staurosporin, cerulenine, trifluoperazine and compactin. The *SNQ2* gene, highly homologous with *PDR5*, was originally isolated as the gene that confers resistance to the mutagen 4-nitroquinoline *N*-oxide [8]. Further analysis indicated that both Pdr5p and Snq2p are involved in PDR with distinct specificity, sharing overlapping specificity toward several other drugs [7]. In the present study, we demonstrate that the yeast multidrug resistance ABC transporters also contribute to the tolerance to the cations such as Na^+, Li^+ and Mn^{2+}.

2. Materials and methods

2.1. Strains, plasmids and growth conditions

Isogenic *S. cerevisiae* strains W303-1A (wild type), DHR5-4C (*pdr5::LEU2*), DHR5-2C (*snq2::HIS3*) and DHR5-1C (*pdr5::LEU2 snq2::HIS3*) described in our previous paper [7] were used. The composition of the yeast media YPD and SD was described previously [9].

2.2. Growth inhibition assay

Sensitivity of yeast strains to various metal salts was assayed in two ways. (i) Solid medium assay: Yeast cells suspended in water (2×10^7 cells/ml) were inoculated using a cell applicator at a cell density of about 4×10^4 cells/spot (3 mm in diameter) on YPD solid medium containing various concentrations of NaCl, LiCl or $MnCl_2$. The plates were incubated at 28°C for 2–3 days. (ii) Liquid culture assay: Cells were inoculated to fresh YPD liquid medium containing various concentrations of the metal salts at a cell concentration of 5×10^5 cells/ml, grown at 28°C for 12 and 20 h with shaking, and their A_{600} was measured.

2.3. Measurement of cellular cation content

The preparation of the cell extract and the measurement of the content of manganese and sodium by the PIXE (particle-induced X-ray emission) method and isotachophoresis, respectively, were performed as described previously [10–13]. Cellular lithium content was measured by a similar procedure as used for sodium [11]. Cation content is expressed as the amount (μg) of metal per mg of total cellular proteins. The rate of efflux of sodium, lithium and manganese was measured as described previously [10,11].

2.4. Northern blot analysis of PDR5 and SNQ2 expression

Northern blot hybridization was done as described in our previous paper [7], except that YPD medium was used instead of SD medium. The concentrations of the metal salts used were as follows: 0.85 M NaCl, 110 mM LiCl and 5 mM $MnCl_2$.

3. Results and discussion

3.1. Null mutants of the PDR5 and SNQ2 genes exhibit increased sensitivity to NaCl, LiCl and $MnCl_2$

Null mutants (Δ*pdr5*, Δ*snq2* or Δ*pdr5* Δ*snq2*) lacking either one or both of the multidrug resistance ABC transporters exhibit an increased sensitivity to various cytotoxic drugs [7]. To investigate if these transporters have additional physiological roles, we searched for the phenotype (other than drug sensitivity) of the double null mutant under various stress conditions. Growth disadvantage of the mutant in comparison with the wild-type strain was noted on YPD solid medium containing NaCl, LiCl or $MnCl_2$ (Fig. 1A), but not other metal salts, which included $CaCl_2$, $MgCl_2$, $ZnCl_2$, $CuCl_2$, NiCl and $CdCl_2$ (data not shown).

On solid medium containing NaCl (1.2 M), LiCl (90 mM) or $MnCl_2$ (5 mM), strain Δ*snq2* grew poorly. Although Δ*pdr5* disruption by itself had no detectable effect on growth under the same conditions, the Δ*pdr5* mutation further increased the cation sensitivity of the Δ*snq2* mutant (Fig. 1A). These results suggest that Pdr5p and Snq2p have redundant functions in the

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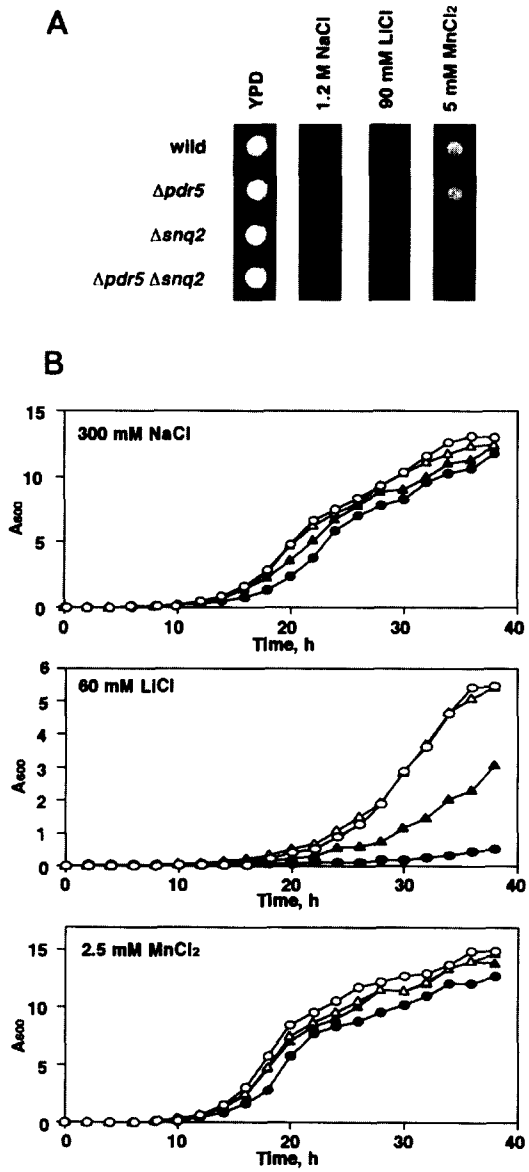

Fig. 1. The sensitivity of various mutants lacking Pdr5p and Snq2p to NaCl, LiCl or $MnCl_2$. A: The strains W303-1A (wild-type, ○), DHR5-4C (*Δpdr5*, △), DHR5-2C (*Δsnq2*, ▲) and DHR5-1C (*Δpdr5 Δsnq2*, ●) were grown on YPD solid medium containing 1.2 M NaCl, 90 mM LiCl or 5 mM $MnCl_2$. The plate was incubated at 28°C for 3 days (no addition) to 4 days (in the presence of metal salts). B: The growth profiles of various strains in liquid YPD medium containing NaCl (300 mM), LiCl (60 mM) and $MnCl_2$ (2.5 mM). The A_{600} values were measured at an interval of 2 h. The experiment was done three times and the results were reproducible. The result presented is from a single representative experiment.

resistance to high concentrations of metal salts, and the contribution of Snq2p in the resistance is greater than that of Pdr5p.

We next examined the effect of the metal salts in liquid culture by comparing the growth profiles of various strains (wild type, *Δpdr5*, *Δsnq2* and *Δpdr5 Δsnq2*) in liquid medium containing NaCl (0.2–1.2 M), LiCl (50–120 mM) or $MnCl_2$ (2–5 mM). The concentrations of the metal salts that caused a difference in the growth rate in liquid culture assay were lower than those for the plate assay. The most remarkable difference in growth rates was observed with 60 mM LiCl (Fig. 1B). Of the two transporter genes disrupted individually, *Δsnq2* disruption increased the sensitivity to Li^+ significantly, while *Δpdr5* disruption had no detectable effect on Li^+ sensitivity. However, *Δpdr5* disruption markedly enhanced the Li^+ sensitivity of strain *Δsnq2*. Consistent with the results obtained on solid medium, these results show the functional redundancy of Pdr5p and Snq2p in the resistance to Li^+. A smaller but reproducible effect on the growth rate by the *Δpdr5* and *Δsnq2* mutations was seen in liquid culture in the presence of 300 mM NaCl or 2.5 mM $MnCl_2$ (Fig. 1B). In the case of $MnCl_2$, however, the two single null mutations (*Δpdr5* and *Δsnq2*) resulted in a similar degree of sensitivity to $MnCl_2$ (Fig. 1B).

The null mutation of *STE6*, which encodes an ABC transporter involved in α-factor secretion, but not in PDR [14,15], exhibited cation sensitivity similar to that of the wild-type strain, indicating that Ste6p is not important for the resistance to these cations. Further, disruption of *YOR1*/*YRS1* [16,17], encoding a MRP-type ABC transporter, had no detectable effect on the sensitivity to these cations (data not shown). According to the *S. cerevisiae* genome sequences made available to the public recently, the yeast has additional genes *PDR10*, *PDR11*, *PDR12* and *PDR15*, highly homologous with *PDR5* and *SNQ2*. The involvement of the putative drug transporters encoded by these newly discovered genes in metal resistance remains to be established.

3.2. *Pdr5 and Snq2 contribute to cellular cation homeostasis against cationic stresses*

The poor growth of the mutant in the medium containing high levels of metal salts could be due to the inability to maintain cellular cation homeostasis under ionic stress conditions. The cation content of the cells grown in the presence of various metal salts was measured (Table 1). The sodium level of *Δpdr5 Δsnq2* cells was 1.5-fold that of the wild-type strain. The cellular sodium levels of strains *Δpdr5* and *Δsnq2* were intermediate between the wild-type and double null mutant strains (Table 1). Similar differences among the various mutants in cellular cation content were observed in cells grown in the presence of LiCl and $MnCl_2$, suggesting that Pdr5p and

Table 1
Comparison of the metal content of various strains grown in medium containing a high concentration of metal salt

Strain	Metal content (μg/mg protein)		
	Sodium	Lithium	Manganese
W303-1A (wild type)	10.5 ± 1.5	0.83 ± 0.06	6.2 ± 0.3
DHR5-4C (*Δpdr5*)	11.1 ± 1.2	1.02 ± 0.05	6.8 ± 1.0
DHR5-2C (*Δsnq2*)	12.6 ± 1.2	1.05 ± 0.15	7.6 ± 0.8
DHR5-1C (*Δpdr5 Δsnq2*)	15.6 ± 0.9	1.27 ± 0.08	9.1 ± 0.8

The cells were grown in YPD medium containing NaCl (0.85 M), LiCl (60 mM) or $MnCl_2$ (2 mM) for 3 h and the cellular metal content was determined as described in Section 2.

Snq2p are involved in the regulation of cellular cation homeostasis under cationic stress conditions (Table 1).

To further examine if the higher metal content of the cells lacking Pdr5p and Snq2p is due to a defect of cation efflux, we measured cation export activities of wild-type and *Δpdr5 Δsnq2* cells. Cells grown in YPD medium containing 0.85 M NaCl, 60 mM LiCl or 2 mM $MnCl_2$ for 3 h were transferred to fresh YPD medium, and the cellular metal content was measured periodically. The results with $MnCl_2$ are shown in Fig. 2. Nearly half of the total manganese accumulated in wild-type cells was rapidly extruded from the cells on incubation in manganese-free medium, reaching a constant level in 10 min. In contrast, manganese efflux from *Δpdr5 Δsnq2* cells was much slower. The difference in efflux rates indicates that wild-type yeast grown in high manganese medium is able to transport excess manganese out of the cell, and this activity can be explained for the greater part, if not totally, by Pdr5p and Snq2p. Manganese retained in the cells (nonexchangeable manganese) after incubation in manganese-free medium, by contrast to that extruded (exchangeable manganese), is assumed to reflect stored manganese in the organelles (e.g. vacuoles and endoplasmic reticulum), presumably in the form of insoluble manganese phosphate [18].

We also compared the efflux rate of preloaded Na^+ or Li^+ from *Δpdr5 Δsnq2* and wild-type cells. However, no significant difference in the rate of cation efflux was observed (data not shown). The contribution of Pdr5p and Snq2p transporters in Na^+/Li^+ transport may be hardly detectable under these conditions, due to the potent activity of P-type Na^+/Li^+-transporting ATPase, such as Ena1p [19–21].

The mechanism of action of the Pdr5p and Snq2p transporters in cation resistance still remains elusive. The question whether the cations are exported directly by the transporters, or transported by other machinery whose activity is regulated by the ABC transporters, still remains to be answered.

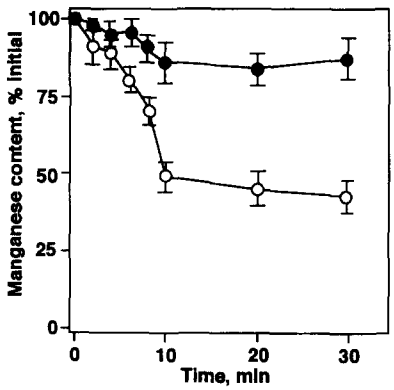

Fig. 2. Time course of manganese efflux from wild-type and *Δpdr5 Δsnq2* mutant cells. Wild-type (W303-1A, ○) and DHR5-1C (*Δpdr5 Δsnq2*, ●) cells were grown in YPD medium containing 2 mM $MnCl_2$ for 2 h at 28°C. After washing once with YPD medium at 4°C, the cells were suspended in fresh YPD medium (no manganese added) and incubated at 28°C with shaking. An aliquot (1 ml) of the cell suspension was taken at various times of incubation and the cells were washed by centrifugation twice with cold YPD medium. Manganese content of cell extract was determined by the PIXE method [10]. Data are presented as the mean ± S.E. of four independent experiments.

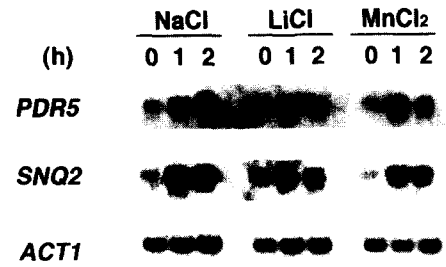

Fig. 3. Northern blot analysis of the induction of *PDR5* and *SNQ2* expression by the metal salts. The cells were incubated at 28°C in YPD medium containing 0.85 M NaCl, 110 mM LiCl or 5 mM $MnCl_2$. The RNA samples (30 μg per slot) extracted from the cells grown in the medium containing each metal salt for various periods of time were applied to the gel. The filter was hybridized with radioactive probes containing *YDR1*, *SNQ2* and *ACT1* (as internal standard).

3.3. The expression of PDR5 and SNQ2 is induced by metal ions

The expression of the *PDR5* and *SNQ2* genes is induced in response to the stress imposed by drugs and heat shock [7,22]. We next examined if the expression of these genes can be induced by metal salts. Cells grown in YPD medium were shifted to medium containing NaCl (0.85 M), LiCl (0.11 M) or $MnCl_2$ (5 mM) and the mRNA levels of the *PDR5* and *SNQ2* genes were compared by Northern blot hybridization. The expression of the *PDR5* and *SNQ2* genes was clearly induced by these metal salts in 1 h of shift to the medium containing the metal salts (Fig. 3).

Previously, we demonstrated that heat shock-induced activation of *PDR5* and *SNQ2* transcription is dependent on the AP-1-like transcriptional activators (yAP-1 and yAP-2) [22] encoded by *YAP1* and *YAP2* [9,23–25]. Because double disruption of these genes (*Δyap1 Δyap2*) had no effect on cation sensitivity (data not shown), the involvement of yAP-1 and yAP-2 in cation-induced activation of *PDR5* and *SNQ2* transcription is unlikely. The transcription factors Pdr5p and Snq2p are involved in the expression of *PDR5* and *SNQ2* genes [26,27]. Whether the Pdr1p and Pdr3p transcription factors are involved in the cation-induced *PDR5* and *SNQ2* activation remains to be seen.

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