Molecular Microbiology (2016) 100(6), 928–930

doi:10.1111/mmi.13395
First published online 29 April 2016

MicroCommentary

A novel pathway of arsenate detoxification

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Summary

Microorganisms have evolved various mechanisms to detoxify arsenic, an ubiquitous environmental toxin. Known mechanisms include arsenite efflux, arsenate reduction followed by arsenite efflux and arsenite methylation. In this issue, Chen *et al.* describe a novel mechanism for arsenate detoxification via synergistic interaction of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and a major facilitator superfamily protein (ArsJ). They propose that GAPDH catalyzes the formation of 1-arseno-3-phosphoglycerate, which is then extruded out of the cell by ArsJ. The significance of this pathway and questions for further research are discussed.

Arsenic is ubiquitous in the environment; it is the twentieth most abundant element in the Earth's crust, with an average concentration of approximately 3 mg kg^{-1} (Cullen and Reimer, 1989). Because arsenic is toxic to all living organisms, various mechanisms for arsenic resistance have evolved. When life first arose in the primordial Earth approximately 3.7 billion years ago, the Earth's atmosphere was devoid of oxygen and arsenic would be expected to exist as arsenite [As(III)] under the anoxic conditions. As(III) has high affinity for sulfydryl groups and can bind to reduced cysteines in peptides and proteins, affecting their structures or catalytic functions and inactivating up to 200 enzymes (Shen *et al.*, 2013). It is thought that organisms evolved mechanisms for As(III) resistance first (Zhu *et al.*, 2014). The most common mechanism of As(III) resistance is As(III) efflux mediated by membrane permeases such as ArsB or ACR3 (Chen *et al.*, 1986; Wu *et al.*, 1992; Wysocki *et al.*, 2003), which help keep the cellular concentration of As(III) at low levels.

Later as the Earth gradually became oxygenated and As(III) was oxidized to As(V), organisms faced a different challenge of arsenic toxicity in the form of arsenate [As(V)]. As(V) is a chemical analogue of phosphate, and can be taken up by phosphate transporters due to imperfect selectivity (Rosenberg *et al.*, 1977; Bun-ya *et al.*, 1996). As(V) can also participate in phosphorylation reactions, forming arsenic esters which are much less stable than phosphate esters and hydrolyze quickly, a process termed 'arsenolysis' that uncouples phosphorylation (Byers *et al.*, 1979). Arsenolysis is the primary mode of As(V) toxicity as it interferes with glucose, energy and phosphate metabolism. To detoxify As(V), organisms employ As(V) reductases to reduce As(V) to As(III) (Gladysheva *et al.*, 1994; Mukhopadhyay and Rosen, 1998); the latter can then be extruded from the cells via the existing As(III) efflux systems or methylated to produce volatile arsenicals (Qin *et al.*, 2006) (Fig. 1). These As(V) detoxification mechanisms have the benefit of getting rid of arsenic without losing phosphate, a nutrient essential to life.

In this issue, Chen *et al.* (2016) report a novel mechanism of As(V) resistance in microorganisms. They found that two genes, *gapdh* and *arsJ* encoding the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase and a major facilitator superfamily (MFS) protein, respectively, occur together as a pair in the arsenic resistance (*ars*) operons in many organisms. When the two genes from *Pseudomonas aeruginosa* are expressed together in *Escherichia coli*, they confer resistance to As(V) but not to As(III) or a range of organic arsenical compounds, indicating that resistance is specific to As(V). Expressing either gene alone does not confer As(V) resistance, suggesting that the products of the two genes work synergistically to enhance As(V) resistance. Because ArsJ is a membrane transporter, its role in transporting arsenical compounds is intuitively logical. How does GAPDH, a protein that is thought of as 'cellular heirloom' for its role in glycolysis and many other functions (Sirover, 2011), get involved in As(V) resistance? The hint comes from arsenolysis mentioned above. In glycolysis, GAPDH

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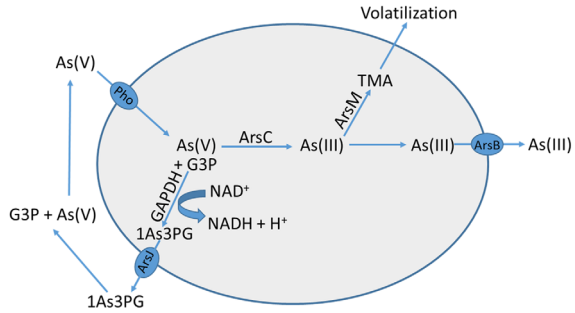

Fig. 1. Schematic representation of arsenic resistance mechanisms in microorganisms. As(V), arsenate; As(III), arsenite; TMA, trimethylarsine; 1As3PG, 1-arseno-3-phosphoglycerate; G3P, D-glyceraldehyde 3-phosphate; Pho, phosphate transporter; ArsB, As(III) efflux transporter; ArsJ, a MFS transporter for 1As3PG; ArsC, As(V) reductase; ArsM, As(III) methyltransferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

catalyzes the oxidative phosphorylation of the substrate D-glyceraldehyde 3-phosphate (G3P) to 1,3-biphospho-D-glycerate (1,3BPG). In the presence of As(V), GAPDH catalyzes the formation of 1-arseno-3-phosphoglycerate (1As3PG), which is unstable with a half life of < 2.5 s (Byers *et al*., 1979). Chen *et al*. (2016) propose that 1As3PG is the substrate for the efflux mediated by ArsJ (Fig. 1). Because 1As3PG is not available, they provide indirect evidence for this hypothesis by measuring arsenic accumulation in everted membrane vesicles prepared from *E. coli* expressing *arsJ* from *P. aeruginosa*. They found that As accumulation occurs only when As(V), a GAPDH enzyme, NAD^+ and G3P are present in the assay medium, i.e. when 1As3PG is expected to form. Once extruded, extracellular 1As3PG spontaneously hydrolyzes into As(V) and G3P. This is the first time that a pathway of As(V) detoxification without involving the step of As(V) reduction has been reported in any organism. Detoxification of As(V) in this way would avoid the reduction step that generates the more toxic As(III). The fact that *gapdh* and *arsJ* are present in tandem in many microorganisms and algae suggests that the mechanism may be employed by diverse organisms. Because the *ars* GAPDH is very similar to the glycolytic GAPDH enzymes (Chen *et al*., 2016), the identification of its involvement in As(V) resistance adds another function to the long list of this multifunctional enzyme (Sirover, 2011; Boradia *et al*., 2014).

The study of Chen *et al*. (2016) opens up a number of questions. First, presumably *arsJ* and *gapdh* in the *ars* operons have evolved specifically for As(V) resistance. It would, therefore, be interesting to determine the substrate selectivity of ArsJ, whether it is permeable to 1As3PG only or to both 1As3PG and 1,3BPG. If ArsJ selectively transports 1As3PG, the cost of As(V) detoxification in terms of energy and phosphate losses would be substantially smaller than if there is no such selectivity. Related to this question is the specificity of the *ars* GAPDH towards As(V) and phosphate. It would be physiologically more useful if the enzyme preferred As(V) to avoid the competitive inhibition of phosphate. Second, is G3P recycled into the cell? If not, it would represent a considerable loss of energy and phosphate for the organism. This may be a necessary tradeoff for As(V) detoxification. Third, is expression of *gapdh* and *arsJ* regulated by the transcriptional repressor ArsR that regulates other arsenic resistance genes in the *ars* operons? ArsR represses transcription of *ars* genes by binding to the promoter regions of the genes in the absence of As(III). In the presence of As(III), the repressor binds As(III) and dissociates from the promoter, resulting in expression of As(III) resistance (Wu and Rosen, 1991; Shi *et al*., 1994). If expression of *gapdh* and *arsJ* is regulated by ArsR, it implies a need of As(III) for the activation of the As(V) detoxification mechanism enacted by GAPDH and ArsJ. This is analogous to the As(V) reductase ArsC being transcriptionally induced by As(III) (Carlin *et al*., 1995). Fourth, how important is the GAPDH-ArsJ As(V) detoxification mechanism compared to the As(V) reduction – As(III) efflux mechanism, as genes required for both are present in the *ars* operons of *P. aeruginosa* and some other organisms (Fig. 1 in Chen *et al*., 2016)? It is possible that the GAPDH-ArsJ detoxification mechanism may provide additional resistance when the As(V) reduction and As(III) efflux mechanism is overwhelmed with high levels of As(V). Although As(V) resistance was clearly demonstrated in *E. coli* expressing *gapdh* and *arsJ*, this was done with a strong promoter (Chen *et al*., 2016). Deletion of specific genes in *P. aeruginosa* is the way forward to address these questions. Future studies should also address the issue of the transport substrate for ArsJ.

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