ArsD: A Novel Metallochaperone for an Arsenic Detoxification Pump

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ABSTRACT

Arsenic is a metalloid toxicant that is widely distributed throughout the earth's crust and causes a variety of health and environment problems. As an adaptation to arsenic-contaminated environments, organisms have developed resistance systems. *E. coli* plasmid R773 carries the well-studied *arsRDABC* operon. ArsA is an ATPase that is the catalytic subunit of the ArsAB As(III) extrusion pump. ArsD was shown to have weak repressor activity, but this may not be its physiological function. Most *ars* operons contain only three genes, *arsRBC*. Five gene operons have two additional genes, *arsD* and *arsA*, and these are usually adjacent to each other. Obviously *arsD* and *arsA* were co-evolved, suggesting a related function for the two gene products.

Recently metallochaperones have been identified for a number of metals. Metallochaperones prevent inappropriate metal interactions with other cellular components. Thus, these ubiquitous proteins have a critical biological function: to deliver metals in the cytoplasm to the site of utilization or export. We report here that ArsD is an arsenic chaperone that transfers trivalent metalloids to the ArsA ATPase. Through protein-protein interactions, ArsD increases the affinity of the ATPase for As(III) and results in increased efflux and resistance. This is the first report of an arsenic chaperone and suggests that cells can regulate the intracellular concentration of free arsenite to prevent toxicity. Supported by NIH grant AI45428.

INTRODUCTION

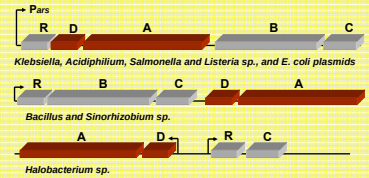

Fig. 1: Evolution of *ars* operons in prokaryotes. In five-gene *ars* operons, the *arsD* and *arsA* genes are always found together. This observation led us to propose that five-gene *arsRDABC* operons evolved from three-gene *arsRBC* operons by insertion of the *arsD* and *arsA* genes as a unit. The linkage of these two genes leads us to consider the possibility that ArsD and ArsA might have associated functions in arsenic detoxification.

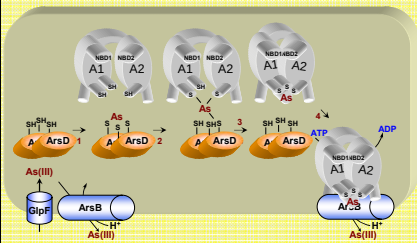

Fig2: Model of ArsD-ArsA interaction. As(III) enters cells by aquaglyceroporins such as GlpF, where it is bound by ArsD through two or three cysteine residues (Cys12, Cys13 and/or Cys18). As(III) is then transferred to Cys113, Cys172 and Cys422 in the metal binding domain of ArsA in a step-wise manner. ArsD and ArsB are proposed to bind to the same site on ArsA sequentially in a cycle of metal transfer from ArsD to ArsA to ArsB concomitant with ATP binding and then hydrolysis by ArsA.

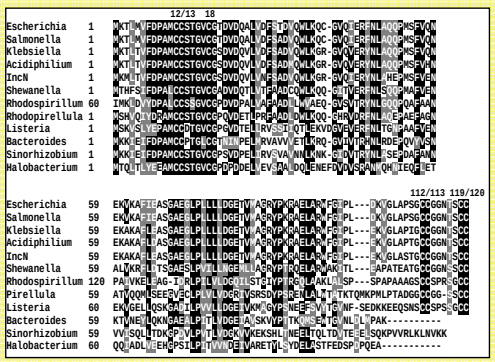

Fig. 3: Multiple alignment of ArsD homologues. ArsD homologues are shown from 12 archeal and bacterial organisms. Cysteine residues are indicated. The multiple alignment was calculated with CLUSTAL W27.

METHODS & RESULTS

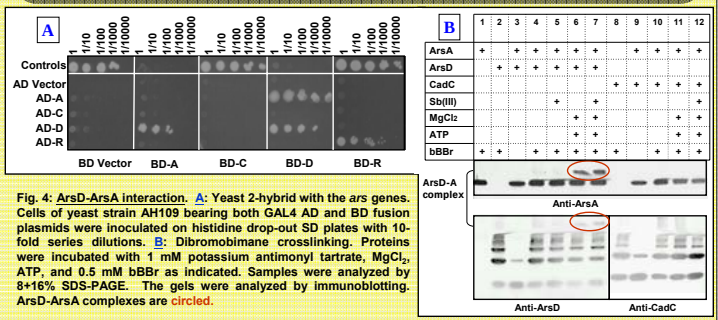

Fig. 4: ArsD-ArsA interaction. A: Yeast 2-hybrid with the *ars* genes. Cells of yeast strain AH109 bearing both GAL4 AD and BD fusion plasmids were inoculated on histidine drop-out SD plates with 10-fold series dilutions. B: Dibromobimane crosslinking. Proteins were incubated with 1 mM potassium antimonyl tartrate, $MgCl_2$, ATP, and 0.5 mM bBBr as indicated. Samples were analyzed by 8+16% SDS-PAGE. The gels were analyzed by immunoblotting. ArsD-ArsA complexes are circled.

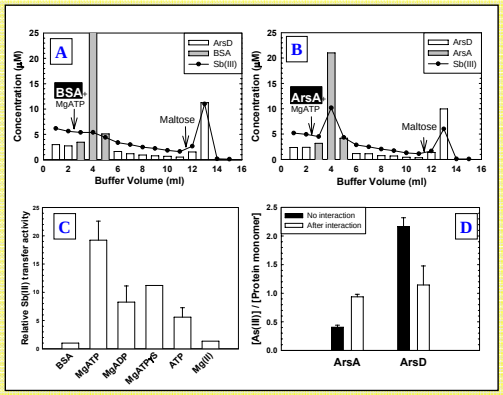

Fig. 5: ArsD-ArsA metal transfer. A-B: Sb(III) transfer. Sb(III)-bound MBP-fused ArsD was bound to an amylose column, which was then loaded with purified BSA (A) or ArsA (B) + 1 mM ATP and $MgCl_2$, as indicated. After thorough washing, ArsD was eluted with 10 mM maltose, and the fractions were analyzed for the proteins and Sb(III). C: Effect of nucleotides. The transfer assay was performed in the presence of the indicated nucleotides. Transfer activity was calculated as ([Sb(III) $_{ArsA}$]/[ArsA]) /([Sb(III)$_{ArsD}$]/[ArsD]). The values were normalized to BSA. D: As(III) transfer. Binding of As(III) to ArsA and ArsD was assayed in the presence of MgATPγS without the partner protein (black bar); and after interaction with the partner protein (white bar).

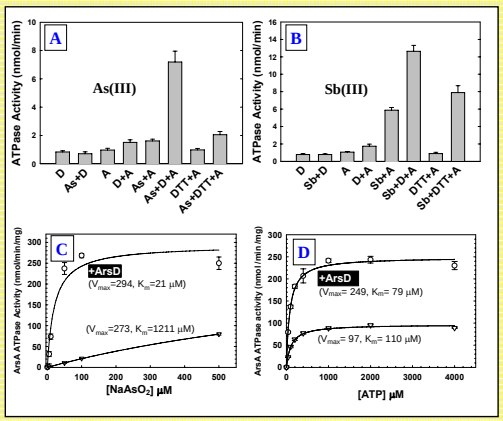

Fig. 6. ArsD increases the affinity of ArsA for metalloid. A: ArsD increases the stimulation of ArsA ATPase activity by As(III). ATPase activities were measured in different combinations of 3 μM ArsD, 0.3 μM ArsA, 10 μM DTT and 100 μM sodium arsenite. B: Stimulation by Sb(III). 10 μM potassium antimonyl tartrate was used to replace sodium arsenite in "A". C: Effect of ArsD on the Km for As(III). ATPase activities were measured in the absence and presence of ArsD at varying concentrations of sodium arsenite. D: Effect of ArsD on Km for ATP. ATPase activity was assayed at 0.5 mM sodium arsenite and varying concentrations of ATP.

METHODS & RESULTS

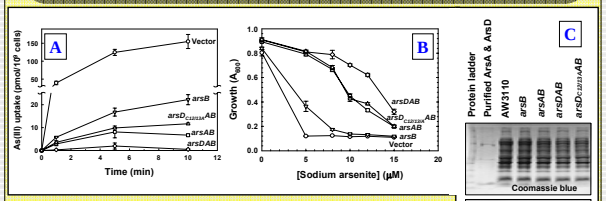

Fig. 7: Effect of the *arsD* gene on arsenical transport and resistance confered by the *arsAB* genes. Cells of *E. coli* strain AW3110 (Δ*arsRBC*) harboring vector plasmids pSE380 and pACYC184 (○) or plasmids with *arsB* (▽), *arsAB* (□), *arsDAB* (◇) or *arsD*$_{C12A/C13A}$*AB* (△) were grown and assayed. A: As(III) transport. The cells were assayed with 10 μM sodium arsenite in varying incubation time. B: As(III) resistance. The cells were assayed for resistance at various concentrations of sodium arsenite. C: ArsD does not affect expression of ArsA. Protein expression levels were determined by immunoblotting using anti-ArsA and anti-ArsD.

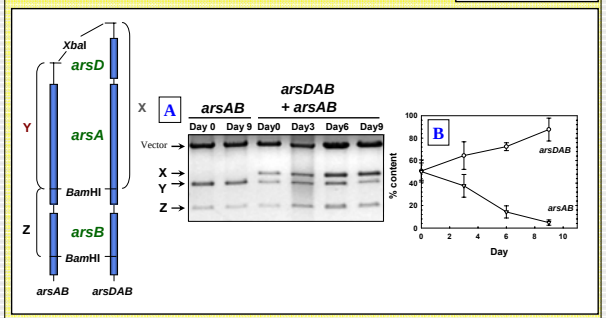

Fig. 8. The *arsD* gene confers a competitive advantage for cells with *arsAB* genes. A: Plasmid analysis. Cells of *E. coli* strain AW3110 bearing either *arsAB* or *arsDAB* were grown in a mixture culture. The mixture was 1000-fold diluted daily in LB medium containing 10 μM sodium arsenite. The plasmids were extracted and analyzed by restriction analysis with *Xba*I and *Bam*HI. B: Cells with only *arsAB* are lost from the population. The percentage of the cells with each plasmid were calculated as following: *arsDAB*: X/(vector + Z); *arsAB*: Y/(vector + Z).

CONCLUSIONS

- We have identified the first chaperone for metalloids, the product of the *arsD* gene of the *Escherichia coli* plasmid R773 *arsRDABC* operon.
- Through protein-protein interactions, ArsD transfers As(III) to ArsA, increasing the affinity of the ATPase for As(III). Thus, at low concentrations of As(III), cells with *arsDAB* have increased efflux of and resistance to As(III).
- Cells with *arsDAB* have increased ability to regulate intracellular free As(III), preventing toxicity and thus providing a competitive advantage.