Aquaporins in *Saccharomyces*

GENETIC AND FUNCTIONAL DISTINCTIONS BETWEEN LABORATORY AND WILD-TYPE STRAINS*

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Mélanie Bonhivers‡, Jennifer M. Carbery§, Stephen J. Gould, and Peter Agre¶

From the Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Aquaporin water channel proteins mediate the transport of water across cell membranes in numerous species. The *Saccharomyces* genome data base contains an open reading frame (here designated *AQY1*) that encodes a protein with strong homology to aquaporins. *AQY1* from laboratory and wild-type strains of *Saccharomyces* were expressed in *Xenopus* oocytes to determine the coefficients of osmotic water permeability (P_f). Oocytes injected with wild-type *AQY1* cRNAs exhibit high P_f values, whereas oocytes injected with *AQY1* cRNAs from laboratory strains exhibit low P_f values and have reduced levels of Aqyp1p due to two amino acid substitutions. When the *AQY1* gene was deleted from a wild-type yeast and cells were cultured in *vitro* with cycled hypo-osmolar or hyper-osmolar stresses, the *AQY1* null yeast showed significantly improved viability when compared with the parental wild-type strain. We conclude that *Saccharomyces cerevisiae* contains at least one aquaporin gene, but it is not functional in laboratory strains due to apparent negative selection pressures resulting from *in vitro* methods.

Aquaporin water channel proteins have been characterized in animals, plants, and insects and are composed of two subgroups: one is permeable only to water (orthodox aquaporins) and a second is permeable to water, glycerol, and other small uncharged molecules (aquaglyceroporins) (for review, see Ref. 1). The three-dimensional structure of human red cell AQP1 at 6 Å resolution revealed the putative aqueous pore (2); however, the structure-function relationships and the physiological roles of the other members of the aquaporin family are still poorly understood (for review, see Ref. 3).

Aquaporins have more recently been recognized in bacteria and other microorganisms where their physiological roles are now being explored. *Dictyostelium discoideum* contains *waca*, a related gene with a developmentally regulated pattern of expression; however, disruption of the gene did not reveal phenotypic differences in spore formation or osmotic challenge (4). In addition to *gLpF*, the well-recognized glycerol facilitator, *Escherichia coli* contains *aqpZ*, a second related gene with a monocistronic organization (5) encoding a functionally defined aquaporin (6). When a wild-type *E. coli* parental strain and an *aqpZ* null mutant were directly compared, the latter showed reduced growth when cultured in hypo-osmolar medium and under conditions of maximum growth rate (7). A highly related gene, *smx*, has been identified in *Synechocystis* (8), but its function and null phenotype have not been defined.

Because of powerful genetic and molecular approaches, *Saccharomyces cerevisiae* has been extensively used for studies of osmoregulation (9) and thus may be a good microorganism for studies of possible physiological roles of aquaporins. The *S. cerevisiae* genome (10) is now accessible through the *Saccharomyces* genomic data base and has been found to contain open reading frames (ORFs) related to the aquaporins (11). *FPS1* has been characterized previously as a glycerol facilitator homolog (12, 13), and the deduced amino acid sequence of a second ORF, *YPL054C*, is highly homologous (Fig. 1). Additional ORFs were found in the genome with DNA sequences more closely related to aquaporin water channels than to bacterial glycerol facilitators or mammalian aquaglyceroporins (Fig. 1). The ORF *YPR192c* is located on chromosome XVI (here designated *AQY1* for aquaporin from yeast). The contiguous ORFs *YLL052* and *YLL053* reside on chromosome XII and may correspond to fragments of an aquaporin gene (here designated *AQY2*).

Laboratory yeast strains are the result of interbreeding wild-type *S. cerevisiae* and related species, as well as extensive and sometimes incompletely documented growth selection and genetic modifications of the initial strain (14). Many laboratory strains of *S. cerevisiae* were derived from the parental strain, S288C. For example, the strains used for the European Union Yeast Genome Sequencing Program (*Saccharomyces* genomic data base) were isogenic to S288C (10, 15, 16) but may contain genetic modifications induced by selection, growth, and storage conditions. To explore the potential significance of aquaporins in microorganisms, we have genetically evaluated the putative water channel gene *AQY1* from *S. cerevisiae*, functionally characterized its water transport capacities, and directly evaluated the null phenotype. Our studies reveal that wild-type and laboratory strains contain coding differences within *AQY1* that result in marked alterations of function.

MATERIALS AND METHODS

Yeast Strains and Culture Conditions—The yeast strains used in this study are listed in Table I. Cells were routinely grown in YPD media (1% yeast extract and 2% bactopeptone (Difco) with 2% dextrose) and were plated on YPD media supplemented with 1.8% bactoagar. Selection for genetic resistance was made with YPD liquid media or plates supplemented with 200 mg/liter of G418 sulfate (Life Technologies, Grand Island, NY). The *S. cerevisiae* strain *S288C* and its congeners were maintained in YPD media and were plated at 30 °C.

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1. The abbreviations used are: ORF, open reading frame; PCR, polymerase chain reaction; bp, base pair(s); MOPS, 4-morpholinepropanesulfonic acid.

2. Unless otherwise stated, the *S. cerevisiae* strain *S288C* was used as our standard wild-type. Wild-type strains GRF5 and NRRL-Y-12632 were also studied.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EBI Data Bank with accession number(s) AF053981.

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¶ To whom correspondence should be addressed: Dept. of Biological Chemistry, Johns Hopkins School of Medicine, 725 North Wolfe St., Baltimore, MD 21205-2185. Tel.: 410-955-7049; Fax: 410-955-3149; E-mail: pagre@jhmi.edu.

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Inc.) One laboratory strain FY86 (S. cerevisiae, MATα, ura3-52, his3Δ200, leu2Δ11) and three wild-type strains, NRRY-L12632 (S. cerevisiae, MATα, Northern Regional Research Laboratories), GRF5 (S. norbensis, MATa), and Σ1278b (S. cerevisiae, MATα), were kindly provided by J. Boeke. Alignments were made with sequences from the yeast genome database (10, 15).

PCR Amplifications—Oligonucleotide primers used in this study are listed in Table I. All PCR reactions were performed with the Expand High Fidelity System (Boehringer Mannheim) with 10× buffer 2 containing MgCl2. Reactions contained 0.2 μm primers, 160 μm dNTPs, 1× buffer 2, and 50 ng of DNA template. Polymerase (0.75 μl per 50-μl reaction) was added after denaturation for 5 min at 95°C. Thirty cycles were performed with the following conditions: denaturation for 1 min at 94°C, annealing for 1 min at a temperature specific for each pair of primers, elongation for 1 min at 72°C. To complete the final strand, the last step was allowed to run 11 min at 72°C. Aqs1 (18). After transformation, cells the one-step gene replacement method (22) using the High Fidelity System (Boehringer Mannheim) with 10 μl of polymerase and 10 μl of template DNA was added to the reaction. The reactions were incubated a total of 27 h, and the final 50 μl of PCR product was amplified from the plasmid pUG6 kindly provided by N. Shani (18) using the primers 5′ KOKanY1 and 3′ KOKanY1 after denaturation for 5 min at 95°C followed by five cycles of amplification: 1 min at 94°C, 1 min at 55°C, 1 min at 72°C. This was followed by 20 cycles: 1 min at 94°C, 1 min at 65°C, 1 min at 72°C. To complete the final strand, the last step ran 11 min at 72°C. To generate a genomic Southern probe, a 650-bp fragment was amplified from Σ1278b genomic DNA using primers SCAQP3 and SCAQP4 specific to the S288C Aqy1p 5′-flanking region with annealing at 63°C.

Plasmid Construction—Plasmids used in this study are listed in Table I and were generated with standard cloning methods (19). Constructs were checked by restriction digestion and double strand sequencing. The SCAQP1–5/SCAQPI–3′ PCR products amplified from genomic DNA of strains FY86, NRRY-L12632, GRF5, and Σ1278b were cloned into pCR2.1 (Invitrogen) yielding pFY86103, pGN18102, p912103, and p912102. The SCAQP1–5/SAQY1–3′ PCR product from strain Σ1278b was cloned into pCR2.1 yielding p912103. For expression of Aqy1p in oocytes, BgII/BglII fragments containing the Aqy1 ORF from pFY86103, pGN18102, and p912103 were cloned into the BglII site of pEG202 to form pFY86105, pGN18106, and p912105. The expression vector was linearized at EcoRI and XhoI. The expression vector was linearized at the MluI and AvaII sites. Membranes were probed with the affinity-purified polyclonal anti-Aqy1p antibody overnight at 4°C. Then, horseradish peroxidase conjugated to donkey anti-rabbit IgG (Amersham Pharmacia Biotech) was added and the band was visualized by chemiluminescence. Phenotypic Analyses—Studies routinely employed 200 μl of YPD cultures of Σ1278b and MELY11 inoculated with single colonies from fresh YPD or YPD/G418 plates. For the growth curve experiments, 50-ml cultures of Σ1278b or MELY11 were grown in 200 ml of YPD, 20% NaCl. At time zero, 100 μl of each culture was diluted 100 times in YPD, and five 20-μl aliquots of Σ1278b or MELY11 were plated on YPD or YPD/G418 plates. OD was determined, and aliquots were diluted 200 times (after incubation periods of 2 and 4 h) and 1,000 times (after incubation periods of 6 and 8 h) and plated. After 8 h, the cells grown in 20% YPD were spun down at 1,500 × g for 5 min and resuspended in fresh 20% YPD to restore nutrients. Cultures were grown under these conditions for an additional 24 h. Protein analysis was performed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting using goat polyclonal antibodies to Aqy1p or Aqy2p (23). Blots were incubated in 5% nonfat dry milk in Tris-buffered saline followed by incubation with biotin-conjugated secondary antibodies and reaction with horseradish peroxidase-conjugated streptavidin (Pierce). Membranes were probed with the affinity-purified polyclonal anti-Aqy1p antibody overnight at 4°C. Then, horseradish peroxidase conjugated to donkey anti-rabbit IgG (Amersham Pharmacia Biotech) was added and the band was visualized by chemiluminescence.
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RESULTS

DNA Sequence Analysis of Putative Aquaporins—The complete genomic DNA sequence from the S. cerevisiae laboratory strain S288C (10) was found to contain four genes related to the aquaporin family of membrane water channels. The known gene FPS1 encodes a protein related to bacterial glycerol facilitators (12), and a second related gene YFL054 was identified. These homologs are genetically close to the aquaglyceroporins, the group of mammalian proteins that transport water, glycerol, and other small molecules (aquaglyceroporins, stippled area). The bar represents the genetic distance of the branch length (24).

A second variant of AQY1 ORF was found in two wild-type strains, Σ1278b and GRF5 (Fig. 2B), which both share a C terminus distinctly different from the first variant ORFs. These AQY1 genes encode 327-amino acid polypeptides with a calculated molecular mass of 35.4 kDa (Σ1278b) and structural domains and residues highly conserved among mammalian and plant aquaporins, including Val-121 and Pro-255. The deduced amino acid sequences were otherwise identical except for three polymorphisms: R42K, V53A, and P308S. The major difference between these Aqy1 polypeptides and those with the first variant is the C-terminal domain. In the 984-bp ORF, a series of seven adenines beginning at position 872 (instead of eight adenines) lead to translation from the reading frame beginning with Val-293 (Fig. 2B, arrows). Thus, when compared with FY86, S288C, and NRRL-Y-12632 strains, the Aqy1p from wild-type strains Σ1278b and GRF5 each possess a longer C terminus with an 18-residue hydrophobic stretch, which may represent a seventh transmembrane segment.

Functional Analysis of Water Permeability—Functional demonstration of yeast aquaporin homologs was established by calculating the coefficients of osmotic water permeability, Pᵣ (21), from rates of hypo-osmotic swelling measured from Xenopus oocytes injected with cRNA synthesized from AQY1 constructs prepared from multiple laboratory strains or wild-type strains of Saccharomyces (described in Tables I and II). Oocytes expressing Aqy1p from laboratory strain FY86 failed to show an increase in Pᵣ (Table II, Fig. 3A). In contrast, oocytes expressing Aqy1p from wild-type strain FY86 failed to show an increase in Pᵣ (Table II, Fig. 3A). In contrast, oocytes expressing Aqy1p from wild-type strains Σ1278b and GRF5 each exhibited more than a 10-fold increase in Pᵣ (127.6×10⁻⁴ cm/s) when compared with the water-injected oocytes (10.5×10⁻⁴ cm/s, Fig. 3A). Consistent with the lack of a cysteine preceding the second NPA motif (28), no inhibition of Pᵣ was achieved by preincubating oocytes in up to 1 mM HgCl₂ (128×10⁻⁴ cm/s).

To determine whether all cRNA injections resulted in Aqy1p expression, an antibody was raised to a synthetic peptide corresponding to 20 amino acids of the Aqy1p N terminus. Immunoblot analysis revealed that oocytes expressing increased Pf (127.6×10⁻⁴ cm/s) contained significant amounts of the 35-kDa polypeptide. In contrast, oocytes injected with cRNAs that conferred no increase in Pf contained more than a 10-fold increase in the Pf (127.6×10⁻⁴ cm/s) compared with FY86, S288C, and NRRL-Y-12632 strains, the Aqy1p from wild-type strains Σ1278b and GRF5 each possess a longer C terminus with an 18-residue hydrophobic stretch, which may represent a seventh transmembrane segment.

The most notable sequence difference in Aqy1p between lab-
oratory and wild-type strains is at the C terminus (Fig. 2). To establish the functional importance of this variation, we constructed two DNAs encoding chimeric proteins (Table II, Fig. 2). The first chimera, Aqy1pCh1, is a fusion of \( \Sigma 1278b \) wild-type Aqy1p (M1-K292) with the C terminus of FY86 laboratory strain Aqy1p (M1-121V) with the C terminus of FY86 laboratory strain of yeast (FY86) were directly compared with a laboratory strain of yeast (FY86) containing the D47N mutation.

To explore other possible explanations for reduced water permeability by oocytes expressing Aqy1p from laboratory strains, a series of site-directed mutants was prepared with each of the amino acid substitutions replaced individually or in pairs by the corresponding residues in Aqy1p from the wild-type strain \( \Sigma 1278b \). This study

### Yeast strains, plasmids, and oligonucleotides

| Strain | Genotype | Source, reference |
|--------|----------|------------------|
| \( \Sigma 1278b \) | S. cerevisiae, MATa | J. Boeke |
| FY86 | S. cerevisiae, MATa, ura3–52, his3Δ200, leu2Δ1 | J. Boeke |
| NRRL-Y-12632 | S. cerevisiae, MATa | J. Boeke; North. Reg. Res. Labs. |
| GRF5 | S. norbensis, MATa | J. Boeke |
| MELY11 | \( \Sigma 1278b \) MATa, aqy1::loxP-KanMX-loxP | This study |

The table includes a variety of yeast strains, plasmids, and oligonucleotides used in the study. The table lists the strains, genotypes, and their sources or references. The strains include \( \Sigma 1278b \), FY86, NRRL-Y-12632, and GRF5, each with specific genetic markers. The plasmids listed include p912102, p912103, pX799102, and others, each with unique descriptions. The oligonucleotides are also listed, including those used for primer synthesis.

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### Yeast strains, plasmids, and oligonucleotides

| Primer | Sequence |
|--------|----------|
| ScAQPI–1′ | 5’ GGGAGATCTCACCATGAGTTCAAGATGAGGACTGAGATAGTATAGC 3’ |
| ScAQPI–3′ | 5’ GGGAATCTGAGGGAGAAAAAATGACCTACAAGC 3’ |
| LAQY1–3 | 5’ GGGAATCTGACAATTTATCTAGGATTTTGGCC 3’ |
| Aqy1p-AviI | 5’ CCCCTGCTGGTTGCTGTCGTG 3’ |
| Aqy1p-LT | 5’ CCCATGCCTTCTACGTCAGCCACAGGAGGAACGTGGCGCCGCTGCTG 3’ |
| Aqy1p-D47N | 5’ CGGAGGAAATGTTTTCAGCGGGTGGC 3’ |
| Aqy1p-D121V | 5’ CACAGGAGAACGGAAACGAGATCTGTCGTGGCC 3’ |
| Aqy1p-T255P | 5’ CTAATGTTGCTACCCAGGAAACGAGATCTGTCGTGGCC 3’ |
| Scal-Mlu | 5′ CTGTTGACTGAGGGCGGCTCAGGACGTGC 3’ |
| Scal-Mlu | 5′ CTGTTGACTGAGGGCGGCTCAGGACGTGC 3’ |
| Scal-Mlu | 5′ CTGTTGACTGAGGGCGGCTCAGGACGTGC 3’ |
| Scal-Mlu | 5′ CTGTTGACTGAGGGCGGCTCAGGACGTGC 3’ |
| Scal-Mlu | 5′ CTGTTGACTGAGGGCGGCTCAGGACGTGC 3’ |
| Scal-Mlu | 5′ CTGTTGACTGAGGGCGGCTCAGGACGTGC 3’ |
| Scal-Mlu | 5′ CTGTTGACTGAGGGCGGCTCAGGACGTGC 3’ |
| Scal-Mlu | 5′ CTGTTGACTGAGGGCGGCTCAGGACGTGC 3’ |
| Scal-Mlu | 5′ CTGTTGACTGAGGGCGGCTCAGGACGTGC 3’ |
| Scal-Mlu | 5′ CTGTTGACTGAGGGCGGCTCAGGACGTGC 3’ |
| Scal-Mlu | 5′ CTGTTGACTGAGGGCGGCTCAGGACGTGC 3’ |
| Scal-Mlu | 5′ CTGTTGACTGAGGGCGGCTCAGGACGTGC 3’ |

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### Sensitivity to Osmotic Stress by Laboratory and Wild-Type Strains

The identification of AqY1 with two amino acid substitutions that impair protein biogenesis or function in multiple laboratory strains of Saccharomyces suggests that the yeast are under selective pressure not to express functional aquaporins during laboratory procedures. Membranes from a laboratory strain of yeast (FY86) were directly compared with a wild-type strain (\( \Sigma 1278b \)) by immunoblotting with anti-Aqy1p (Fig. 4A). Although the lab strain contained a weakly detectable band of ~35 kDa, the wild-type yeast contained a much stronger signal. To determine whether deletion of the AqY1 gene from \( \Sigma 1278b \) cells would produce a similar selective advantage, it was removed by the gene replacement technique (18). Genomic DNAs from five geneticin-resistant clones were digested with HindIII and EcoRV + SpeI and probed with the...
PCR-amplified fragment specific to the S288C AQY1 5′-flanking region (Fig. 4B). Hybridization signals of 630 bp after the HinClII digestion and 2270 bp after EcoRV digestion were detected in four clones (MELY11–14), confirming the recombination between the loxP-KanMX-loxP cassette and AQY1. AQY1 deletion clones were also confirmed by PCR using the AQY1 ORF-specific primers SCAQP1–5 and SCAQP1–3 (not shown).

To test the phenotype of the AQY1-deleted yeast, the growth rate of parental wild-type strain S1278b and clone MELY11 were compared in hypo-osmolar media (20% YPD), iso-osmolar media (YPD), and hyper-osmolar media (YPD 1 M NaCl). In all growth conditions, wild-type and MELY11 cells exhibited similar growth rates (Fig. 5A). Wild-type and MELY11 cells were similarly sensitive to hyper-osmolarity, as their growth rates were equivalently reduced in presence of 1 M NaCl (Fig. 5A). Likewise, they both exhibited equivalently brisk growth rates in YPD and 20% YPD. Wild-type and MELY11 cells were then subjected to a series of osmotic stress-growth cycles. Growth in hypo-osmolar buffer with hyper-osmolar washes produced a clear advantage for the MELY11 cells, with incremental differences becoming more pronounced after six cycles (Fig. 5B). Equivalent results were achieved in nine successive experiments. The converse experiment was performed by culturing the cells in hyper-osmolar media followed by hypo-osmolar washes. After the first cycle, the number of surviving cells had greatly declined, but the MELY11 cells exhibited a 4-fold survival advantage (Fig. 5C). Although the number of surviving cells further declined after each cycle, the MELY11 cells were found to be relatively spared with 6.4-, 6.8-, and 9.2-fold greater survival after two, four, and six cycles (Fig. 5C). Equivalent results were achieved in eight successive experiments. To confirm that the plating on geneticin did not affect the survival rates, MELY11 cells were plated on YPD plates.
without or with the antibiotic, and survivals were similar in the presence of geneticin (Fig. 5, B and C). Thus, we conclude that increased survival under these conditions is significantly enhanced by deletion of AQY1.

DISCUSSION

The recent availability of completely sequenced genomes has greatly facilitated recognition of genes homologous to known sequences; however our experience illustrates some complexities encountered when taking this information to the laboratory bench. Computer screening of the genome from S. cerevisiae (10) revealed that the widely expressed aquaporin family of membrane water channels is represented in yeast. As expected, we found the gene FPS1, which is known to encode a glycerol transporter with sequence similarities to aquaglyceroporins (12), and a second sequence-related ORF was found, which most likely also encodes a glycerol transporter (Fig. 1). Another sequence-related ORF was recognized in the S. cerevisiae genome, and our studies indicate that AQY1 represents an aquaporin gene.

Surprisingly, different laboratory and wild-type strains of S. cerevisiae were found to have specific sequence differences with functional consequences. AQY1 from two laboratory strains and one wild-type strain encode a 305-residue polypeptide that fails to confer increased osmotic water permeability when expressed in Xenopus oocytes. In contrast, AQY1 from two other wild-type strains encodes a 327-residue polypeptide that is functional in oocytes. To our knowledge, these observations represent the first demonstration of a functional aquaporin from yeast and are supported by preliminary studies made independently by other scientists (26).

The failure of Aqy1p from laboratory strains to transport water is not due to the C terminus. The single nucleotide insertion before codon 293 of AQY1 from laboratory strains results in a frameshift with termination after codon 305. Although we initially suspected this to be an explanation for the lack of function, creation of an Aqy1p chimeric molecule with the laboratory strain C terminus on wild-type Aqy1p did not cause reduced activity but actually resulted in a significant 70% increase in Pf. In addition, creation of a chimeric Aqy1p with the wild-type C terminus replaced by a non-natural peptide sequence also increased the Pf. Although not yet conclusive, these observations suggest that the C terminus of the wild-type sequence containing hydrophobic stretches with spaced, charged residues may serve as a negative regulator of Aqy1p.

Differences within the coding region of Aqy1p from laboratory and wild-type strains revealed genetic explanations for the lack of function. Three amino acid substitutions were found when the
Aqy1p sequences of wild-type and laboratory strains were compared, and two led to loss of water transport activity when expressed in oocytes. The V121M substitution lies within loop B (Fig. 2) and may occlude the channel possibly destabilizing the protein, since in the hourglass model it is proposed that loops B and E fold back in the membrane bilayer forming a single aqueous pathway (27). The P255T substitution occurs at the site of a critical bend in the sixth membrane-spanning α-helix (3) and therefore may induce a conformational change impairing stability of the protein. Immunoblot analysis of oocytes expressing these mutant forms of Aqy1p indicated that cRNA-injected oocytes with low Pf values contained only low levels of the protein and some higher molecular weight aggregates (Fig. 4B). Since translation should be similar for all injected AQY1 cRNAs, the low level of protein indicates that the Aqy1p from the laboratory strains may be misfolded and unstable in the oocytes. Although this may possibly represent an artifact of the oocyte system (30), these results are consistent with the reduced level of endogenous Aqy1p in the laboratory yeast strain FY86 compared with the wild-type strain Σ1278b (Fig. 4A). Studies of mutant forms of AQP1 (28) and AQP2 (29) demonstrated the failure to traffic to the outer membrane because of apparent misfolding.

Our studies demonstrate that Aqy1p is not essential for life, and standard laboratory conditions exert negative selection pressure on yeast expressing the protein. This is particularly well supported by identification of two separate mutations in AQY1 leaving minimal chance for spontaneous reversal. When compared by survival in conditions representing exaggerations of laboratory procedures, we found that osmotic stress during growth cycles was much better tolerated by AQY1 null yeast than by the parental wild-type yeast.

Our studies do not define the physiological role of Aqy1p in a natural environment; however, a role in osmoregulation seems likely. Current models describing how cells sense osmotic stress do not delineate the very first step in the process, which is rapid flow of water among intracellular compartments. It is known that in response to a hyper-osmotic stress, yeast cells lose cytoplasmic water; this is followed by an efflux of water from the vacuole to the cytoplasm. Finally, the cells accumulate glycerol at high concentrations. In response to hypo-osmotic stress, yeast cells cease to accumulate glycerol and undergo activation of a mitogen-activated protein kinase cascade, which is regulated by Pkc1p (for review, see Ref. 31). We speculate that Aqy1p may mediate rapid movements of water preceding the osmotic responses.

It has recently been shown that the wild-type strain Σ1278b is capable of pseudohyphal growth under nitrogen starvation conditions, whereas laboratory strain S288C is unable to form pseudohyphae (32, 33). The authors demonstrated a point mutation in FLO8 from strain S288C leading to a block in growth of pseudohyphae. Because many wild-type yeast strains are filamentous, pseudohyphal formation may contribute to their ability to survive in their normal environments. Many laboratory strains are defective in pseudohyphal formation and carry the same FLO8 mutation, leading to the conclusion that these mutations were selected during laboratory cultivation. Thus, comparisons of data between laboratories using different putative wild-type strains must be undertaken with the utmost care.

Although our studies predict that Aqy1p will function to facilitate osmotically directed movements of water, firm identification of the physiological need for this process may not be achieved directly. The E. coli homolog AqpZ was also found to be nonessential for life; however, the null phenotype only became obvious under conditions sustaining maximum growth rates (7). The known sequence-related protein Fps1p was predicted by gene sequence comparisons to be a glycerol facilitator (11). Bacterial glycerol facilitators have been defined in oocytes for their ability to transport glycerol (34) and are related to aquaglyceroporins. Much effort was needed to demonstrate the function of Fps1p in releasing glycerol (an osmolyte) in response to hypo-osmotic stress (12). Nevertheless, Fps1p is not essential for life, and other functions are still being studied including possible roles in phospholipid biogenesis (13) or in maintenance of osmotic balance, which regulates cell fusion during mating (35).

Elucidation of the normal functions of Aqy1p will require additional studies. It is possible that Aqy1p may be involved in specific phases of the cell cycle. If control of the osmotic balance is important for mating, it may also be critical for processes like budding, pseudohyphal formation, or sporulation. It has been already shown that laboratory strains, industrial strains, and wild-type strains of yeast may have physiological differences in osmosensitivity, but the genetic explanation is not yet known (36). Our studies showing that wild-type and laboratory strains of S. cerevisiae contain distinct AQY1 sequences, resulting in major functional differences that may provide an explanation for these differences.
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REFERENCES

1. Agre, P., Bonhivers, M., and Borgnia, M. J. (1998) J. Biol. Chem. 273, 14659–14662
2. Walz, T., Teruhisa, H., Kazuyoshi, M., Heymann, J. B., Mitsuoka, K., Fujiiysahi, Y., Smith, B. L., Agre, P., and Engel, A. (1997) Nature 387, 624–627
3. Heymann, J. B., Agre, P., and Engel, A. (1998) J. Struct Biol. 121, 191–206
4. Flick, K. M., Shaulsky, G., and Loomis, W. F. (1997) Gene (Amst.) 195, 127–130
5. Calamita, G., Kempf, B., Bishai, W. R., Bremer, E., and Agre, P. (1997) Biol. Cell 89, 321–329
6. Calamita, G., Bishai, W. R., Preston, G. M., Guggino, W. B., and Agre, P. (1995) J. Biol. Chem. 270, 29063–29066
7. Calamita, G., Kempf, B., Bonhivers, M., Bishai, W., Bremer, E., and Agre, P. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 3627–3631
8. Kashiwagi, S., Kanamaru, K., and Mizuno, T. (1995) Biochim. Biophys. Acta 1237, 189–192
9. Posas, F. and Saito, H. (1997) Science 276, 1702–1705
10. Goffeau, A., Barrell, B. G., Bussey, H., Davis, R. W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J. D., Jacq, C., Johnston, M., Louis, E. J., Mewes, H. W., Murakami, Y., Philipppens, P., Tettelin, H., and Oliver, S. G. (1996) Science 274, 546–547
11. Park, J. H., and Saier, M. H. J. (1996) J. Membr. Biol. 153, 171–180
12. Luyten, K., Albertyn, J., Skibbe, W. F., Prior, B. A., Ramos, J., Thevelein, J. M., and Hohmann, S. (1995) EMBO J. 14, 1360–1371
13. Sutherland, F. W. C., Laguë, R., Lucas, C., Luyten, K., Albertyn, J., Hohmann, S., Prior, B. A., and Kilian, S. G. (1997) J. Bacteriol. 179, 7790–7795
14. Mortimer, R. K., and Johnston, J. R. (1986) Genetics 113, 35–43
15. Dujon, B. (1993) Cold Spring Harbor Symp. Quant. Biol. 58, 357–366
16. Winston, F., Dolliard, C., and Ricupero-Hovasse, S. L. (1995) Yeast 11, 53–55
17. Kozak, M. (1991) J. Biol. Chem. 266, 19867–19870
18. Guldener, U., Heck, S., Fiedler, T., Beinhauer, J., and Hegemann, J. H. (1996) Nucleic Acids Res. 24, 2519–2524
19. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
20. Preston, G. M., Carroll, T. P., Guggino, W. B., and Agre, P. (1992) Science 256, 385–387
21. Zhang, R., Logee, K. A., and Verkman, A. S. (1990) J. Biol. Chem. 265, 15375–15378
22. Orr-Weaver, T. L., Szostak, J. W., and Rothstein, R. J. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6354–6358
23. Laemmli, U. K. (1970) Nature 227, 680–685
24. Higgins, D., Thompson, J., and Gibson, T. (1996) Methods Enzymol. 266, 383–402
25. Rost, B., Casadio, R., Fariselli, P., and Sander, C. (1995) Protein Sci. 4, 521–533
26. Laize, V., Roudier, N., Rousselet, G., Ripoche, P., and Tacnet, F. (1998) FASEB J., 12, 2540 (abstract)
27. Jung, J. S., Preston, G. M., Smith, B. L., Guggino, W. B., and Agre, P. (1994) J. Biol. Chem. 269, 14648–14654
28. Preston, G. M., Jung, J. S., Guggino, W. B., and Agre, P. (1993) J. Biol. Chem. 268, 17–20
29. Deen, P. M., Croes, H., van Aubel, R. A., Ginsel, L. A., and van Os, C. H. (1995) J. Clin. Invest. 95, 2291–2296
30. Lagrère, V., Pellerin, I., Hubert, J.-F., Tacnet, F., Le Cahére, F., Roudier, N., Thomas, D., Gouranton, J., and Deschamps, S. (1998) J. Biol. Chem. 273, 12422–12426
31. Varela, J. C. S., and Mager, W. H. (1996) Microbiology 142, 721–731
32. Liu, H., Styles, C. A., and Fink, G. R. (1996) Genetics 144, 967–978
33. Kron, S. J. (1997) Trends Microbiol. 5, 450–454
34. Maurel, C., Reizer J., Schroeder, J. I., Chrispeels, M. J., and Saier, M. H. (1994) J. Biol. Chem. 269, 11869–11872
35. Philips, J., and Herskowitz, I. (1997) J. Cell Biol. 138, 961–974
36. Blomberg, A. (1997) Yeast 13, 529–539

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