Arsenite transport by mammalian aquaglyceroporins AQP7 and AQP9

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Much is known about the transport of arsenite and antimonite into microbes, but the identities of mammalian transport proteins are unknown. The *Saccharomyces cerevisiae FPS1* gene encodes a membrane protein homologous to the bacterial aquaglyceroporin GlpF and to mammalian aquaglyceroporins AQP7 and AQP9. Fps1p mediates glycerol uptake and glycerol efflux in response to hypoosmotic shock. Fps1p has been shown to facilitate uptake of the metalloids arsenite and antimonite, and the *Escherichia coli* homolog, GlpF, facilitates the uptake and sensitivity to metalloid salts. In this study, the ability of mammalian aquaglyceroporins AQP7 and AQP9 to substitute for the yeast Fps1p was examined. The *fps1*Δ strain of *S. cerevisiae* exhibits increased tolerance to arsenite and antimonite compared to a wild-type strain. Introduction of a plasmid containing AQP9 reverses the metalloid tolerance of the deletion strain. AQP7 was not expressed in yeast. The *fps1*Δ cells exhibit reduced transport of ^{73}As(III) or ^{125}Sb(III), but uptake is enhanced by expression of AQP9. *Xenopus laevis* oocytes microinjected with either AQP7 or AQP9 cRNA exhibited increased transport of ^{73}As(III). These results suggest that AQP9 and AQP7 may be a major routes of arsenite uptake into mammalian cells, an observation potentially of large importance for understanding the action of arsenite as a human toxin and carcinogen, as well as its efficacy as a chemotherapeutic agent for acute promyelocytic leukemia.

Fps1p | GlpF | acute promyelocytic leukemia

The U.S. Environmental Protection Agency has classified arsenic as a human carcinogen (1). Conversely trivalent arsenic is an effective chemotherapeutic agent for the treatment of acute promyelocytic leukemia (2). For a complete understanding of the mechanisms of arsenic toxicity and carcinogenesis as well as its ability to serve as a chemotherapeutic agent, it is necessary to identify the routes of arsenite uptake and efflux from cells (3, 4). Arsenic is bioavailable in either of two oxidation states, As(V) (arsenate) or As(III) (arsenite). Pentavalent arsenic is transported into cells via phosphate transport systems in both prokaryotes (5) and eukaryotes (6). It is reduced to the more toxic trivalent arsenite by arsenate reductases (7–9). Arsenite can be detoxified either by extrusion from cells or by sequestration within intracellular organelles as thiol conjugates (3, 4, 10). In *Saccharomyces cerevisiae*, Acr3p is an arsenite extrusion system (11, 12), and the ABC transporter Ycf1p catalyzes uptake of $As(GS)_3$ into the vacuole (12).

The routes of uptake of arsenite are less well defined. In *Escherichia coli*, we showed that disruption of the *glpF* gene led to increased tolerance for antimonite, which is chemically similar to arsenite (13). GlpF facilitates glycerol uptake (14, 15) and is a bacterial member of the aquaporins, a family of membrane proteins that includes water-selective pores (aquaporins) and multifunctional channels (aquaglyceroporins), which also transport organic polyols and urea (16). It is likely that GlpF recognizes the un-ionized form of antimonite, $Sb(OH)_3$, transporting it as the inorganic equivalent of a polyol. With a pKa of 11.8, this protonated form would predominate in neutral solutions. Although the *glpF* mutant did not exhibit increased arsenite tolerance, we postulated that GlpF also transports $As(OH)_3$, the primary ionization state of arsenite in neutral solutions, but that redundancy in arsenic transporters dampens the effect of the *glpF* mutation (13). Recently the GlpF homolog Fps1p, a *S. cerevisiae* aquaglyceroporin (17), was clearly shown to be involved in uptake of arsenite (18). Deletion of the *FPS1* gene resulted in increased tolerance to both arsenite and antimonite, and *fps1*Δ cells exhibited decreased uptake of radioactive arsenite. Although *E. coli* GlpF has a physiological role for uptake of glycerol, yeast Fps1p functions primarily as a glycerol efflux pathway for osmoregulation (19).

The transporters for uptake of arsenite and antimonite into mammalian cells are unknown. Passive permeability of the neutral $As(OH)_3$ and $Sb(OH)_3$ species is energetically unfavorable, implying that one or more transport proteins must exist. Given the ubiquity of aquaglyceroporins and their ability to transport arsenite into microorganisms, it was reasonable to investigate the metalloid transport properties of mammalian aquaglyceroporins. The mammalian aquaglyceroporin subfamily includes AQP3, AQP7, and AQP9, of which AQP9 has broadest solute permeability (16). In this report the *Rattus norvegicus* AQP9 (20) cDNA was cloned into an *S. cerevisiae* expression vector under control of the *GAL4* promoter, and its ability to substitute for Fps1p in metalloid uptake was examined. A strain of *S. cerevisiae* was constructed from which the genes encoding *FPS1* and the two arsenite efflux transporters, Acr3p and Ycf1p, were deleted. Compared with a wild-type strain, an *acr3*Δ *ycf1*Δ strain is hypersensitive to both arsenite and antimonite (12). Deletion of *FPS1* in the *acr3*Δ *ycf1*Δ strain increased tolerance to both metalloids, in agreement with the results of Wysocki *et al.* (18). Galactose-inducible expression of membrane-bound AQP9 was observed, and AQP9 expression made the triple deletion strain more sensitive to arsenite and antimonite in a galactose-dependent fashion. Cells of the *acr3*Δ *ycf1*Δ strain accumulate more $^{73}As(OH)_3$ than a wild-type strain because they are unable to extrude arsenite (12). Cells of the *fps1*Δ *acr3*Δ *ycf1*Δ strain accumulated less arsenite than the *acr3*Δ *ycf1*Δ strain, consistent with Fps1p facilitating arsenite uptake (18). AQP9 expression resulted in increased transport of both $^{73}As(OH)_3$ and $^{125}Sb(OH)_3$, demonstrating that AQP9 can functionally substitute for Fps1p in the transport of metalloids. In contrast, when the *Mus musculus* AQP7 cDNA was cloned into the same yeast vector, no AQP7 was expressed. However, *Xenopus* oocytes microinjected with either AQP7 or AQP9 cRNA exhibited a 10-fold increase in $^{73}As(OH)_3$ permeability, indicating that both aquaglyceroporins recognize and transport arsenite.

Materials and Methods

Strains and Plasmids. Plasmids and *S. cerevisiae* strains used in this study are described in Table 1. *E. coli* strain JM109 [*recA1*

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Table 1. Strains and plasmids

Strains/plasmids	Genotype/description	Source
S. cerevisiae strains		
W303-1B	*MATα ade2-1 his3-11,15 leu2-3,112 ura3-1 trp-1*	Ref. 44
DTY167	*MATα ura3-52 his6 leu2-3,112 his3-Δ,200 trp1-901 lys2-80 suc2̃Δycf1::hisG*	Ref. 45
MG102	*MATα ura3-52 his6 leu2-3,112 his3-Δ,200 trp1-901 lys2-801 suc2̃Δycf1::hisG acr3::URA3*	Ref. 12
HD9	*MATα ura3-52 his6 leu2-3,112 his3-Δ,200 trp1-901 lys2-801 suc2̃Δycf1::hisG acr3::URA3 fps1::leu2*	This study
Plasmids		
pGEM-T	*E. coli* cloning vector, Ap^r	Promega
pYES3	*S. cerevisiae–E. coli* shuttle vector, Ap^r, *TRP3*	Invitrogen
pGEM-T-FPS1	1,941-kbp PCR fragment containing *FPS1* cloned in pGEM-T	This study
pUC18	*E. coli* cloning vector, Ap^r	Ref. 46
pUC18-LEU2	3.0-kbp *Bgl*II fragment containing *LEU2* ligated into *Bgl*II site of pUC18	This study
pX-βG-ev1	Cloning vector	Ref. 26
pX-βG-ev1-AQP9	*Rattus norvegicus AQP9* gene cloned into the *Bgl*II site of pXβG-ev1	This study
pAQP9	1.1-kbp *Hin*dIII–*Kpn*I fragment containing AQP9 cloned into *Hin*dIII–*Kpn*I-digested pYES3	This study
pX-βG-ev1-AQP7	*Mus musculus AQP7* gene cloned into the *Bgl*II site of pXβG-ev1	This study
pAQP7	1.2-kpb *Eco*RI fragment containing AQP7 cloned into *Eco*RI-digested pYES3	This study

supE44 endA1 hsdR17 gyrA96 relA1 thi Δ*(lac-proAB) F′ (traD36 proAB*$^+$ *lacI*^{q} *lacZ*Δ*M15*] and JM110 [*rps* (Str^r) *thr leu thi-1 lacY galK galT ara tonA tsx dam dcm supE44* Δ*(lac-proAB)* [*F′ traD36 proAB lacIqZ*Δ*M15*] were used for molecular cloning.

Media. *S. cerevisiae* strains were grown at 30° C in complete yeast extract-peptone-dextrose (2%) or yeast extract-peptone-glycerol (2%) medium (21). Alternatively, the minimal SD medium (composition of the medium may be found in ref. 21) supplemented with either 2% galactose or 2% glucose and with auxotrophic requirements. *E. coli* cells were grown in LB medium (22) supplemented when necessary with 125 μg/ml ampicillin.

DNA Manipulations. Plasmid purification, restriction digestion, endonuclease and exonuclease digestions, gel electrophoresis, PCR, ligation, dephosphorylation, and *E. coli* transformations were carried out as described (22, 23). Transformation of yeast cells was carried out by using a Geno easy-transform kit (Geno Technologies, St. Louis, MO). Yeast genomic DNA was isolated by using QIAamp spin column according to the manufacturer's directions (Qiagen).

Deletion of the *FPS1* Gene. The *FPS1* gene was deleted by a one-step gene replacement method (24). A 1,941-bp fragment of yeast genomic DNA containing *FPS1* was PCR amplified with a forward primer 5′-ATGAGTAATCCTCAAAAAGC-3′ that hybridizes with the start of the *FPS1* gene and a reverse primer 5′-TCATGTTACCTTCTTAGC-3′ that hybridizes to the end of the coding sequence. The fragment was ligated into vector pGEM-T and transformed into *E. coli* JM110. The resulting plasmid, pGEM-T-FPS1, was isolated and digested with *Stu*I, which produced a 1,245-bp fragment that was 427 bp from the start and 269 bp from the end of the FPS1 gene. The 3-kbp *LEU2* gene was isolated from plasmid pUC18-LEU2 by digestion with *Pst*I and *Alw*NI to cut out the *LEU2* gene and with *Sma*I to digest the remaining vector DNA. The *FPS1* and *LEU2* fragments were ligated together, and the resulting plasmid was digested with *Pst*I and *Sph*I. The 3.7-kbp fragment was isolated, purified, and transformed into yeast strain MG102 (*acr3*Δ *ycf1*Δ), producing the *fps1*Δ *acr3*Δ *ycf1*Δ strain HD9 by homologous recombination. Recombinants were selected for growth in the absence of leucine and were screened for arsenite sensitivity. Deletion of *FPS1* was verified by PCR by using the forward and reverse primers described above.

Cloning AQP7 and AQP9 into the *S. cerevisiae* Expression Vector pYES3. AQP7 cDNA was amplified by reverse transcription–PCR of mouse adipocyte total RNA by using forward primer 5′-GGGGAATTCAGATCTATGGCCCCCAGGTCTGT-GCTG-3′ and reverse primer 5′-GGGGAATTCAGATCTT-TAGAAGTGCTCTAGAGGCAC-3′. AQP9 cDNA was amplified by reverse transcription–PCR from rat liver total RNA from Ambion by using a forward 5′-GGGAGATCTGAAT-TCATGCCTTCTGAGAAGGACGGTGC-3′ and reverse primer 5′-GGGAGATCTGAATTCCTACATGATGACA-CTGAGCTCG-3′. PCR products were digested with *Bgl*II and cloned into *Bgl*II-digested vector pXβG-ev1, producing plasmids pXβG-ev1-AQP7 and pXβG-ev1-AQP9. The AQP7 and AQP9 genes were sequenced, and an A93V polymorphism was found in AQP9, which was corrected to the published Ala-93 by site-directed mutagenesis. Plasmid pXβG-ev1-AQP9 was digested with *Hin*dIII and *Kpn*I. A 1.1-kbp fragment containing the AQP9 gene was ligated into *Hin*dIII–*Kpn*I digested plasmid pYES3. The ligation mixture was transformed into *E. coli* strain JM109, and the resulting plasmid, pAQP9, was isolated and transformed into yeast strain HD9. The transformants were selected on minimal SD medium without tryptophan. Plasmid pAQP7 was prepared similarly. Plasmid pXβG-ev1-AQP7 was digested with *Hin*dIII and *Spe*I. The linearized 1.2-kbp fragment containing AQP7 was made blunt by using large fragment of DNA polymerase I. Vector plasmid was digested with *Eco*RI, and the ends were made blunt by using large fragment of DNA polymerase I. The two blunt-end DNAs were ligated together, and the ligation mixture was transformed into *E. coli* strain JM109. A plasmid with the correct insert, termed pAQP7, was isolated and transformed into yeast strain HD9. The transformants were selected on minimal SD medium without tryptophan. The constructs for pAQP7 and pAQP9 were sequenced to confirm that they retained the reported DNA sequences.

Metal Ion Resistance Assays. Strains were grown overnight at 30°C in liquid SD medium with either 2% glucose or 2% galactose and the appropriate supplements. The cultures were diluted into minimal media to an OD_{600} of 0.1 in presence of varying concentrations of the indicated metalloid salts, incubated for an additional 20 h, following which the growth was estimated from OD_{600}. For growth on solid media, cells were streaked from single colonies and incubated for 3–4 d at 30°C.

Transport Assays. *In vivo* metalloid uptake assays were performed as described (12). In brief, cells were grown to exponential phase

at 30°C in either YPG medium or SD medium with 2% galactose in place of glycerol. The cells were harvested, washed twice with degassed transport buffer consisting of 75 mM Hepes, 150 mM KCl, and 1 mM $MgCl_2$ (pH 7.3), and suspended to a density of 2×10^9 cells/ml in the same buffer, all at 4°C. To initiate the assay, 0.1 ml of cells was diluted with 0.9 ml of transport buffer containing 0.1 M glucose. After 30 min at 30°C, $Na_2{}^{73}AsO_2$ or K(^{125}Sb)tartrate were added to a final concentration of 5 μM. When used as an inhibitor, glycerol was added to a final concentration of 200 mM 20 min before the radioactive metalloid. Portions (0.1 ml) were withdrawn at intervals and filtered through nitrocellulose filters (0.2-μm pore size; Whatman). The filters were washed with 5 ml of room temperature transport buffer and dried, and the radioactivity was quantified in liquid scintillation counter. $Na_2{}^{73}AsO_2$ and K(^{125}Sb)tartrate were prepared by reduction of radioactive arsenate or antimonate (25). ^{73}As was obtained from Los Alamos National Laboratories. ^{125}Sb was purchased from New England Nuclear.

Transport in *Xenopus laevis* oocytes was performed as described (26). Plasmids pXβG-ev1-AQP7 and pXβG-ev1-AQP9 were linearized with *Not*I and *Xba*I, respectively. Capped cRNAs were synthesized in *in vitro* reactions by using the linearized plasmids. Oocytes from *Xenopus laevis* were defolliculated and injected with 5 ng of AQP7 cRNA, 25 ng of AQP9 cRNA, or 50 nl of water. They were then incubated at 18°C for 3 d in Barth's solution (26). For transport assays the oocytes were incubated with 0.5 ml of Barth's solution containing 3 μM $^{73}As(OH)_3$. After 90 sec the assay solution was removed, and the oocytes were washed with same solution without labeled arsenite. The oocytes were then solubilized with 0.2 ml of 10% SDS, and radioactivity was quantified in liquid scintillation counter.

Immunological Detection of AQP7 and AQP9 Expression. Membranes for immunoblot analysis of AQP7 and AQP9 expression were prepared as described previously (12). The membranes were analyzed for AQP7 and AQP9 expression by PAGE and immunoblotting. Samples were dissolved in 0.1 ml of SDS sample buffer, incubated for 2 h at room temperature and analyzed by SDS/PAGE (27) by using a 12% polyacrylamide gel. Proteins were electrophoretically transferred to a nitrocellulose membrane (0.2-μm pore size) and immunoblotted overnight at 12 mV and 4° C with an Ab directed against either AQP7 or AQP9 (Alpha Diagnostic, San Antonio) at a dilution of 1:5,000 or 1:4,000, respectively. A chemiluminescent assay was used to detect the antigen-Ab reaction. The filter was incubated with 10 ml of the enhanced chemiluminescence solution (New England Nuclear) and exposed on x-ray film for 10 sec at room temperature.

Results

AQP9 Complements the Metalloid Resistance Phenotype of an *fps1*Δ Strain of *S. cerevisiae*. Two microbial aquaglyceroporins have been shown to facilitate metalloid uptake, GlpF in *E. coli* (13) and Fps1p in *S. cerevisiae* (18). The most closely related mammalian homologs to the two microbial proteins are AQP7 and AQP9. *R. norvegicus* AQP9 is 32% identical and 52% similar to Fps1p. *M. musculus* AQP7 is slightly more distant, with 28% identity and 44% similarity to Fps1p. Both mammalian proteins are more distant from the prokaryotic aquaglyceroporin, but AQP7 and AQP9 are more closely related to each other, sharing 52% identity and 68% similarity.

The ability of AQP7 and AQP9 to substitute for Fps1p in yeast was examined. Strain MG102 (*acr3*Δ *ycf1*Δ) is hypersensitive to metalloids because the genes for Acr3p and Ycf1p have been deleted (12). MG102 was used to construct a triple *fps1*Δ *acr3*Δ *ycf1*Δ strain, HD9. The two mammalian cDNAs were cloned into the yeast expression vector pYES3 under control of the *GAL4* promoter, and galactose-dependent expression was examined.

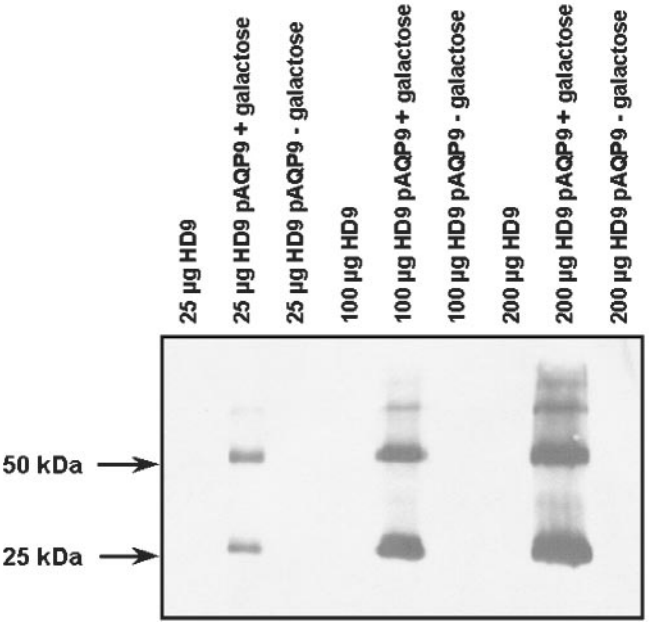

Fig. 1. Expression of AQP9 in membranes of *S. cerevisiae*. Membranes were prepared from cells of strain HD9 or HD9 pAQP9 grown in the absence or presence of 2% galactose, as indicated. Membrane proteins were separated by SDS/PAGE and immunoblotted with anti-AQP9 as described in *Materials and Methods*.

When pAQP9 was introduced into strain HD9, cell membranes contained bands at ≈25 and 50 kDa when induced with galactose (Fig. 1). As is often observed (16), the immunologically reacting bands migrated slightly faster than expected for the mass of a monomer and dimer of the 32-kDa AQP9. In contrast, no immunologically reacting material was found in membranes from cells transformed with pAQP7 (data not shown).

Compared to strain MG102, strain HD9 was resistant to arsenite (Fig. 2*A*). HD9 was also relatively resistant to antimonite (Fig. 2*B*) but not arsenate (Fig. 2*C*) or cadmium (Fig. 2*D*). This result is consistent with the proposal that Fps1p facilitates metalloid uptake in yeast (18). Strain HD9 was used as the host for analysis of AQP7 and AQP9 function. Expression of AQP9 reversed the arsenite (Fig. 2*A*) and antimonite (Fig. 2*B*) resistance phenotype of strain HD9. Reversal of the *fps1*Δ phenotype by expression of AQP9 from the *GAL4* promoter required induction with galactose (Fig. 2*A*). AQP9 had no effect on sensitivity of strain HD9 to either arsenate (Fig. 2*C*) or cadmium (Fig. 2*D*). On the whole, these results clearly demonstrate that AQP9 can replace the function of Fps1p in trivalent metalloid sensitivity. Even though there was not enough AQP7 produced to be detected by Western blotting, it was possible that there was enough to complement the FSP1 deletion. However, introduction of AQP7 on a plasmid had no effect on resistance to either arsenite or antimonite.

AQP9 Facilitates Uptake of $^{73}As(OH)_3$ and $^{125}Sb(OH)_3$ in *S. cerevisiae*. The ability of AQP9 to transport arsenite into yeast also was investigated. Strain MG102 lacks arsenite extrusion and, hence, accumulates arsenite (12). When *FPS1* was deleted in this background, the rate of transport of ^{73}As(III) was reduced (Fig. 3*A*). These results demonstrate that Fps1p facilitates uptake of $^{73}As(OH)_3$, which should be the predominant form at neutral pH. Because aquaporins are channels and do not exhibit energy-dependent concentration of solutes, the apparent accumulation probably reflects intracellular binding of arsenite. Cells expressing AQP9 accumulated more $^{73}As(OH)_3$ than the parental strain

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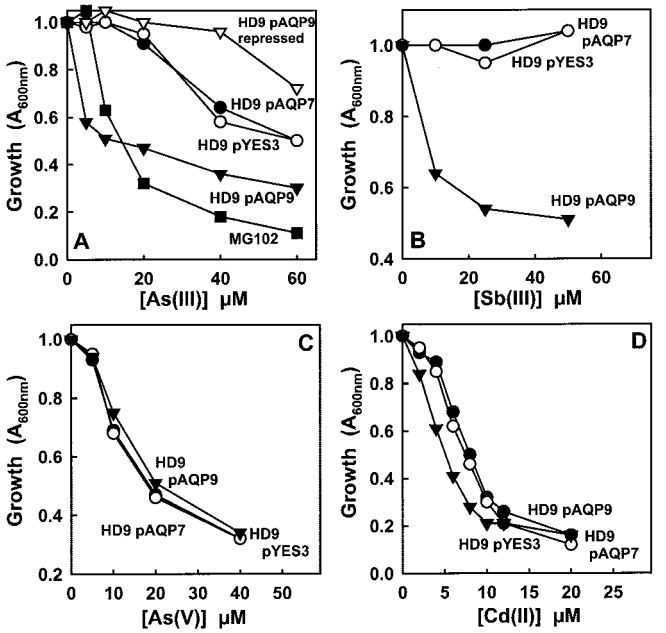

Fig. 2. AQP9 functionally complements the metalloid sensitivity of *S. cerevisiae fps1*Δ. Growth was measured in liquid SD minimal medium with 2% galactose (or, if repressed, glucose) in the presence of the indicated concentrations of sodium arsenite (*A*), potassium antimonyl tartrate (*B*), sodium arsenate (*C*), or cadmium chloride (*D*). Strains were: ■, MG102 (*acr3*Δ *ycf1*Δ); ○, HD9 (*fps1*Δ *acr3*Δ *ycf1*Δ) pYES3; ●, HD9 pAQP7 (induced); ▼, HD9 pAQP9 (induced); and ▽, HD9 pAQP9 (repressed).

MG102. Transport of ^{73}As(III) and ^{125}Sb(III) (see below) consistently exhibited a lag, with increased intracellular arsenite observed at later time points, but the basis for this observation is unknown. Glycerol is the primary substrate of AQP9, so competition between glycerol and arsenite would be expected, and 0.2 M glycerol reduced the uptake of 5 μM arsenite (Fig. 3*B*). In growth assays, 0.2 M glycerol also produced a small reversal of the AQP9-related arsenite sensitivity (data not shown).

Transport of Sb(III) has been inferred from *in vivo* resistance assays, but direct measurement has not been possible because of the unavailability of radioactive antimony. For this study ^{125}Sb was custom synthesized. Cells of the triple deletion *fps1*Δ *acr3*Δ *ycf1*Δ strain HD9 exhibited low rates of transport of $^{125}Sb(OH)_3$ compared to HD9 expressing AQP9, demonstrating that AQP9 facilitates transport of Sb(III) as well as As(III) (Fig. 3*C*).

Both AQP7 and AQP9 Facilitate $^{73}As(OH)_3$ Permeability in *Xenopus* Oocytes. Because AQP7 was not expressed in yeast, the ability of the aquaglyceroporins to increase arsenite permeability in *Xenopus* oocytes was examined. Water, AQP7, or AQP9 cRNA prepared *in vitro* was microinjected into *Xenopus* oocytes. Either aquaglyceroporin increased the uptake of $^{73}As(OH)_3$ ≈10-fold (Fig. 4). These results suggest that both aquaglyceroporins can facilitate uptake of arsenite.

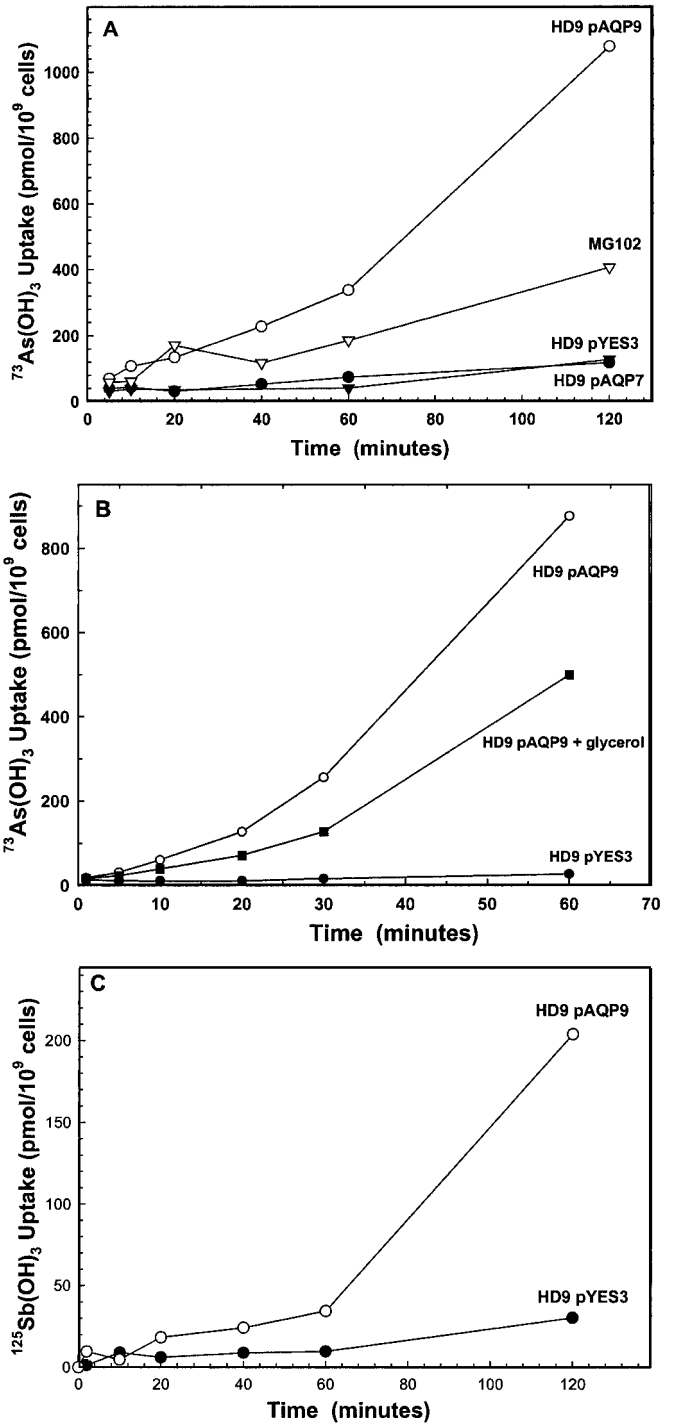

Fig. 3. AQP9 facilitates uptake of $^{73}As(OH)_3$ and $^{125}Sb(OH)_3$. (*A*) Transport of $^{73}As(OH)_3$. (*B*) Inhibition of $^{73}As(OH)_3$ uptake by glycerol. (*C*) Transport of $^{125}Sb(OH)_3$. Strains were: ●, HD9 pYES3; ○, HD9 pAQP9; ▼, HD9 pAQP7; ▽, MG102; and ■, HD9 pAQP9 + 0.2 M glycerol.

Discussion

Aquaglyceroporins have been shown to facilitate metalloid uptake in both *E. coli* (13) and *S. cerevisiae* (18). Without these transporters, cells are unusually resistant to arsenite and/or antimonite. The closest mammalian homologs to *E. coli* GlpF and yeast Fsp1p are AQP7 and AQP9. In this report, we demonstrate that AQP9 can transport arsenite into yeast. AQP9 exhibits the broadest solute permeation, including carbamides, polyols, purines, and pyrimidines (20). AQP7, which is expressed in testis (28) and kidney (29) and has a narrower range of substrate specificity, transporting primarily glycerol and urea (28), was not expressed in yeast. However, in *Xenopus laevis* oocytes AQP7 and AQP9 both increased arsenite permeability. These results indicate that both AQP7 and AQP9 may be routes of arsenite uptake into mammalian cells (Fig. 5).

The structure of aquaporin AQP1 (30, 31) and aquaglyceroporin GlpF (32) have recently been determined at atomic

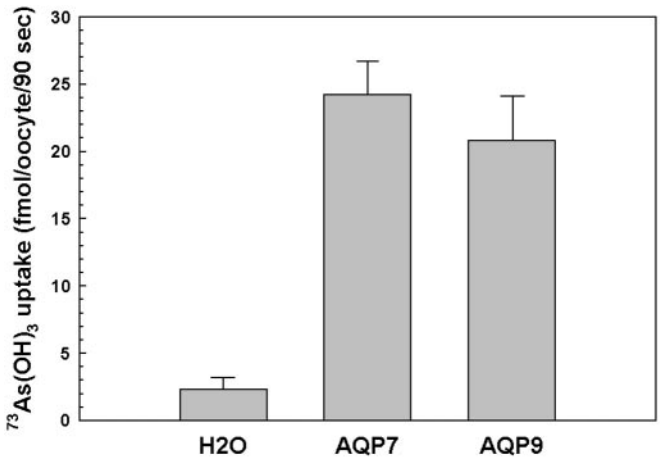

Fig. 4. $^{73}As(OH)_3$ permeability in *Xenopus* oocytes expressing AQP7 or AQP9. Oocyte transport of $^{73}As(OH)_3$ was assayed for 90 sec as described in *Materials and Methods*. Each bar represents the average of three assays. Oocytes were injected with H_2O, AQP7 cRNA, or AQP9 cRNA.

resolution, and the spacing at the narrowest region of the pore (the aromatic/arginine ring) is significantly wider in the aquaglyceroporin. Molecular dynamics simulation may now reveal the permeation mechanism for metalloid transport by AQP7 and AQP9. Arsenite and antimonite are commonly considered oxyanions. Indeed, transport of arsenite by the bacterial ArsB carrier is driven by the membrane potential, suggesting anionic substrates (33, 34). However the trivalent arsenic and antimony acids have pKa values of 9.2 and 11.8, respectively, so that at neutral pH they would be primarily present in solution as neutral species, $As(OH)_3$ or $Sb(OH)_3$. We predict that these neutral polyhydroxylated species have sufficient molecular similarity to glycerol to be substrates of aquaglyceroporins.

Arsenic is classified as a carcinogen by the Environmental Protection Agency (1). Possible mechanisms of carcinogenesis include genetic instability resulting from inhibition of telomerase transcription (35) or by acting as a cocarcinogen to inhibit DNA repair (36). Exposure to arsenic in drinking water is associated with increased risk of multiple cancers. According to the Natural Resources Defense Council, millions of Americans are consuming tap water every day that poses unacceptable cancer risks (http://www.nrdc.org/water/drinking/qarsenic/asp). Over 56 million Americans in the 25 reporting states consumed water from systems containing arsenic at levels presenting a potentially fatal cancer risk. Based on reports by the U.S. National Research Council (37), the Environmental Protection Agency has amended the Safe Drinking Water Act to lower the standard for arsenic in drinking water from 50 to 10 μg/liter; however these standards are based on the assumption that individuals respond uniformly to arsenic. Uniform arsenic uptake is very unlikely because levels of rat AQP9 expression in liver are known to exhibit wide variations because of age, gender, and nutritional status (ref. 38; J.M.C. and P.A., unpublished data). Thus, humans would be expected to respond differently to arsenic in the water supply, and safety standards need to reflect the likelihood that some individual are at higher risk for toxicity.

Most of the epidemiological studies have been conducted in countries such as Taiwan, Bangladesh, and Chile, where arsenic exposure is endemic (39, 40). Chronic effects of arsenic in the water supply include skin hyperpigmentation and keratoses of the hands and feet that frequently progress to skin cancers. In approximately 10% of these cases, exposure is associated with a very high incidence of lung, bladder, and other cancers. However, there is considerable variation in individual responses to arsenic. Some family members in arsenic-contaminated regions of Bangladesh exhibit acute arsenicosis and various forms of cancer, whereas other siblings show few or no adverse effects. Knowledge of the aquaglyceroporin status of these individuals, including DNA sequence and levels of expression, could illuminate reasons for these variations because individuals with increased expression of AQP7 or AQP9 may be more sensitive to environmental arsenic. Thus, the levels of AQP7 and/or AQP9 expression may be a biomarker for arsenic exposure.

AQP9 is primarily expressed in human lung, liver, and leukocytes (41). Interestingly, liver is an organ where arsenite toxicity is found, and some malignancies of the leukocyte lineage respond to arsenite chemotherapy. A century ago, arsenic was recommended as an antileukemic agent; however its use was abandoned because of toxicity. More recently, arsenic trioxide, the anhydrous form of $As(OH)_3$ (Trisenox, Cell Therapeutics, Seattle) has been approved as a chemotherapeutic agent for the treatment of acute promyelocytic leukemia (39, 40). Our study suggests that AQP9 may be responsible for the chemotherapeutic effects of arsenite by facilitating diffusion of the agent into the leukemia cells. As has been found with most chemotherapeutic

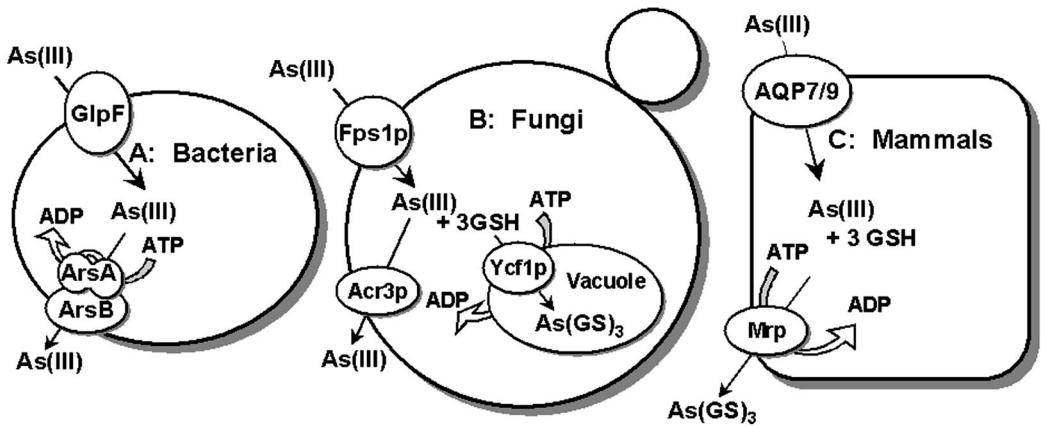

Fig. 5. Metalloid transporters in prokaryotes and eukaryotes. The model shows a comparison of arsenite transport pathways in *E. coli* (*A*), *S. cerevisiae* (*B*), and a generalized mammalian cell (*C*). In each of three, arsenite uptake is facilitated by an aquaglyceroporin: the bacterial GlpF, the yeast Fps1p, or the mammalian AQP7 or AQP9. In *E. coli*, arsenite is extruded from the cytosol by the ArsAB pump. In yeast, arsenite extrusion from the cytosol is catalyzed by Acr3p or into the vacuole by the ATP-coupled Ycf1p pump as $As(GS)_3$. In mammalian cells, various isoforms of the multidrug resistance associate protein (MRP), a Ycf1p homolog, pump out $As(GS)_3$.

agents, resistance eventually arises with continued use (42), and arsenic trioxide resistance will eventually occur. Emergence of AQP9 negative clones of promyelocytic leukemia could signal the need to change therapy. Conversely, individuals with increased expression of AQP9 in liver may have altered sensitivity to arsenic trioxide. In a recent study of 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients treated with arsenic trioxide, seven of the newly diagnosed patients exhibited hepatic toxicity (including two deaths), whereas the relapsed patients had only minor reactions (43). It will be of considerable interest to examine the expression of AQP9 in such patients to determine whether there is a correlation between higher AQP9 levels in the promyelocytic leukemia cells or in the liver and increased sensitivity to arsenic trioxide therapy or toxicity. If so, the AQP9 status of patients with promyelocytic leukemia may be predictive of the success of arsenic trioxide treatment.

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