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The Saccharomyces cerevisiae SOP1 and SOP2 Genes, Which Act in Cation Homeostasis, Can Be Functionally Substituted by the Drosophila lethal(2)giant larvae TumorSuppressor Gene*

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By complementation of a salt-sensitive mutant of Saccharomyces cerevisiae, we cloned the SOP1 gene, encoding a 114.5-kDa protein of 1033 amino acids. Cells deleted for SOP1 exhibited sensitivity to sodium stress, but showed no sensitivity to general osmotic stress. Following exposure of sop1Δ cells to NaCl stress, the intracellular Na⁺ level and the Na⁺/K⁺ ratio rose to values significantly higher than in wild type cells. Deletion of SOP2, encoding a protein sharing 54% amino acid identity with Sop1p, produced only slight Na⁺ sensitivity. Cells carrying a sop1Δsop2Δ double deletion became, however, hypersensitive to Na⁺ and exhibited increased sensitivity also to Li⁺ and K⁺, suggesting involvement of both SOP1 and SOP2 in cation homeostasis. The predicted amino acid sequences of Sop1p and Sop2p show significant homologies with the cytoskeletal-associated protein encoded by the Drosophila lethal(2)giant larvae tumor suppressor gene. Immunolocalization of Sop1p revealed a cytoplasmic distribution and cell fractionation studies showed that a significant fraction of Sop1p was recovered in a sedimentable fraction of the cytosol material. Expression of a Drosophila l(2) g cDNA in the sop1Δsop2Δ strain partially restored the Na⁺ tolerance of the cells, indicating a functional relationship between the Sop proteins and the tumor suppressor protein, and a novel function in cell homeostasis for this family of proteins extending from yeast to human.

Ions are continuously transported across the cell membrane, the net flux being adjusted to satisfy the requirement for a cytosol rich in potassium and scarce in sodium. Control of the intracellular concentration of these major monovalent cations is crucial to generate a biochemically-functional intracellular milieu. Since in natural environments Na⁺ is generally abundant and K⁺ scarce, transport must occur against concentration gradients. Genetic analysis of salt tolerance in Saccharomyces cerevisiae has identified a number of cation transporters which interact with multiple regulatory components in a largely unidentified fashion (1). In particular, a major system involved in K⁺ uptake is constituted by the TRK1- and TRK2-encoded membrane proteins (2–4), which appear to contribute to the uptake of K⁺ in symport with protons (1). The proton gradient providing the driving force for secondary transport is generated by the PMA1-encoded plasma membrane ATPase, a major membrane protein whose activity shows little sensitivity to high extracellular NaCl concentration (5). The TRK1/TRK2-dependent transport system also permits influx of Na⁺, while under NaCl stress, the uptake system has the capacity of increasing its selectivity for K⁺ over Na⁺ (6).

In yeast cells, influx of Na⁺ is counteracted by Na⁺ efflux, the primary pathway being mediated by the P-type ATPase encoded by the PMR2A gene (also known as ENAI) (6, 7). The PMR2A gene is part of a gene cluster, containing tandem repeats of 2–5 nearly identical genes (8). However, only PMR2A appears to be significantly expressed (7, 8), and transcription of this gene is induced in cells subjected to Na⁺ or Li⁺ stress or cells exposed to alkaline pH (7). An additional sodium transporter encoded by the NHA1 gene and acting as a putative Na⁺/H⁺ antiporter was recently identified in S. cerevisiae (9). Disruption of the NHA1 gene displays only minor effects in wild type cells but elicits increased Na⁺ sensitivity in S. cerevisiae cells lacking the PMR2 genes.

To identify components that are crucial for salt tolerance, the isolation of recessive, salt-sensitive mutants is an obvious approach. However, the only S. cerevisiae mutant characterized so far by this procedure is the calcineurin-defective strain isolated by Mendoza et al. (10). These authors demonstrated that the protein phosphatase calcineurin, is involved in Na⁺ tolerance and is required for (i) induced expression of the PMR2A gene and (ii) modulating the K⁺ uptake system to display increased K⁺ versus Na⁺ discrimination. Further evidence that protein phosphorylation and dephosphorylation regulate Na⁺ tolerance in S. cerevisiae is provided by the increased cellular tolerance to sodium ions following inactivation of the PPZ1 and PPZ2-encoded serine-threonine phosphatases (11). In addition, increased dosage of the YCK1 or YCK2 gene, encoding yeast homologues of casein kinase I, enhances sodium tolerance (12), while cells defective in either of the a or b subunits of the yeast casein kinase II homologue become specifically sensitive to high concentrations of Na⁺ (13).

By isolation and functional complementation a NaCl-sensitive mutant, we cloned the SOP1 gene. Here we report the initial characterization of the gene product and show that the predicted sequence of Sop1p lacks apparent membrane spanning regions or other characteristics of previously isolated determinants for Na⁺ tolerance, and displays significant homology with the Drosophila p127 protein encoded by the lethal(2)giant larvae (l(2)g) tumor suppressor gene and its homologues in mouse and man. Our results demonstrate that

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SOP1 and a second related gene, designated as SOP2, are involved in regulating cation homeostasis in *S. cerevisiae*. Furthermore, we demonstrated that the expression of a *Drosophila* l(2)gl cDNA sequence in the *S. cerevisiae* sop1Δsop2Δ double mutant can partially restore Na⁺ tolerance. This data provide evidence for functional conservation between the *Drosophila* p127 protein and its yeast homologues, and reveal involvement of l(2)gl proteins in a new aspect of cell homeostasis.

**EXPERIMENTAL PROCEDURES**

**Strains, Media, and Yeast Genetic Methods—** *S. cerevisiae* strains and genotypes are listed in Table I. Cells were routinely grown at 30 °C in either YEPD medium supplemented with 120 μg/ml adenine or in a synthetic yeast nitrogen base (YNB) medium (Difco) supplemented with 2% glucose and necessary amino acids and nucleotides, to a final concentration of 120 μg/ml for each.

Procedures used to isolate the *osg4* mutant have been described previously (14). Cell density was estimated by measuring optical density at 610 nm in 1-cm cuvettes (OD₆₁₀). Standard yeast genetic methods were used throughout (15, 16).

**Escherichia coli** DH5α (17) was used for cloning and amplification of DNA. Bacterial cultures were grown in 2× LB medium (18).

**Plasmids—** The Yep50-based yeast genomic library (19) was used as a complementation of the *osg4* mutant. Transformants were screened on YNB agar plates containing 1.4 M NaCl, and plasmids from complemented cells were amplified in *E. coli* restriction fragments of the PCR product. A 4.7-kb *HindIII/BamHI* fragment containing the complementing capacity was subcloned into pBluescript KSⅡ- and *E. coli* BamHI fragment) at the *BstI* site. The cell suspension was centrifuged and the supernatant stored at -70 °C. The procedure used to isolate the *osg4* mutant has been described previously (14). Cell density was estimated by measuring optical density at 610 nm in 1-cm cuvettes (OD₆₁₀). Standard yeast genetic methods were used throughout (15, 16).

Deletion of the SOP2 gene was accomplished by the long flanking homology PCR-targeting technique (24, 25). In the first step, a set of 5′- and 3′-homology PCR primers was used to amplify 377-bp fragment immediately downstream of the SOP2 ORF stop codon. The end of the primers adjacently to the insertions homologous to the 5′ and 3′ regions of the his5MX6 disruption cassette of plasmid pFA6a-his5MX6 (26). In the second PCR reaction, pFA6a-his5MX6 was used as template and the 5′- and 3′-homologous regions of the first PCR reaction were fused to the disruption cassette by serving as primers, together with the upstream forward and downstream reverse primers of the flanking regions, thus producing the ORF targeting cassette. This cassette was transformed into a diploid *S. cerevisiae* strain, and independent transformants were selected for sporulation and verification of SOP2 replacement. Diploid transformants able to grow on his− plates were sporulated, and the progeny from a complete tetrad was examined by PCR for correct integration of the disruption cassette into one of the *SOP2* alleles. A set of primers (forward, 5′-GGGTTACCTTGTTGGAACAAAAACACACTTTA-3′; reverse, 5′-TCTCGAGGATTCTGTTTTGCGT-3′) hybridizing upstream and downstream W303 strain, respectively, of the disruption cassette were used to amplify chromosomal DNA. The length of the PCR products was verified by agarose-gel electrophoresis, as was the length of the *SpfI* and *XbaI* restriction fragments of the PCR product.

**Determination of Stress Tolerance—** Tolerance to salt or osmotic stress was examined by spotting 10-fold dilutions (10⁻²) of an overnight culture diluted to OD₆₀₀ ≈ 1.0 onto YEPD plates (pH 6.9, unless otherwise indicated). Plates were incubated at 30 °C for sporulation and verification of *SOP2* replacement. Diploid transformants able to grow on his− plates were sporulated, and the progeny from a complete tetrad was examined by PCR for correct integration of the disruption cassette into one of the *SOP2* alleles. A set of primers (forward, 5′-GGGTTACCTTGTTGGAACAAAAACACACTTTA-3′; reverse, 5′-TCTCGAGGATTCTGTTTTGCGT-3′) hybridizing upstream and downstream W303 strain, respectively, of the disruption cassette were used to amplify chromosomal DNA. The length of the PCR products was verified by agarose-gel electrophoresis, as was the length of the *SpfI* and *XbaI* restriction fragments of the PCR product.

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Subcellular Fractionation of Sop1p—Strains harboring plasmid YEpH/Bmyc or pH/B316myc and control plasmids without insert were grown to OD600 0.5–1.0 in YEPD medium. Cells were washed by centrifugation and resuspended in twice the pellet volume of resuspension buffer (R-buffer), containing 50 mM Tris (pH 7.5), 5 mM EDTA, complete protease inhibitor mixture (Boehringer Mannheim; 1 tablet/50 ml of cell extract), and either 0.1 M or 1 M KCl. Four volumes of glass beads were added, and each suspension was vortexed four times consecutively for 30 s at 4 °C, with incubation on ice between each vortex. Unlysed cells were removed by centrifugation at 500 × g for 5 min at 4 °C. The lysate was then centrifuged at 100,000 × g for 1 h in a Beckman TL-100 ultracentrifuge. The pellets were resuspended in 100 mM KCl R-buffer and the lysate desalted by gel filtration (Microcolumns, Bio-Rad). Aliquots containing equal amounts of cell material were boiled for 1 min in Laemmli SDS buffer (16).

Western Blot Analysis—Proteins were separated by SDS-PAGE in 10% acrylamide gels at 125 V for 1.5 h using a Mini-PROTEAN II electrophoresis system (Bio-Rad) and transferred overnight to Hybond membranes (Amersham Pharmacia Biotech), according to the manufacturers’ protocols. The membranes were then incubated for 1 h at room temperature in 10 mM Tris (pH 7.4), 0.9% NaCl, 0.5% Tween 20 (Buffer A), supplemented with 1% milk powder and then for 2 h with mouse anti-c-Myc antibody (Boehringer Mannheim) diluted 1:5000 in Buffer A with 1% milk powder. The anti-p127 C39 antibodies were as described by Strand et al. (30). Membranes were washed once for 5 min in Buffer A, twice for 5 min in 0.2% SDS, 0.5% Triton X-100, 0.9% NaCl (Buffer B), and twice for 5 min again in Buffer A. The membranes were probed for 1 h with 1:20,000 dilution of anti-mouse peroxidase-linked antibodies (Amersham Pharmacia Biotech) in Buffer A containing 5% milk powder. The blots were washed as before, and antibody detection was performed using ECL labeling system (Amersham Pharmacia Biotech).

Immunofluorescence—Cells were fixed in growth media by the addition of formaldehyde to a final concentration of 3.7%, and cells were prepared for immunofluorescence according to Rose et al. (31). Mouse anti-c-Myc antibodies and sheep anti-mouse Ig-fluorescein, F(ab′)2 fragment secondary antibodies were from Boehringer Mannheim. Glass slides were coated with poly-L-lysine, Mγ >300,000 (Sigma), to promote cell adhesion. Anti-c-Myc antibody concentration was 0.1 mg/ml, and the concentration of anti-mouse Ig-fluorescein was 0.02 mg/ml. Cells were stained with diaminophenylindole (1 mg/ml) for 1 min and covered by mounting medium (50 mg of p-phenylenediamine in 5 ml of phosphate-buffered saline (pH 9) and 45 ml of glycerol). Coverslips were then applied and sealed with nail polish.

Results

Complementation of a Salt-sensitive Yeast Mutant Identifies the SOP1 Gene.—We previously reported the isolation of a set of osmosensitive (osg) mutants of S. cerevisiae (14). Among these mutants we also identified a strain (osg4) that proved specifically sensitive to sodium chloride rather than being generally osmosensitive. Transformation of this mutant with a YCP50-based yeast genomic library (19) resulted in the isolation of clones that restored growth of the mutant at high salinity (Fig. 1A), and restriction endonuclease analysis identified a 4.7-kb HindIII/BamHI DNA fragment, which fully complemented the mutation. Subcloning, partial sequencing, and searching of DNA data bases tentatively identified a single large open reading frame, assigned YPR032W by the Yeast Genome Sequencing Project (EMBL accession no. Z49274). The 3102-bp open reading frame is localized to the left arm of chromosome XVI and encodes a putative protein of 1033 amino acids with a predicted molecular mass of 114.5 kDa. The codon bias (0.44) predicts a low expression of the gene, as indicated by the weakness of signal observed on a Northern blot (data not shown). Since this gene restored the salt sensitivity of the mutant, it was named SOP1 for sodium protection.

The Predicted Sop1p Shows Homology to Another S. cerevisiae Protein and to the p127 Protein Encoded by the l(2)gl Tumor Suppressor Gene of Drosophila—Homology searches revealed that Sop1p possesses 54% amino acid identity with another S. cerevisiae protein, encoded by the open reading frame YBL106C (EMBL accession no. Z35867). We designated this gene SOP2, since it shows structural and functional (see below) similarities to SOP1. Significant similarity scores were also recovered (Fig. 2) between the yeast proteins and the p127 protein encoded by the Drosophila l(2)gl tumor suppressor gene (22, 32), and its homologues from mouse (MGL) (33) and man (HUGL) (34). A well conserved feature in this family of proteins is the presence of WD-40-like motifs in the NH2-terminal half of the proteins (Fig. 2). These motifs were first described for the b subunit of trimeric GTP-binding proteins (35, 36), and might represent domains for protein-protein interactions.

The sopΔ mutant Displays No General Stress Sensitivity but Is Specifically Sensitive to Na+ Stress—To determine the phenotype of a cell lacking the SOP1 gene, null alleles were constructed by one-step gene disruption (37). A BglII/Nhel fragment of the SOP1 open reading frame was replaced by the LEU2 (Fig. 1B) or URA3 marker gene, and the HindIII/SpiI fragment of the resulting constructs was used to disrupt the SOP1 locus of the W303–1A and W303–1B strains. Southern blot analysis of the genomic DNA prepared from the putative disruptants confirmed the correct replacement of the SOP1 gene with the marker gene constructs (data not shown).

The sopΔ mutant was screened for osmotic sensitivity on agar plates supplemented with NaCl, KCl, LiCl, or sorbitol. Of these solutes, only NaCl strongly restricted the growth of the null mutant (Fig. 3), whereas KCl and the non-ionic agent sorbitol caused a significant decrease in the growth of the mutant compared with that of the wild type. Since Li1+ and Na+ ions are relatively similar, it is generally believed that these ions share the same uptake and efflux systems in the cell (6). Interestingly, we found that SOP1 inactivation conferred only minor changes in Li1+ tolerance (Fig. 3), suggesting that SOP1 is highly specific for Na1+ ion homeostasis of S. cerevisiae.
FIG. 2 Alignment of the SOP1 and SOP2 predicted amino acid sequences with the sequence of the p127 protein encoded by the Drosophila l(2)gl gene (DLGL) as well as the mouse (MGL1) and human (HUGL) homologues. The comparison was generated by the BestFit and Gap programs of the GCG Wisconsin package. Two WD-40-like motifs are indicated by solid bars.
To examine whether the sop1Δ strain was sensitive to other forms of stress, we compared the response of mutant and wild type cells to heat stress, nitrogen starvation, oxidative stress, and high and low pH. None of these conditions produced any growth difference between wild type and mutant cells, indicating that a loss of the SOP1 gene causes no general stress response defect (data not shown). However, the Na⁺ sensitivity of the sop1Δ mutant proved strongly pH-dependent, as indicated by a considerably stronger tolerance to Na⁺ at pH 4.5 than at 6.9 (Fig. 3A).

**SOP1 Expression Is Not Controlled by Environmental Salinity, and Na⁺ Tolerance Is Not Enhanced by Increased Gene Dosage**—As SOP1 inactivation confers sensitivity to NaCl, it was of interest to examine whether the expression of the gene is controlled by environmental salinity. Northern blot analysis revealed a low abundance of SOP1 transcript in cells grown in basal medium and no increased amount of transcript under salt stress conditions (data not shown). We also observed that overexpression of SOP1 gene from its own promoter on a YEplac195 multicopy vector caused no increase in Na⁺ tolerance by comparison to control cells, carrying a vector without insert (data not shown). Likewise, overexpression of SOP1 exerted no effect on the Na⁺ tolerance of a pmr2Δ null mutant lacking the Na⁺ extruding ATPase activity, or could not bypass the salt sensitivity of either cnb1 cells (10) lacking calcineurin or cmd1-5 cells (38) carrying a mutant form of calmodulin unable to bind Ca²⁺.

**Na⁺ Accumulates in sop1Δ Cells at High Extracellular NaCl Concentrations**—To examine the effect of SOP1 inactivation on Na⁺ and K⁺ homeostasis, the intracellular levels of these ions were measured in mutant and wild type cells after conditioning the cells for 6 h in basal medium or in the same medium containing either 0.7 m or 1.0 m NaCl. The intracellular Na⁺ concentration in the mutant cells increased strongly with salinity to become about 3 times higher (0.4 m) than in wild type cells maintained in medium containing 1 m NaCl (Fig. 4). The K⁺ concentration in sop1Δ cells decreased proportionally so that the internal K⁺ level reached 40% (0.06 m) of the wild type level at the highest salinity. Thus, under these conditions, not only the Na⁺ concentration but also the Na⁺/K⁺ ratio became unfavorably high, reaching a ratio of 5 in the mutant cells, while staying at about 1 in wild type cells. These observations suggest a defective ion transporting potential of the mutant at high salinity.

**Possible Genetic Interaction between SOP1 and PMR2A**—Since the PMR2A gene is reported to be the most important determinant of Na⁺ tolerance in S. cerevisiae (1, 6, 39), we examined whether we could detect a genetic interaction between SOP1 and PMR2A. For this purpose, a sop1Δ::LEU2 strain was mated to a pmr2Δ::HIS3 strain and the resultant diploid cells were sporulated and dissected. Half of the 36 tetrads dissected produced four viable spore clones, including double null mutant segregants. The NaCl sensitivity of the sop1Δ::LEU2 pmr2Δ::HIS3 clones proved identical to that of a pmr2Δ single mutant strain (Fig. 5). The absence of enhancement of the NaCl sensitivity of a pmr2Δ strain by a simultaneous inactivation of SOP1 indicates that SOP1 may contribute to the same transport mechanisms as PMR2.

**Cytoplasmic Distribution of Sop1p**—To determine the subcellular localization of Sop1p, three tandem repeats encoding the c-Myc epitope were fused in frame near the COOH terminus of Sop1p and expressed from a single-copy (pH/B316myc) or a multicopy plasmid (YEplacH/myc). The SOP1::myc derivative fully complemented the salt sensitivity of the sop1Δ null mutant, indicating that the c-Myc-tagged Sop1p is functional. Immunofluorescence staining of cells expressing Myc-tagged Sop1p from pH/B316myc showed that Sop1p is present in the cytoplasm of the yeast cells (Fig. 6A), and as revealed by dia-
midinophenylindole staining absent from the nucleus (data not shown). When examining the stronger signal obtained from the YEpl/3Bmyc plasmid, the protein appeared to be preferentially localized in the cytoplasm (data not shown). Isolated sop2Δ:HIS3 clones were verified by PCR and examined for sensitivity to high concentrations of Na+, Li+, K+, sorbitol, or glycerol. This examination revealed a slight Na+-specific sensitivity of the sop2Δ mutant that was much less pronounced than for the sop1Δ strain (Fig. 3). However, unlike sop1Δ cells, mutants lacking SOP2 displayed obvious sensitivity to Li+.

To produce a sop1Δsop2Δ double null mutant strain, a sop1Δ::LEU2 mutant was mated with a sop2Δ::HIS3 strain and the resultant diploid sporulated and subjected to tetrad dissection. His+ Leu+ segregants were isolated and the expected deletion of the sop1Δ and sop2Δ loci verified by PCR. All of the confirmed sop1Δsop2Δ mutated cells grew more slowly than wild type cells in basal YEPE medium and showed hypersensitivity toward Na+ (Fig. 3). The double mutant also exhibited sensitivity toward solutes other than NaCl, as demonstrated by the attenuated growth at increased concentrations of LiCl and KCl. However, the tolerance toward the non-ionic osmoticum sorbitol of sop1Δsop2Δ was identical to that of wild type cells. These observations clearly indicate that both SOP1 and SOP2 play a role in cation homeostasis in S. cerevisiae.

Complementation of the sop1Δ and the sop1Δsop2Δ Phenotype by the Drosophila l(2)gl Gene—The strong salt sensitivity of the sop1Δ and the sop1Δsop2Δ strains provided an assay to explore the functional relationship between the SOP1 and SOP2 gene products and the p127 protein encoded by the l(2)gl tumor suppressor gene of Drosophila. To this end we introduced the cDNA Ee173 encoding the p127 protein (22) in the multicopy pYX212 plasmid under control of the constitutive yeast TPI promoter, and determined whether the l(2)gl cDNA would complement the sop1Δ or sop1sop2Δ mutations. As shown in Fig. 7A, Western blot analysis confirmed that p127 is expressed by the l(2)gl+ -transformed sop1Δsop2Δ S. cerevisiae cells. Only slight complementation by p127 of the yeast sop1Δ mutation was obtained (data not shown), whereas p127 clearly decreased the Na+ sensitivity of the sop1Δsop2Δ cells (Fig. 7, B and C). Three independently isolated sop1Δsop2Δ clones were transformed with the l(2)gl cDNA, all being complemented to a similar extent, while mutants transformed with an empty vector showed no improved salt tolerance. The complementation of the yeast double mutant by the Drosophila l(2)gl gene indicates functional conservation of this family of proteins across species borders.

**DISCUSSION**

Role of Sop1p in Cellular Tolerance toward Na+—By complementing the NaCl sensitivity of a previously isolated mutant, we cloned the SOP1 gene, encoding a 114.5-kDa cytosolic protein that is required for growth at high Na+ concentrations. Although deletion of SOP1 strongly decreases Na+ tolerance, SOP1 overexpression results in no protection toward increased concentration of Na+. In this respect, SOP1 differs from the series of HAL genes (1, 40, 41), which improve Na+ tolerance in a dose-dependent fashion. SOP1 differs also from the genes involved in the general osmoregulatory response. The sop1Δ cells remain osmoreistant, and the production and accumulation of the compatible solute, glycerol, is similar to that of wild type cells at high salinity (data not shown).

The strict Na+ sensitivity of sop1Δ cells and the enhanced Na+ levels detected in a null mutant subjected to exogenous NaCl stress suggest that inactivation of SOP1 leads to an increased influx and/or a decreased efflux of Na+, due to defective transporter function(s). Considering the drastic effect on cellular Na+ sensitivity caused by the SOP1 deletion, it is possible that that Sop1p may contribute to the transporter system controlled by the major determinant for Na+ tolerance.

**FIG. 4.** Intracellular concentration of Na+ (open bars) and K+ (shaded bars) in the sop1Δ mutant and the W303-1A wild type strain after conditioning for 6 h in YEPE or YEPE plus 0.7 M or 1.0 M NaCl. Triplicate samples taken from each culture varied within ±10%.

**FIG. 5.** Genetic interaction between SOP1 and PMR2A. A wild type strain, a pmr2-2 mutant, a sop1Δ mutant, and a sop1Δpmr2-2 double mutant were cultured overnight in liquid YEPE medium. The OD600 was adjusted to 1 and serial 10-fold dilutions spotted onto YEPE plates supplemented with 0.1 M NaCl or 0.3 M NaCl, as indicated. Plates were incubated for 2 days at 30 °C prior to photography.
in *S. cerevisiae*, the PMR2-encoded system. This conjecture agrees with the observation that deletion of *SOP1* in an *pmr2A* background does not aggravate the Na⁺ tolerance profile of the *pmr2A* mutant (Fig. 5), suggesting direct or indirect interactions of Sop1p with the Na⁺-pumping ATPase. Furthermore, the strong pH dependence of the Na⁺ sensitivity of *sop1Δ* mutants indicates that the cells might have become dependent upon the alternative Na⁺ exporting system, the *NHA1*-encoded Na⁺/H⁺ antiporter (9). Since the activity of this system becomes gradually inhibited by increasing pH, cells with a defective *PMR2A* function will display a drastic decrease of the Na⁺ tolerance at high pH.

The coordinate regulation of both *PMR2A*-encoded Na⁺ efflux system and *TRK1*-encoded Na⁺/K⁺ influx system is controlled by a signaling pathway involving Ca²⁺/calmodulin and the protein phosphatase, calcineurin (8, 10). This signaling system appears to play a crucial role in modulating intracellular Na⁺ and K⁺ concentrations following exposure of cells to NaCl stress, and it is conceivable that Sop1p may interact with components of this signaling system. However, overexpression of *SOP1* was unable to rescue the Na⁺ sensitivity of *cmd1–5* or *cnb1Δ* mutants, indicating that Sop1p does not operate downstream from these components in the salt stress signaling system controlled by these genes.

Relationship between the Yeast SOP Encoded Proteins and Drosophila p127 Protein Encoded by the l(2)gl Tumor Suppressor Gene—The deduced amino acid sequences of Sop1p and its close yeast homologue Sop2p show a significant about 50% similarity, extending over the entire coding sequence, with the p127 protein encoded by the *l(2)gl* tumor suppressor gene of *Drosophila* and its homologues from mouse (MGL) and man (HUGL). Biochemical investigations and cell fractionation studies have previously shown that both p127 and HUGL are intracellular proteins diffusely distributed in the cytoplasm and associated with the cytoskeletal matrix underlying the plasma membrane (30, 34, 42). Neoplastic transformation of *Drosophila* larvae resulting from the inactivation of the *l(2)gl* gene may stem from a partial disruption of the cytoskeletal network, leading to reduced potential for signal processing and alteration in the maintenance of cell polarity and cell architecture (43). Biochemical and immunological evidence show that p127 forms high molecular mass complexes made of homooligomers (44) with which are associated other proteins. Among the proteins interacting with p127, a few have been identified, including non-muscle myosin II (45) and a putative serine kinase, whose activation leads to a specific phosphorylation of p127, resulting in the dissociation of myosin II from p127 without affecting p127 oligomerization (46). Several potential motifs for protein–protein interaction, such as repeated heptad units of hydrophobic amino acids (42) and motifs showing partial homology to the WD-40 repeats are evolutionary conserved among the members of the *l(2)gl* protein family. The conservation of two putative WD-40 motifs in p127, as well as in the other homologues, suggests that the Sop1p, like p127 and HUGL, may be a component of large protein complexes. Our subcellular fractionation studies revealing that a significant fraction of Sop1p is associated with a particulate fraction of the yeast cells support this contention. In particular, the sedimentable Sop1p proteins could be readily solubilized by treatment with 1 M KCl, suggesting that Sop1p association within this material is dependent upon electrostatic interactions.

Additional information on possible functional overlaps between Sop1p and p127 was provided by the immunolocalization studies, which indicated a predominantly cytoplasmic localization of Sop1p, transformed with YEpH/Bmyc, containing 100 mM or 1 M KCl were centrifuged at 100,000 × g for 1 h at 4 °C. Samples from the resulting supernatant and pellet were subjected to SDS-PAGE and transferred to Hybond membranes, and the blot incubated with anti-c-Myc antibody and visualized by ECL. A *sop1Δ* mutant transformed with an empty vector gave no band at the SOP1::myc position (data not shown). Pellet and supernatant refer to the two fractions obtained after the 100,000 × g centrifugation.

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**FIG. 6. Immunofluorescence localization of epitope-tagged Sop1p.** The *sop1Δ* strain transformed with a *SOP1::myc* centromeric vector (A, left) and the wild type W303–1A harboring a vector without insert (A, right) were grown to early exponential phase in YEPD medium, harvested, fixed with formaldehyde, and processed for immunofluorescence microscopy using anti-c-Myc and fluorescein isothiocyanate-conjugated anti-mouse antibodies. B, subcellular localization of Sop1p. Cleared cell lysates of the *sop1Δ* mutant, transformed with YEpH/Bmyc, containing 100 mM or 1 M KCl were centrifuged at 100,000 × g for 1 h at 4 °C. Samples from the resulting supernatant and pellet were subjected to SDS-PAGE and transferred to Hybond membranes, and the blot incubated with anti-c-Myc antibody and visualized by ECL. A *sop1Δ* mutant transformed with an empty vector gave no band at the SOP1::myc position (data not shown). Pellet and supernatant refer to the two fractions obtained after the 100,000 × g centrifugation.
plus pYX212 (').

Indication for a true functional relationship of the cell. The overall intracellular distribution of Sop1p, with a preferential distribution to the periphery of the cell. The overall intracellular distribution of Sop1p is highly reminiscent to that previously described for p127 (30) and HUGL (34). Indication for a true functional relationship between the yeast and Drosophila proteins derives, however, from the results obtained by analyzing double mutant cells in which SOP1 was deleted together with its isogene SOP2. While deletion of SOP2 alone produced only slight Na⁺/Li⁺ sensitivity, the sop1Δsop2Δ mutant exhibited a dramatic salt sensitivity, which was partly restored by expressing the Drosophila l(2)gl gene in the double mutant cells. In addition, the much stronger phenotype of the double mutant, as compared with the single mutants, indicates synergistic effects and functional relationships in cation homeostasis between Sop1p and Sop2p. Plausibly, SOP1 and SOP2 are involved in different but overlapping functions in ion homeostasis; SOP1 is able to compensate well for loss of SOP2, while SOP2 is unable to adequately correct for a loss of SOP1.

A Link between Cytoskeletal Organization and Cation Homeostasis—A possible relationship between the Sop proteins and the yeast cytoskeleton is suggested by a recent entry in the SGD data base of the SRO7 gene, which corresponds to SOP1. The SRO7 gene is one of the nine different isolated multicyclic suppressors of a rho3 cell polarity defect (47). The GTP-binding Rho proteins are implicated in regulating various actin-based events that are involved in cytoskeletal polarity (48). At non-permissive temperature, conditional rho3 mutants lose cell polarity during bud formation and display randomized actin and delocalized chitin (49). These findings were interpreted as an involvement of Rhod in the organization of the actin cytoskeleton for proper surface growth of the yeast bud. A plausible mechanism for explaining the suppression of the rho3 defect by increased dosage of SRO7/SOP1 would be that Sop1p acts downstream of Rhod by stabilizing the polarized actin skeleton. Among additional genes that were also identified as rho3 multicyclic suppressors (47), the CDC-42 and BEM1 genes deserve special mention because BEM1 encodes a bud site assembly protein that binds to Cdc24p, a guanine nucleotide exchange factor of the CDC42 encoded GTPase (48). A further indication of a possible link between the polar organization of the cytoskeletal elements and ion homeostasis stems from the recent observation that certain temperature-sensitive alleles of CDC42 exhibit specific sensitivity to Na⁺ and Li⁺ (50). Interestingly, there are precedents in higher eukaryotes for a close relationship between ion transporters and the underlying cytoskeleton. In particular, the gastric parietal cell H⁺/K⁺ ATPase (51) and the anion exchanger of the red cell (52) are associated with the membrane cytoskeleton via ankyrin, a component of the plasma membrane cytoskeleton. There is also evidence that interaction between the cadherin-catenin complex and the membrane cytoskeleton is required for localization of Na⁺/K⁺ ATPases to sites of cell adhesion in epithelial cells (53, 54). In addition, Na⁺/K⁺ exchangers of the NHE1 subtype are reported to be downstream targets of Cdc42p in fibroblasts (55), and, interestingly, neoplastic transformation of these cells is correlated with an increased Na⁺/K⁺ exchange activity (56). Although the consequences of the establishment of cell polarity may be different in yeast and metazoan cells, the general mechanisms controlling cell polarity and cell architecture may be highly conserved among eukaryotes, even for phylogenetically distant cells (48). Our results showing a functional complementation of the sop1Δsop2Δ defect by the Drosophila l(2)gl tumor suppressor gene provide evidence for phylogenetic conservation of a previously unrecognized function of a family of proteins linked to maintenance of the cytoskeletal architecture. The Na⁺ sensitivity of strains lacking SOP1, or both SOP1 and SOP2, provides an avenue for further exploring the physiological role of these molecules and uncovering new biological functions.

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FIG. 7. A, immunoblot showing expression of the Drosophila p127 protein in the S. cerevisiae transformants. Cell lysates were subjected to SDS-PAGE and transferred to Hybond membranes, and the blot was incubated with anti-p127 antisera and immunoreactive species detected by ECL. B, complementation of the NaCl sensitivity of the sop1Δsop2Δ double mutant. The W303–1A wild type strain and the sop1Δsop2Δ mutant were transformed with the Drosophila l(2)gl cDNA inserted in the multicopy pYX212 plasmid, or the same plasmid without insert. Serial dilutions were produced as described in Fig. 3 and spotted onto YEPD plates of 0 M and 0.3 M NaCl. C, complementation shown in liquid medium. The transformed W303–1A wild type strain and sop1Δsop2Δ mutant were grown overnight in YNB medium. Sidearm flasks containing 25 ml of YEFD medium plus 0.1 M NaCl were inoculated with overnight cultures to give a final OD610 of 0.1. The flasks were incubated with agitation at 30 °C. Growth was monitored by sidearm measurement of OD610 of W303–1A + pYX212 (○), sop1Δsop2Δ plus pYX212 containing the l(2)gl cDNA construct (□), and sop1Δsop2Δ plus pYX212 (△).
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The *Saccharomyces cerevisiae* *SOP1* and *SOP2* Genes, Which Act in Cation Homeostasis, Can Be Functionally Substituted by the *Drosophila lethal(2)giant larvae* Tumor Suppressor Gene
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