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Synergistic interaction of glyceraldehydes-3-phosphate dehydrogenase and ArsJ, a novel organoarsenical efflux permease, confers arsenate resistance

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Summary

Microbial biotransformations are major contributors to the arsenic biogeocycle. In parallel with transformations of inorganic arsenic, organoarsenicals pathways have recently been recognized as important components of global cycling of arsenic. The well-characterized pathway of resistance to arsenate is reduction coupled to arsenite efflux. Here, we describe a new pathway of arsenate resistance involving biosynthesis and extrusion of an unusual pentavalent organoarsenical. A number of arsenic resistance (*ars*) operons have two genes of unknown function that are linked in these operons. One, *gapdh*, encodes the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase. The other, *arsJ*, encodes a major facilitator superfamily (MFS) protein. The two genes were cloned from the chromosome of *Pseudomonas aeruginosa*. When expressed together, but not alone, in *Escherichia coli, gapdh* and *arsJ* specifically conferred resistance to arsenate and decreased accumulation of As(V). Everted membrane vesicles from cells expressing *arsJ* accumulated As(V) in the presence of purified GAPDH, D-glceraldehylde 3-phosphate (G3P) and NAD^+. GAPDH forms the unstable organoarsenical 1-arseno-3-phosphoglycerate (1As3PGA). We propose that ArsJ is an efflux permease that extrudes 1As3PGA from cells, where it rapidly dissociates into As(V) and 3-phosphoglycerate (3PGA), creating a novel pathway of arsenate resistance.

Introduction

The toxic metalloid arsenic is ubiquitous in the environment, coming from both natural and anthropogenic sources (Hughes *et al.*, 2011). Exposure to high levels of arsenic causes acute toxicity, but chronic lower level exposure is related to carcinogenesis and other health effects (Mandal and Suzuki, 2002). Environmental arsenic occurs mainly as inorganic species, and association with minerals or organic substances in soils limits its mobility (Akter *et al.*, 2005). Inorganic trivalent As(III) is considerably more toxic than pentavalent As(V), in large

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

part because the trivalent species reacts with small molecule thiols and protein sulfhydryl groups, thereby affecting their function (Hughes *et al.*, 2011). The lower toxicity of arsenate results primarily from its chemical similarity to phosphate. It acts as a phosphate analog in phosphorylation reactions, but the products are less stable and dissociate rapidly (Long and Ray, 1973; Byers *et al.*, 1979; Moore *et al.*, 1983).

As a result of continual exposure to arsenic, most organisms, from bacteria to humans, have evolved arsenic detoxification pathways (Rosen, 2002). In bacteria arsenate is taken up by phosphate transport systems (Rosenberg *et al.*, 1977), where it is reduced to arsenite by arsenate reductases (Mukhopadhyay and Rosen, 2002). Subsequent arsenic resistance mechanisms use arsenite as a substrate for efflux (Yang *et al.*, 2012) or methylation (Ajees and Rosen, 2015). It appears counterintuitive to convert the less toxic pentavelent inorganic arsenic to the more toxic trivalent species for detoxification. We have postulated that the detoxification pathways evolved when the atmosphere was neutral, and most arsenic was present as reduced As(III) (Liu *et al.*, 2013). Only an As(III) efflux system would be required for resistance, and so nearly every extant prokaryote has one of two unrelated As(III) efflux permeases, either ArsB or Acr3 (Yang *et al.*, 2012). Once the atmosphere became oxidizing, arsenic would have become predominately pentavalent As(V). At least three families of arsenate reductase enzymes arose, apparently by convergent evolution, that would transform As(V) to As(III), the substrate of the efflux permeases (Mukhopadhyay and Rosen, 2002).

Yet an arsenate efflux system would have several advantages. First, it would not require cellular reductants or reductases. Second, it would not require biotransformation to more reactive and toxic trivalent species. However, to date, no As(V) efflux systems have been observed. Here, we report the existence of a two-gene system encoded by an *ars* operon in *P. aeruginosa* DK2 that creates, in effect, an arsenate efflux pathway. The first gene, *gapdh*, encodes the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase. The other gene, which we have named *arsJ*, encodes a putative MFS transporter. When *gapdh* or *arsJ* were co-expressed, cells were resistant to As(V) but not As(III) or methylarsenate (MAs(V)) or methylarsenite (MAs(III)). Those cells accumulated less As(V) than control cells without the two genes, suggesting either reduced uptake or increased efflux of an arsenate-containing compound. Everted membrane vesicles prepared from cells expressing *arsJ* accumulated As(V) but not As(III) only when purified rabbit GAPDH and its substrates were present. These results indicate that ArsJ transports an arsenate-containing product of the GAPDH reaction. GAPDH catalyzes formation of 1-arseno-3-phosphoglycerate (1As3PGA), a highly unstable organoarsenical with a half-life of less than 2.5 s (Byers *et al.*, 1979). We predict that 1As3PGA is the true substrate of ArsJ and propose that *in vivo* GAPDH forms 1As3PGA, which is extruded from cells by ArsJ, conferring arsenate resistance. This novel coupling of a glycolytic enzyme and an organoarsenical efflux permease is the first identified transport pathway for arsenate resistance.

Results

Analysis of the ars operons of P. aeruginosa DK2

Arsenic is the most prevalent environmental toxin. As a result of its ubiquity, nearly every prokaryote and archaea has an *ars* operon. A number of *ars* operons include a pair of genes, *gapdh* and *arsJ*, with no known function in arsenic resistance. Representative examples are shown in Fig. 1. For example, in the chromosome of *P. aeruginosa* DK2 (accession number NC_018080.1) there are two *ars* operons from nucleotide 6068254 to nucleotide 6079515, a six-gene operon with *arsR1-orf1-arsA-arsD-arsO-arsN* and a divergently transcribed seven-gene *ars* operon with *arsR2-arsC-acr3-arsH-dspgapdh-arsJ*. Some of the gene products have known functions in arsenic resistance. The ArsRs are As(III)-responsive transcriptional repressors (Shi *et al.*, 1994; Qin *et al.*, 2007; Ordóñez *et al.*, 2008). ArsD is a metallochaperone that transfers As(III) to the ArsA As(III) ATPase (Lin *et al.*, 2006). Acr3 is an As(III) efflux permease that can interact with ArsA to form an ATP-driven efflux pump (Fu *et al.*, 2009). ArsC is an arsenate reductase (Mukhopadhyay and Rosen, 2002). ArsH is a MAs(III) oxidase (Chen *et al.*, 2015). The roles of Orf1, ArsO, ArsN and Dsp (dual specific protein phosphatase) in arsenic resistance are not known.

In this study, we define the roles of the *gapdh* and *arsJ* gene products. The *gapdh* gene product (accession number WP_003109848.1) is a typical glyceraldehyde 3-phosphate dehydrogenase (GAPDH), the cytosolic NAD^+-dependent enzyme (EC 1.2.1.12) found in all organisms so far studied (Fothergill-Gilmore and Michels, 1993). Representative examples are shown in Supporting Information Fig. 1S. The *arsJ* gene product (accession number WP_003109849.1) appears to be a novel 410-residue MFS membrane protein. The transmembrane topology of *P. aeruginosa* DK2 ArsJ was analyzed using the the TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/), which predicts 10 transmembrane spanning segments. In the NCBI database there are 2336 entries for ArsJ sequences, primarily in gammaproteobacteria. We analyzed representative examples (Supporting Information Fig. 2S), and each was in an *ars* operon adjacent to a *gapdh* gene (Fig. 1). There are no related sequences in archaea. A number of eukaryotic algae have related genes. In the alga *Chlamydomonas reinhardtii*, the gene for ArsJ (accession number XP_001702048.1) is also adjacent to genes for GAPDH (accession number XP_001702068.1) and phosphoglycerate kinase (accession number XP_001702049.1). There are also genes for related MFS proteins in the mycorrhizal fungus *Rhizophagus irregularis* DAOM 181602 (accession number ESA15517.1) and in the acorn worm *Saccoglossus kowalevskii* (accession number NP_001171775.1), but neither appears to be adjacent to a *gapdh* gene, and functional relatedness of the gene products to ArsJ is uncertain.

GAPDH and ArsJ synergistically confer As(V) resistance

The physical association of the *gapdh* and *arsJ* genes in *ars* operon suggests a common function. For example, the *arsD* and *arsA* genes are nearly always adjacent to each other in *ars* operons, and ArsD serves as an As(III) chaperone for the ArsA As(III)-translocating ATPase (Lin *et al.*, 2006). The *gapdh* and *arsJ* genes were cloned individually and together into pUC19 under control of the *lac* promoter, creating plasmids pGAPDH, pArsJ and pGAPDH_ArsJ, which were expressed in either in the arsenate sensitive *E. coli* strain

WC3110 (Δ*arsC*) (Mukhopadhyay *et al.*, 2000) or the arsenic-hypersensitive strain AW3110 (Δ*arsRBC*) (Carlin *et al.*, 1995). Resistance to inorganic and organic arsenicals was assayed in these strains. Cells of either strain AW3110 or WC3110 with plasmid pGAPDH_ArsJ were resistant to As(V) compared with the same strain with vector only (Fig. 2A). Cells of strain AW3110 with either pGAPDH or pArsJ remained As(V) sensitive (Fig. 2B), as were cells of WC3110 with the individual genes (data not shown). Cells of AW3110 with both genes was also sensitive to As(III), but WC3110 with plasmid pGAPDH_ArsJ is resistant because it still has an *arsB* gene. Cells of either strain expressing both genes remained sensitive to the trivalent organoarsenicals MAs(III), PhAs(III), Rox(III) and Nit(III) compared with the same strain with vector only (Fig. 3). These results clearly demonstrate that both the *gapdh* and *arsJ* genes synergistically and specifically detoxify inorganic As(V).

GAPDH and ArsJ synergistically reduce cellular As(V) accumulation

ArsJ is predicted to be an MFS membrane protein, suggesting that it could be a permease. The effect of expression of *gapdh* and *arsJ* on accumulation of As(V), As(III) and MAs(III) was examined (Fig. 4). Cells expressing both *gapdh* and *arsJ* accumulated considerably less As(V) compared with cells lacking the two genes. However, there was little difference in uptake of either As(III) or MAs(III) between cells expressing the two genes or vector only. Cells with vector only did not accumulate MAs(V) (data not shown), so it was not possible to determine whether cells of *E. coli* AW3110 expressing the two genes can extrude the pentavalent species. These results indicate that the combination of GAPDH and ArsJ reduce accumulation of As(V). This could be the result of decreased uptake or increased efflux of inorganic As(V) or a compound containing As(V).

ArsJ transports 1-arseno-3-phosphoglycerate

To distinguish between reduced uptake or increased efflux, transport into everted membrane vesicles prepared from cells of *E. coli* AW3110pArsJ was measured. Uptake into everted membrane vesicles is the equivalent of efflux from cells (Rosen and Tsuchiya, 1979). Vesicle transport was assayed in the presence of purified rabbit GAPDH, G3P and NAD^+. Cells expressing *arsJ* accumulated As(V), while cells with vector only had considerably less uptake (Fig. 5A). Accumulated As(V) was released by addition of the uncoupler carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), indicating that uptake is energy dependent. The usual energy source in everted vesicle transport is NADH, which generates a protonmotive force through respiration. We assume that GAPDH generates sufficient NADH to drive the transport reaction.

What is the chemical nature of the substrate for ArsJ? As(V) uptake was observed only when purified GAPDH, G3P and NAD^+ were present (Fig. 5B). Little uptake occurred in the absence of GAPDH, G3P or NAD^+. Thus the GAPDH reaction is required for As(V) accumulation in vesicles. In the presence of As(V), the product of the reaction is 1As3PGA (Byers *et al.*, 1979), and it is reasonable to assume that 1As3PGA is the true substrate of ArsJ. 1As3PGA has a half-life of less than 2.5 s, so it must be generated *in situ*. In addition, ArsJ is specific; there was little uptake when As(V) was substituted with As(III), MAs(III), MAs(V) or DMAs(V), which are not substrates of GAPDH (Fig. 6).

GAPDH activity with arsenate

GAPDH activity was assayed by NADH formation (Supporting Information Fig. 3S). No activity was observed in the absence of inorganic arsenate (Supporting Information Fig. 3SA) or in the presence of As(III), MAs(III), MAs(V) or DMAs(V) (Supporting Information Fig. 4SA). GAPDH activity required G3P, NAD^+ and As(V) (Supporting Information Fig. 4SB) and was dependent on the arsenate concentration, with an apparent Km of 0.46 mM (Supporting Information Fig. 5S). GAPDH has been reported to reduce As(V) to As(III) (Gregus and Nemeti, 2005; Nemeti *et al.*, 2006). However, in our hands, no As(V) reduction was observed either *in vivo* in cells of *E. coli* expressing *gapdh* (Supporting Information Fig. 6SA) or *in vitro* with purified rabbit GAPDH (Supporting Information Fig. 6SB). Cells of neither *E. coli* AW3110 nor WC3110, both of which have the *arsC* gene deleted, reduced significant amounts of As(V). Under the same conditions, the parental strain W3110, which has a chromosomal *arsC* gene, reduced more than half of the As(V) to As(III). Similarly, there was nearly no reduction of arsenate by purified GAPDH even after 40 min. Thus, our results cannot be explained by any adventitious arsenate reductase activity of GAPDH.

When both arsenate and phosphate were assayed at 50 μM, final concentration, the steady state rate of GAPDH activity with phosphate was very low compared with the same concentration of arsenate (Supporting Information Fig. 3SB). Activity was very rapid at phosphate concentrations in the range of 3–25 mM, too fast to acquire initial rates with our plate reader (Supporting Information Fig. 3SC), but the reaction reached a steady state within the first few seconds. The difference in GADPH reactivity with arsenate or phosphate may relate to the fact that the two products have three orders of magnitude difference in stability ($t_{1/2}$ of <2.5 s for 1As3PGA versus 48 min for 1,3-PGA) (Byers *et al.*, 1979). 1,3-PGA builds up and becomes inhibitory, while 1As3PGA never accumulates, which is the basis of the uncoupling effect of arsenate. Thus GAPDH activity correlates with ArsJ activity. These results are consistent with generation of 1As3PGA by GAPDH coupled with transport by ArsJ.

Discussion

Arsenite is more toxic than arsenate. Arsenite inhibits many cysteine-containing enzymes and bind to thiol-containing metabolites such as reduced glutathione and lipoic acid. Arsenate exerts its toxicity as a phosphate analog, where it substitutes for Pi in phosphorylation reactions. Since the arsenylated products are unstable, As(V) uncouples substrate-level phosphorylation reactions in glycolysis (Pillai, 1938) and ATP production in oxidative phosphorylation (Azzone and Ernster, 1961). Yet nearly every microbial protein involved in resistance to arsenicals uses the trivalent species as a ligand or substrate, including the As(III)-responsive transcriptional repressor ArsR (Shi *et al.*, 1994), the As(III) metallochaperone ArsD (Lin *et al.*, 2006), the ArsA As(III) ATPase (Rosen *et al.*, 1988), the ArsB and Acr3 As(III) efflux permeases (Meng *et al.*, 2004; Villadangos *et al.*, 2012), the ArsM As(III) S-adenosylmethionine methyltransferase (Qin *et al.*, 2006). The most obvious exceptions are the arsenate reductases, which reduce As(V) to As(III) in order to become the substrates of the resistance mechanisms (Mukhopadhyay and Rosen, 2002). The ArrAB

respiratory arsenate reductase also transforms the pentavelent species, but for energy production and not resistance (Saltikov and Newman, 2003).

Yet no arsenate efflux carriers or pumps have been identified. In this study, we identify a two-step reaction that creates a pathway for arsenate efflux. There are a number of genes with unknown function in a wide variety of *ars* operons. We began with the assumption that all genes in *ars* operons encode an arsenic-related function. We noted that various *ars* operons have two genes, *gapdh*, which encodes a typical glyceraldhyde-3-phosphate dehydrogenase or GAPDH enzyme, and a second encoding an MFS membrane protein that we have named *arsJ*. GAPDH is an NAD^+-dependent enzyme present in nearly every organism. This cytosolic enzyme plays a major role in glycolysis and gluconeogenesis. The substrates for the glycolytic direction is inorganic phosphate, D-glyceraldehyde-3-phosphate and NAD^+ and the product is 1,3-bisphosphoglycerate. As G3P is a metabolite in glycolysis, it is always present during glucose metabolism. Arsenate can substitute for phosphate, with production of the product 1As3PGA, which is unstable and immediately hydrolyzes to inorganic arsenate and 3PGA, creating a futile cycle that uncouples glycolysis (Byers *et al.*, 1979). ArsJ is an MFS membrane protein with a likely transport function. Since the genes for ArsJ and GAPDH are linked, it seemed reasonable to consider that the ArsJ could be an efflux system for 1As3PGA (Fig. 7). Whether 1,3-PGA is also a substrate was not tested, but it seems unlikely that the function of a gene in an *ars* operon would be to deplete cells of phosphate. Even if it is specific for arsenate, it still creates a potential futile cycle of arsenate uptake and efflux, but the data in Fig. 4 suggests that it functions more like a bilge pump. With a half life of <2.5 s for 1As3PGA, the organoarsenical is sufficiently stable to be transported by ArsJ before it hydrolyzes. Formation of 1As3PGA with intracellular As(V) by GAPDH coupled with extrusion by ArsJ and hydrolysis to extracellular As(V) creates the equivalent of an As(V) efflux system. Although 1As3PGA is not available to add as a substrate, it is generated *in situ* by adding purified GAPDH and the reaction components to an *in vitro* vesicle transport assay. There does not appear to be anything special about the sequence of the *ars* GAPDH that differentiates it from glycolytic GAPDH enzymes, so why does not *gapA* gene product of *E. coli* AW3110 or WC3110 produce sufficient 1As3PGA to confer arsenate resistance or efflux? It is likely the result of high levels of *gapdh* transcription from the strong *ars* promoter under natural conditions or from the *lac* promoter in the recombinant plasmid used in this study. The *gapA* gene of *E. coli* is under control of multiple promoters but is most highly expressed at the beginning of exponential phase or during gluconeogenesis (Charpentier and Branlant, 1994). Under the conditions of this study the *ars gapdh* gene is probably much more highly expressed than the chromosomal *gapA* gene. In summary, the *ars* operon genes *gapdh* and *arsJ* encode a new and novel pentavalent organoarsenical resistance, the only identified pathway for efflux of arsenate.

Experimental procedures

Strains, plasmids, medium and reagents

Escherichia coli Stellar™ (Clontech Laboratories, Mountain View, CA; F^-, *endA1, supE44, thi-1, recA1, relA1, gyrA96 phoA, Φ80d lacZ*Δ M15, Δ(*lacZYA-argF*)U169, Δ(*mrrhsdRMS-mcrBC*), Δ*mcrA*, λ−) was used for plasmid DNA construction and replication. *E. coli*

AW3110(DE3) (Δ*arsRBC::cam* F–IN(*rrn-rrnE*) (Carlin *et al.*, 1995), which is hypersensitive to As(III), and WC3110(DE3) (Δ*arsC*) (Sundaram *et al.*, 2008) were used for complementation studies. For most experiments, cultures of *E. coli* bearing the indicated plasmids were grown aerobically in Luria–Bertani (LB) medium (Sambrook *et al.*, 1989) or low phosphate medium (Oden *et al.*, 1994) at 30 or 37°C supplemented with 125 μg/ml ampicillin or 34 μg/ml chloramphenicol, as indicated. Bacterial growth was monitored by measuring the absorbance at 600 nm (A_{600}). All reagents were obtained from commercial sources. Rabbit GAPDH (CAS: 9001-50-7) and G3P (CAS 591-57-1) were obtained from Sigma-Aldrich (St Louis, MO). Roxarsone (CAS 121-19-7) was obtained from ThermoFisher Acros Organics Division (Waltham, MA). Monomethylarsonic acid (MAs(V)) was obtained from Chem Service (West Chester, PA, USA). MAs(III), Rox(III), Nit(III) were prepared by reduction of the pentavalent forms (Reay and Asher, 1977).

Plasmid construction

For expression of GAPDH (accession number WP_003109848) and ArsJ (accession number WP_003109849) in *E. coli*, the *gapdh* and *arsJ* genes were cloned individually and together from *P. aeruginosa* DK2 genomic DNA (a gift from Søren Molin, Technical University of Denmark) into plasmid pUC19 under control of the *lac* promoter, creating plasmids pGAPDH, pArsJ and pGAPDH_ArsJ. The forward and reverse primers for cloning the *gapdh* and *arsJ* genes together were: 5′-CCCAAGCTTATGGCCATCAAAGTAGGCATC-3′ (*Hind*III site underlined) and 5′-ACGCGTCGACTCAGTTCGGGGTGGTCACCAC-3′ (*Sal*I site underlined), respectively. The PCR fragment was gel purified and digested using the underlined restriction enzymes, and ligated into vector plasmid pUC19, which was also digested with *Hind*III and *Sal*I, generating plasmid pGAPDH_ArsJ. To clone the *gapdh* gene alone, the forward and reverse primers were 5′-CCCAAGCTTATGGCCATCAAAGTAGGCATC-3′ (*Hind*III site underlined) and 5′-ACGCGTCGACTCAGCCAGCCAGGCCGACCAG-3′ (*Sal*I site underlined), respectively. To clone the *arsJ* gene alone, the forward and reverse primers were 5′-CCCAAGCTTATGAAGGCGCTATCGTCGCTCT-3′ (*Hind*III site underlined) and 5′-ACGCGTCGACTCAGTTCGGGGTGGTCACCAC-3′ (*Sal*I site underlined), respectively.

Comparison of GAPDH and ArsJ sequences

Multiple alignment of GAPDH (Supporting Information Fig. 1S) and ArsJ (Supporting Information Fig. 2S) sequences was calculated with CLUSTAL W (Thompson *et al.*, 1994).

Metalloid resistance assays

For metalloid resistance assays in liquid medium, competent cells of either AW3110 or WC3110, as indicated, were transformed with the indicated plasmids. Cells were grown overnight with shaking at 37°C in LB medium with 125 μg ml^{-1} ampicillin. Overnight cultures were diluted 100-fold in low phosphate medium containing various concentrations of either trivalent or pentavalent arsenicals plus 0.3 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and incubated at 37°C with shaking for another 24 h. Growth was estimated from the absorbance at 600 nm.

Metalloid transport assays in whole cells

For *in vivo* uptake assays, *E. coli* cells were grown in low phosphate medium at 37°C to a cell density (A_{600nm}) of 2. The cells were harvested and suspended in 1/5th volume of a buffer solution consisting of 75 mM HEPES-KOH, pH 7.5, 0.15 M KCl and 1 mM $MgSO_4$. To initiate the transport reaction, arsenicals were added at a final concentration of 20 μM to 1 ml of cell suspension. Portions (0.1 ml) were withdrawn at the indicated times, filtered through nitrocellulose filters (0.2 μm pore diameter; EMD Millipore, Billerica, MA) and washed twice at room temperature with 5 ml of the same buffer. The filters were digested with 0.3 ml of concentrated HNO_3 (68–70%) overnight at room temperature. The dissolved filters were incubated for 10 min at 70°C, allowed to cool to room temperature and diluted with HPLC-grade water (Sigma-Aldrich, St. Louis) to produce a final HNO_3 concentration of 2%. Arsenic was quantified by inductively coupled mass spectroscopy (ICP-MS). Standard solutions were made in the range of 0.5–50 ppb in 2% nitric acid using an arsenic standard (Ultra Scientific, N. Kingstown, RI).

Assay of GAPDH activity

GAPDH activity was assayed as described (Heinz and Freimuller, 1982). The reaction was initiated by addition of 0.6 mM G3P, final concentration, to a reaction mixture containing 25 mM Bis-Tris propane (pH 7.0), 0.6 mM NAD^+, 10 mM DTT, 1 unit GAPDH and the indicated concentrations of inorganic phosphate or arsenate at 25°C. The absorbance at 340 nm was measured with a Synergy H4 Hybrid MultiMode microplate reader.

Metalloid uptake assays in everted membrane vesicles

Everted membrane vesicles and transport assays were performed as described (Villadangos *et al.*, 2012). Transport assays were performed in a buffer consisting of 75 mM HEPES-KOH, pH 7.5, 0.15 M K_2SO_4, 1 mM $MgSO_4$ and 0.25 M sucrose. The reaction mixture contained 1 mg ml^{-1} membrane proteins, 5 units of GAPDH, 10 mM DTT, 5 mM NAD^+ and the indicated arsenicals at 5 mM, final concentration, in a final volume of 0.6 ml of the same buffer. The reaction was initiated by addition of 5 mM G3P. The uncoupler carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) was added as indicated. Portions (0.1 ml) were withdrawn at the indicated times, filtered through 0.2 μm pore size nitrocellulose filters and washed twice with 5 ml of the same buffer. The arsenic content was determined by ICP-MS, as described above.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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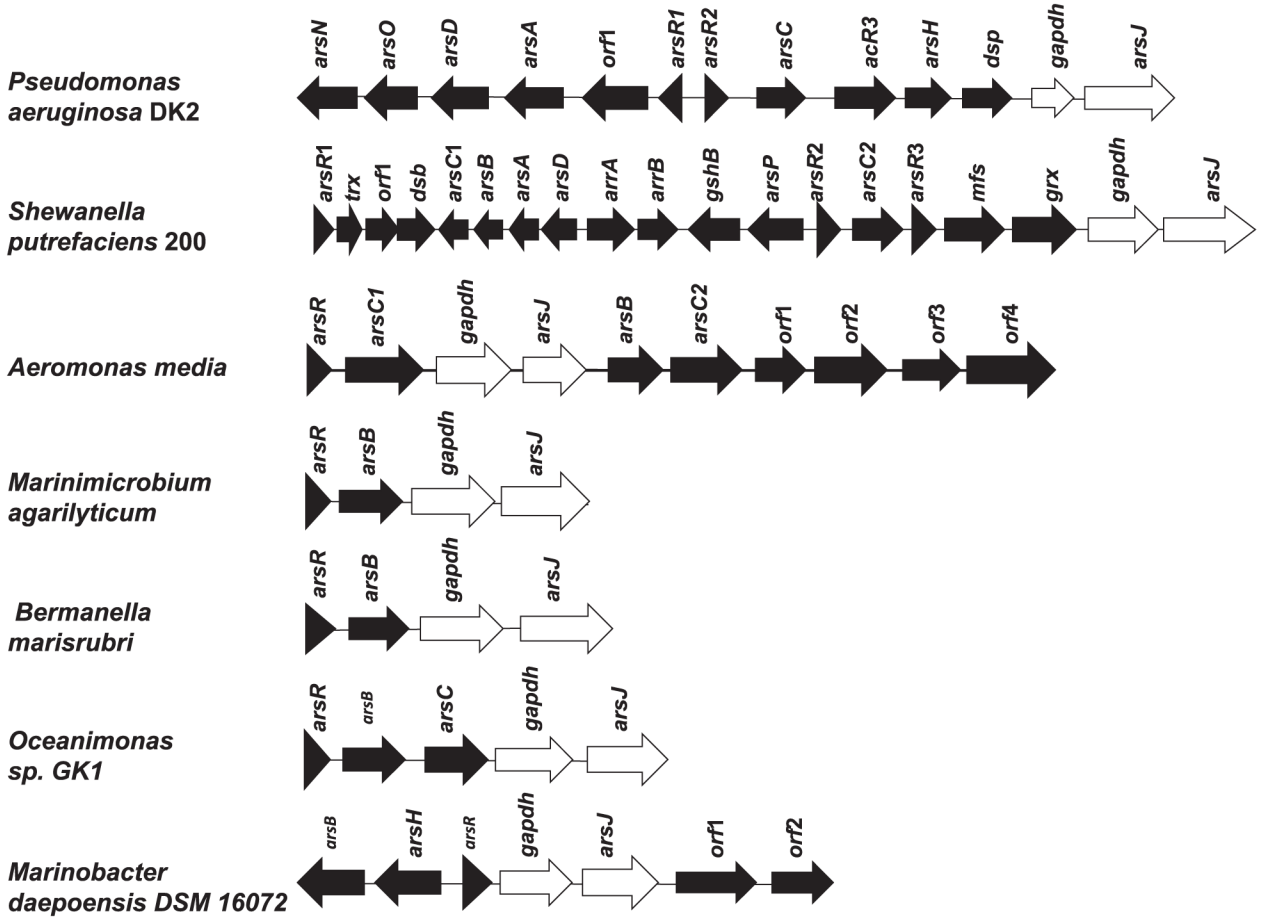

Fig. 1.

The *gapdh* and *arsJ* genes are linked in *ars* operons. Shown are representative *ars* operons containing *gapdh* and *arsJ* genes (white fill). *Pseudomonas aeruginosa* DK2 (accession number NC_018080), *Shewanella putrefaciens* 200 (accession number CP002457), *Aeromonas media* (accession number CP007567), *Marinimicrobium agarilyticum* (accession number AUHU00000000), *Bermanella marisrubri* (accession number AAQH01000000), *Oceanimonas sp*. GK1 (accession number CP003171), *Marinobacter daepoensis* (ATWI01000000).

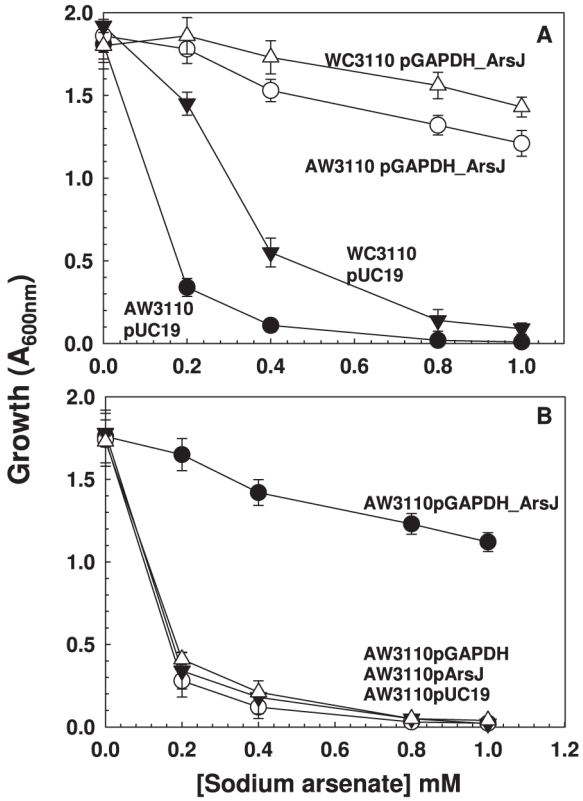

Fig. 2. GAPDH and PaArsJ confer synergistic resistance to arsenate

A. Arsenate resistance in *E. coli* strain AW3110 (Δ*arsRBC*) (▼,Δ) or WC3110 (Δ*arsC*) (●,○) bearing the indicated plasmids was assayed as described in *Experimental Procedures*. (▼,●), vector plasmid pUC19; (Δ,○), pGAPDH_ArsJ.

B. Arsenate resistance in *E. coli* strain AW3110 with (●) pGAPDH_ArsJ, (Δ), pGAPDH, (▼) pArsJ, (○), pUC19. Growth was estimated from the absorption at 600 nm after 24 h at 37°C. Data are the mean±SE (*n*=3).

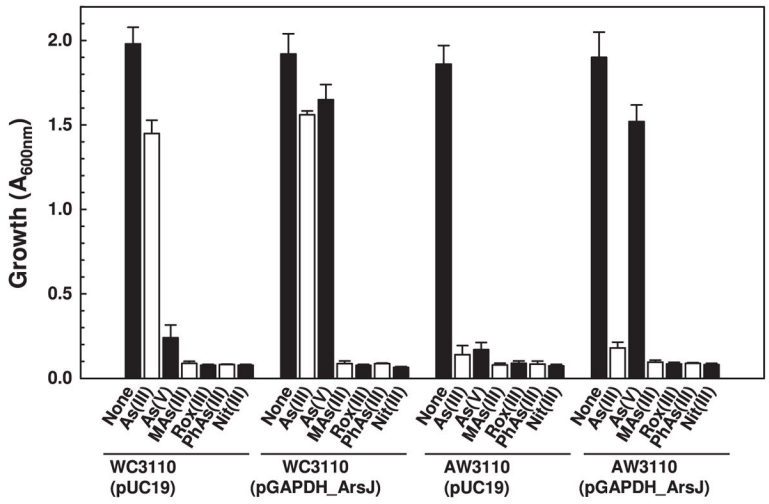

Fig. 3.

Co-expression of *gapdh* and *arsJ* confer resistance to arsenate. Overnight cultures of *E. coli* strain WC3110 or AW3110 bearing either pGAPDH_ArsJ or vector plasmid pUC19 were diluted 100-fold into fresh low phosphate medium containing 0.1 mM As(III), 0.8 mM As(V), 10 μM MAs(III), 0.5 μM PhAs(III), 2 μM Rox(III) or 2 μM Nit(III), as indicated. Expression of the *gapdh* and *arsJ* genes was induced with 0.3 mM IPTG. Growth was estimated from the absorption at 600 nm after 24 h at 37°C. Data are the mean±SE (n=3).

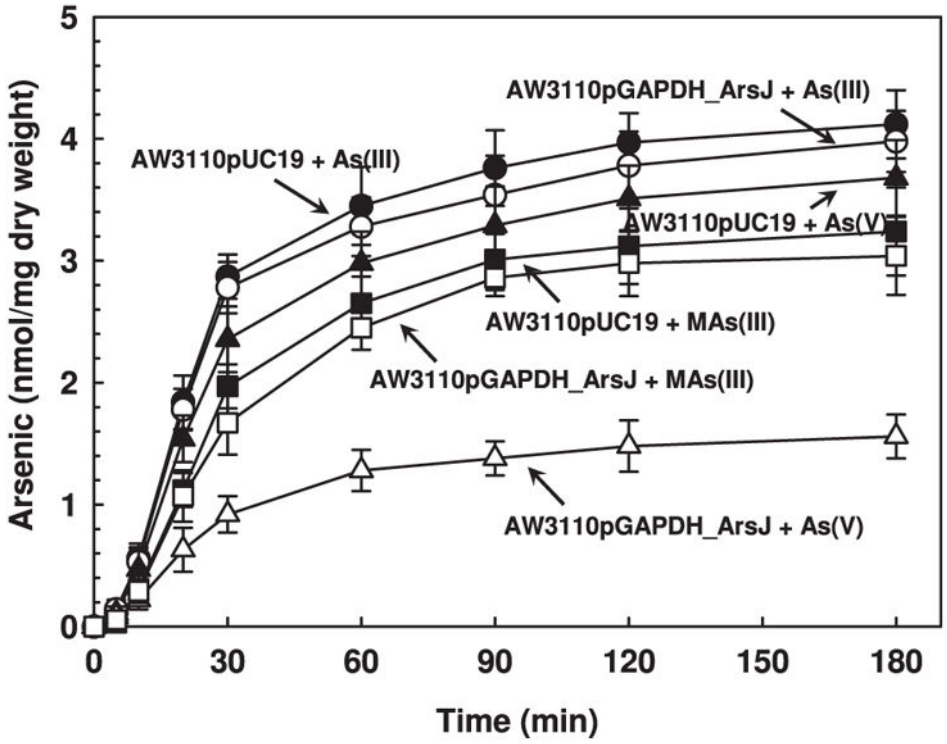

Fig. 4.

GAPDH and ArsJ reduce cellular As(V) accumulation. Uptake of arsenicals by *E. coli* AW3110 carrying either vector plasmid pUC19 (filled symbols) or pGAPDH_ArsJ (open symbols) was assayed with 20 μM, final concentration, of the indicated arsenicals. (Δ,▲) As(V), (○,●), As(III), (□,■) MAs(III), was performed as described in *Experimental procedures*. Data are the mean±SE (n=3).

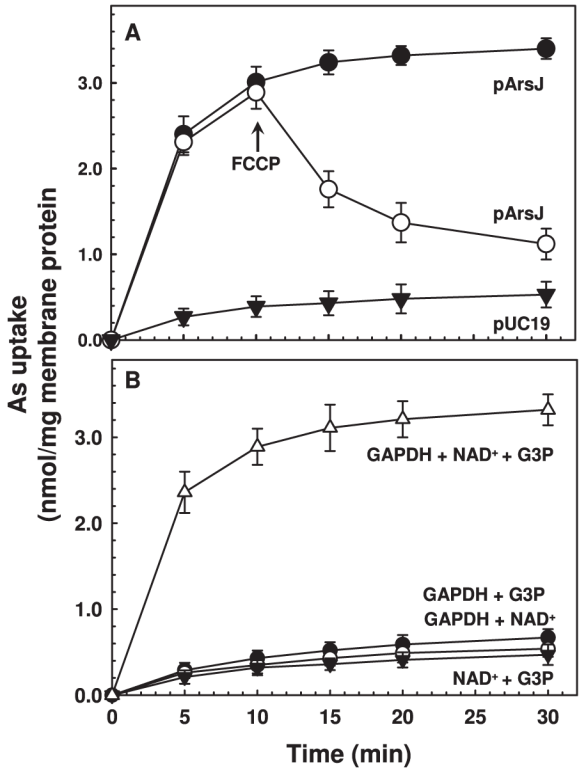

Fig. 5. Uptake of 1-arseno-3-phosphoglycerate in everted membrane vesicles

A. Accumulation of 1As3PGA in everted membrane vesicles prepared from *E. coli* strain AW3110 harboring either plasmid pArsJ (●,○) or vector plasmid pUC19 (▼) was assayed as described in *Experimental procedures* using 1 mg/ml of membrane protein. To produce 1AS3PGA *in situ*, the reaction mixture contained, 5 units of commercial rabbit GAPDH, 10 mM DTT, 5 mM NAD^+ and 5 mM As(V). The reaction was initiated by addition of 5 mM G3P, final concentration. 10 μM FCCP, final concentration, was added at the indicated time (arrow).

B. The requirements for *in situ* generation of 1As3PGA were determined by addition of the components of the GAPDH reaction. (Δ), GAPDH, NAD^+ and G3P; (●), GAPDH and G3P; (○), GAPDH and NAD^+; (▼), NAD^+ and G3P. Vesicular arsenic was determined by ICP-MS. Data are the mean±SE (n=3).

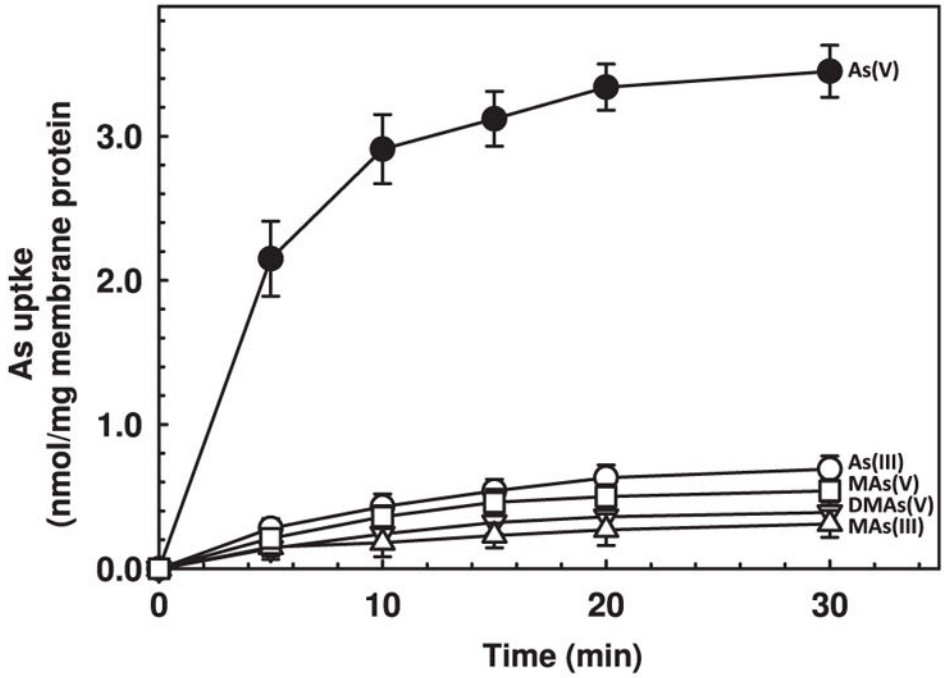

Fig. 6. ArsJ substrate specificity. Transport assays in everted membrane vesicles prepared from *E. coli* strain AW3110pArsJ were performed as described in *Experimental procedures*

Arsenicals were added at 5 mM, final concentration. (●), As(V); (○), As(III); (□), MAs(V); (▽), DMAs(V); (Δ), MAs(III). Vesicular arsenic was determined by ICP-MS. Data are the mean±SE (n=3).

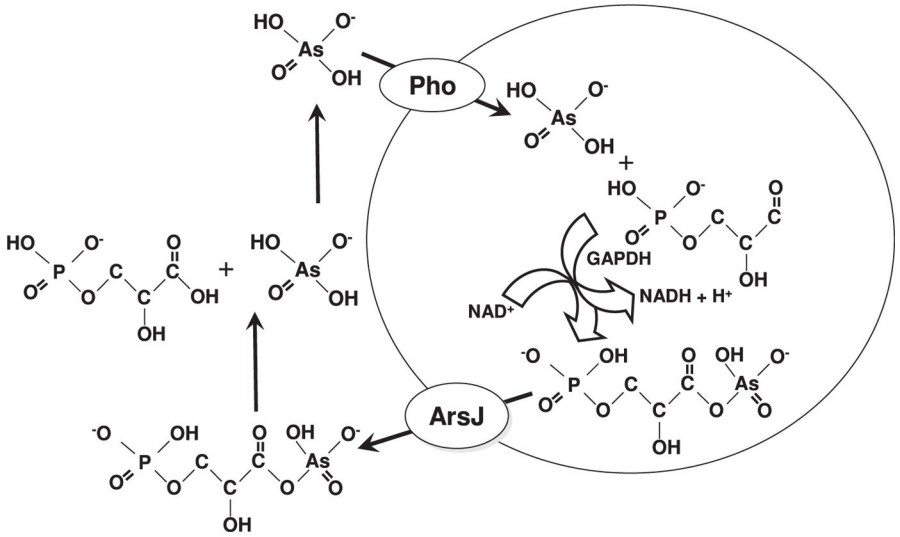

Fig. 7.

Model of synergy between GAPDH and ArsJ in arsenate transport and detoxification. Arsenate is taken up by phosphate transporters. GAPDH catalyzes arsenylation of G3P to form 1As3PGA, which is the substrate of the ArsJ efflux permease. Extracellular 1As3PGA is unstable and spontaneously hydrolyzes into As(V) and 3PGA.