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The organoarsenical biocycle and the primordial antibiotic methylarsenite

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Abstract

Arsenic is the most pervasive environmental toxic substance. As a consequence of its ubiquity, nearly every organism has genes for resistance to inorganic arsenic. In bacteria these genes are found largely in bacterial arsenic resistance (*ars*) operons. Recently a parallel pathway for synthesis and degradation of methylated arsenicals has been identified. The *arsM* gene product encodes the ArsM (AS3MT in animals) As(III) S-adenosylmethionine methyltransferase that methylates inorganic trivalent arsenite in three sequential steps to methylarsenite MAs(III), dimethylarsenite (DMAs(III)) and trimethylarsenite (TMAs(III)). MAs(III) is considerably more toxic than As(III), and we have proposed that MAs(III) was a primordial antibiotic. Under aerobic conditions these products are oxidized to nontoxic pentavalent arsenicals, so that methylation became a detoxifying pathway after the atmosphere became oxidizing. Other microbes have acquired the ability to regenerate MAs(V) by reduction, transforming it again into toxic MAs(III). Under this environmental pressure, MAs(III) resistances evolved, including the *arsI*, *arsH* and *arsP* genes. ArsI is a C-As bond lyase that demethylates MAs(III) back to less toxic As(III). ArsH re-oxidizes MAs(III) to MAs(V). ArsP actively extrudes MAs(III) from cells. These proteins confer resistance to this primitive antibiotic. This oscillation between MAs(III) synthesis and detoxification is a consequential component of the arsenic biogeochemical cycle.

Introduction

Life arose when the atmosphere was anoxic and/or chemically reducing and toxic metal ions were present at higher concentrations than in present-day oceans.¹ To survive in the presence of arsenic, the most ubiquitous of all environmental toxins, the earliest cells must have had mechanisms for detoxifying arsenic. This intense selective pressure was the driving force for the evolution of bacterial arsenic resistance (*ars*) genes and pathways that are found in nearly every extant organism. Without arsenic detoxification systems, life could not have arisen and would not exist today.

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At a minimum *ars* operons encode an As(III)-responsive repressor (ArsR), which senses environmental inorganic arsenic² and one of two As(III) efflux permeases, ArsB³ or Acr3.^{4,5} In addition to an efflux system, there are a variety of *ars* gene products that expand the range or capacity for inorganic arsenic. For example, ArsC is an arsenate reductase that transforms inorganic As(V) to As(III), the substrate of the efflux pumps.⁶ The ArsA As(III)-stimulated ATPase⁷ and the ArsD As(III) metallochaperone⁸ enhance the ability of ArsB to extrude As(III) and increase resistance. The respiratory arsenate reductase, AioBA, and the arsenite oxidase, AoxAB, comprise a redox cycle coupled to energy generation.⁹ These are all mechanisms for sensing, transforming and transporting inorganic arsenic. Only recently has the existence of a parallel cycle for biotransformations and detoxification of organoarsenicals been recognized.

The global biocycle of organoarsenical biotransformations

The revolution in genomics has provided a wealth of sequence information about arsenic resistance genes in thousands of organisms. New genes have been found in *ars* operons with functions that are not obvious. This review will focus on a recently identified global cycle of arsenic methylation, demethylation and detoxification that includes ArsM methyltransferases, the ArsI C-As bond lyase, the ArsH NADPH-FMN dependent oxidoreductases and the ArsP organoarsenical efflux permease (Figure 1).

ArsM and AS3MT: As(III) S-adenosylmethionine (SAM) methyltransferases

The gene for arsenic methylation is widespread in nature, found in many but not all members of every kingdom, from bacteria to humans. The gene product, As(III) S-adenosylmethionine (SAM) methyltransferase (EC 2.1.1.137), which is called ArsM in microbes and AS3MT in animals, methylates arsenite in three sequential steps to the trivalent species methylarsenite (MAs(III)), dimethylarsenite (DMAs(III)) and volatile trimethylarsine (TMAs(III)).^{10,11}

The function of the enzyme is controversial. If the gene arose before the atmosphere became oxidizing, then the products would have remained trivalent, so the enzyme would activate inorganic arsenic into more toxic methylated species such as MAs(III). In extant aerobic microbes, this is clearly a detoxification process because the trivalent products are rapidly oxidized to nontoxic pentavalent species in air. But the ability to reduce MAs(V) is widespread in soil microbes.¹² These bacteria regenerate MAs(III), which can kill off competitors. Wakesman defined an antibiotic as “a chemical substance, produced by micro-organisms, which has the capacity to inhibit the growth of and even to destroy bacteria and other micro-organisms”.¹³ By this definition, MAs(III) is an antibiotic. In response to synthesis of this biological weapon, other environmental microbes evolved the mechanisms described below to detoxify MAs(III), the very definition of antibiotic resistance (Figure 1).

The first identified microbial *arsM* gene was from the soil bacterium *Rhodopseudomonas palustris*,¹⁴ encoding the 283-residue ArsM protein (29,656 Da) (accession number WP_011159102.1). Heterologous expression of *arsM* from *R. palustris* conferred As(III) resistance to an arsenic-sensitive strain of *Escherichia coli*, demonstrating that the present-

day microbial gene detoxifies As(III). Photosynthetic organisms play a significant role in arsenic geochemical cycling by methylation. Methylated arsenic species have been found in many photosynthetic organisms, and *arsM* genes and their protein products have been characterized in cyanobacteria, prokaryotic photosynthetic organisms that are ubiquitous in environments ranging from soil and water to deserts. As the dominant species in toxic algal blooms, *Microcystis* sp. PCC7806, *Nostoc* sp. PCC7120, and *Synechocystis* sp. PCC6803 are typical freshwater cyanobacteria that methylate arsenic.¹¹ Two cyanobacteria ArsM enzymes were purified and were shown to methylate arsenite *in vitro*, with trimethylarsine gas as the end product.

Why do animals methylate arsenic? Is AS3MT an evolutionary fossil that inadvertently produces a toxin? Or does it play a role in arsenic detoxification? Paradoxically both are true, but the truth is more complicated. It is likely that the gene moved from prokaryotes to eukaryotes by horizontal gene transfer after the atmosphere became oxidizing. In eukaryotic microbes the gene confers arsenic resistance, so horizontal gene transfer conferred the ability to detoxify arsenic in lower eukaryotes. Eukaryotic algae such as the thermophilic red alga *Cyanidioschyzon merolae* methylate arsenic rapidly, producing trimethylarsine gas.¹⁵ The *arsM* gene from this alga confers resistance when heterologously expressed in *E. coli*. Similarly, the *arsM* gene from the mesophilic alga *Chlamydomonas reinhardtii* confers resistance in *E. coli*.¹⁶ It also rapidly transforms arsenic into nontoxic arsenosugars.¹⁷ So clearly arsenic methylation in eukaryotic microbes is a detoxification process.

Rapid and high level arsenic biomethylation and biovolatilization has been observed in the aquatic protozoa *Tetrahymena thermophile*, which converted 66% of 40 μ M inorganic arsenic into MAs(V) and DMAs(V) within 48 hr.¹⁸ The invertebrate sea squirt *Ciona intestinalis*, one of the closest living relative of vertebrates, has an AS3MT gene, and this primitive chordate methylates inorganic arsenic.¹⁹ Fungi are prolific arsenic methylators,²⁰ and the genomes of some fungi such as *Aspergillus* have AS3MT genes. In contrast, unlike lower plants such as algae, higher plants do not have an AS3MT gene and do not methylate arsenic. Instead, methylated arsenicals in plants originate from rhizosphere microbes.²¹

In higher eukaryotes the situation is less clear. The first gene for an As(III) SAM methyltransferase, AS3MT, was identified in rat.²² Nonmammalian animals such as fish also methylate arsenic. The zebrafish AS3MT gene is expressed in liver and intestine, where inorganic arsenic is transformed into the mono- and dimethylated species.²³ In mammals the products of liver methylation by AS3MT were thought to be the relatively nontoxic pentavalent species because those species found in urine.²⁴ In support of the idea that methylation is a detoxification process, AS3MT knockout mice are more susceptible to arsenic in drinking water.^{25,26} These results suggest that mammalian AS3MT is protective against acute arsenic exposure. However, more careful studies have shown that the urinary products are actually trivalent that oxidize when left in air.²⁷⁻²⁹ Purified arsenic methyltransferases, both human AS3MT and algal ArsM, produce MAs(III), not MAs(V).^{30,31} AS3MT produces sufficient intracellular MAs(III) to cause arsenic-related diseases such as bladder and other forms of cancer, diabetes and neurological disorders.³²⁻³⁴ In regions of the world with high arsenic in drinking water and food, these diseases are often found in exposed individuals. But most of the population of the world is continually exposed

to low levels of arsenic that do not produce obvious symptoms but increase the probability of arsenic-related diseases later in life.

Since evolution favors survival of the young and is little concerned with disease after the organism's reproductive period is over, the AS3MT gene would be maintained in humans even if it led to long-term increases in arsenic-related diseases. A number of polymorphisms have been identified in the human AS3MT gene, both intronic and in the coding region of the gene,^{35,36} and the distribution of polymorphisms in arsenic endemic regions of the world are associated with increased risk of skin lesions.³⁷ One such intronic polymorphism was identified in inhabitants of the northern Argentinean Andes, an arid region where elevated arsenic concentrations in drinking water is common.³⁸ In a genome-wide association study (GWAS) there was a strong association between an extragenic polymorphism on chromosome 10q24.32 that increases expression of the AS3MT and the amount of methylated arsenicals in urine. This is the first evidence that adaptation to environmental arsenic has likely driven an increase in the frequency of a protective AS3MT polymorphism.

There has been recent progress on the mechanism of As(III) SAM methyltransferases. Alignment of mammalian and algal sequences shows four conserved cysteine residues (Figure 2). The role of the cysteine residues in the catalytic cycle has been explored through a combination of structural, biochemical and molecular biological analyses. The crystal structure of the *C. merolae* CmArsM was solved at 1.6 Å.³⁹ The enzyme has three domains, an N-terminal SAM binding domain, a middle As(III) binding domain, and a C-terminal domain of unknown function. A structure with bound phenylarsenite (PhAs(III)) shows that the two conserved cysteine residues closest to the N-terminus are disulfide bonded with each other, and the other two conserved cysteines bind the arsenic atom (Figure 3).³¹ To explore their role in catalysis, each of the four cysteine residues was mutagenized in both algal and human enzymes.^{30,40} The results were unexpected: all four single mutants lost nearly all methylation activity, but, when either of the first two cysteines was changed, the enzyme lost the ability to methylate inorganic As(III) to MAs(III) (the first methylation step) but could still methylate MAs(III) to DMAs(III) (the second methylation step). These results are consistent with the crystallographic results that the second cysteine pair forms the arsenic binding site. In contrast, the first cysteine pair has a different role in catalysis that is only required during the first methylation (As(III)→MAs(III)) but not the second (MAs(III)→DMAs(III)). We proposed a new and novel reaction mechanism for As(III) SAM methyltransferases.^{30,31} Disulfide bond formation between the first two cysteines is a required step. Transfer of the S-methyl group from SAM oxidizes As(III) to form an enzyme-bound MAs(V) moiety. To proceed to the second methylation step, the arsenic atom must be re-reduced to trivalency, and the first two cysteine residues reduce the pentavalent methylarsenic intermediate during disulfide bond formation. The disulfide bond is then reduced by thioredoxin, a cofactor in AS3MT methylation,⁴¹⁻⁴³ allowing the second methylation step to proceed. Since the first cysteine pair is not required for the second methylation step, that reaction may use a different combination of cysteine residues for reduction of an enzyme-bound DMAs(V) intermediate. Interestingly, a disulfide bond between the second and third cysteine residues is observed in a crystal structure with bound SAM.³⁹ Thus a cascade of two successive disulfide bonds may be a feature of the methylation catalytic cycle.

Pathways for degradation and detoxification of MAs(III)

As mentioned above, we propose that ArsM originally evolved in an anoxic environment to produce toxic MAs(III), which has antibiotic properties. Under anaerobic conditions, MAs(III) is stable. But, once the atmosphere became oxidizing, most environmental MAs(III) would be converted to nontoxic MAs(V), and the function of ArsM became detoxification of inorganic arsenic. However, some soil bacteria such as *Burkholderia* sp. MR1 aerobically reduce MAs(V) to MAs(III), continually regenerating the toxic antibiotic.¹² As described below, other soil bacteria have *arsI*, *arsH* and *arsP* genes that encode proteins that detoxify MAs(III) by degradation, oxidation or efflux. The presence of these genes in extant bacteria would not occur if those bacteria were not under present-day selective pressure of MAs(III) production in soil.

ArsI, a C-As bond lyase

If microbes expressing As(III) SAM methyltransferases are common, why isn't most of the arsenic in the environment methylated? Inorganic arsenic continuously enters the environment from geothermal sources, and some methylated species are degraded abiotically, but, except for areas where it was applied as an herbicide, there is very little environmental MAs(V), which is surprising considering how widespread *arsM* genes are. Thus, continual synthesis of MAs(III) suggests the existence of microbial pathways for its degradation such as the recently identified ArsI MAs(III) demethylase.⁴⁴ Although microbial degradation of environmental organoarsenicals has been documented,⁴⁵⁻⁴⁸ molecular details of the reaction were unknown. Microbial communities in Florida golf course soil were shown to carry out a two-step pathway of MSMA (monosodium methylarsenate or MAs(V)) reduction and demethylation.¹² An environmental MAs(III)-demethylating bacterium *Bacillus* sp. MD1 was isolated from Florida golf course soil, and the *arsI* gene was cloned by selection for resistance to MAs(III).⁴⁴ ArsI (accession number AIA09488.1), is non-heme Fe(II)-dependent dioxygenase with C-As lyase activity that cleaves the C-As bond, converting MAs(III) into As(III). A thermophilic ArsI ortholog was cloned from *Thermomonospora curvata* and crystallized.⁴⁹ The structure of TcArsI was recently solved at a final resolution of 1.46 Å (Figure 4).⁵⁰ The enzyme was crystallized with Ni(II) bound to three amino acids that form the metal binding site in other dioxygenases. The enzyme has a cysteine pair in a flexible loop that was predicted to be the MAs(III) binding site.⁴⁴ Although the enzyme has not been crystallized with bound MAs(III), a structure was determined with 2-mercaptoethanol disulfide bonded to one of the two cysteines at one end and the hydroxyl at the other end interacting with the inner shell of the Fe(II) binding site, consistent with the role of the cysteine pair in binding MAs(III). The enzyme was crystallized in a sequential series of conformations in which the loop could be observed to move from solvent to the metal binding site. These sequential structures suggest a mechanism in which MAs(III) is bound in bulk solvent and progressively moved into proximity of the Fe(II), where one atom of dioxygen could be added to the arsenic atom of MAs(III) and the other oxygen atom to the carbon, forming the products As(OH)₃ and formaldehyde.

ArsI also cleaves the C-As bond of aromatic arsenicals, producing inorganic As(III) and uncharacterized organic molecules.⁴⁴ Large amounts of organic arsenicals have been used as herbicides and growth promoters in animal husbandry,^{45,51} as well as in various industrial activities such as the semiconductor industry. Both inorganic and organic arsenicals have been used in agriculture in the United States for over a century.⁵² For example, MSMA (MAs(V)), was used for decades for weed control and is still in use today as an herbicide for turf maintenance on golf courses, sod farms, highway rights of way and weed control in cotton fields.⁵² DMAs(V) (Agent Blue) was one of the rainbow herbicides used during the war in Vietnam.⁵³ The pentavalent aromatic arsenicals roxarsone (4-hydroxy-3-nitrobenzenearsonic or Rox(V)), nitarosone (4-nitrobenzenearsonic acid or Nit(V)) and atoxyl (*p*-aminophenylarsonic acid or pASA) have been used since the 1940s as antimicrobial growth promoters for chickens, turkeys and pigs to control *Coccidioides* infections, improve weight gain, feed efficiency and meat pigmentation.^{45,46} Pfizer suspended production of aromatic arsenicals in the United States, but they are still produced commercially worldwide, indicating that they are still used in poultry farms in other countries. Aromatic arsenicals are largely excreted intact by animals, and their feces are used as fertilizers.⁴⁵ Pentavalent organoarsenicals are relatively benign and less toxic than inorganic arsenicals. However, both aromatic^{45,46,54} and methylarsenicals^{47,55} are reduced to highly toxic trivalent forms,⁵⁶ which are degraded into toxic inorganic forms in the environment, where they contaminate foods and water supplies. This suggests that ArsI-catalyzed C-As bond cleavage of these aromatic arsenicals by microbial communities transform aromatic arsenicals into inorganic arsenicals that contaminate our food and water supplies.

ArsH, a MAs(III) oxidase

The *arsH* gene is found in *ars* operons, which implies an arsenic-related physiological function. Currently there are more than 7,500 ArsH-related protein sequences in the NCBI database. A vast majority of ArsH sequences are found in bacteria (97.7%), mostly gammaproteobacteria, with a few in fungi (2.2%). The *arsH* gene was originally identified in an *ars* operon in *Yersinia enterocolitica*, but it conferred only a very slight increase in resistance to either arsenite or arsenate.⁵⁷ Deletion of *arsH* in *Serratia marcescens*⁵⁸ and *S. meliloti*⁵⁹ also led to slight increases in arsenic sensitivity. Arsenic resistance in *Thiobacillus ferrooxidans*⁶⁰ and the cyanobacterium *Synechocystis*⁶¹ was unaffected by either a loss of function mutation in or overexpression of *arsH*. Thus no clear inorganic arsenical resistance phenotype or physiologically could be ascribed to ArsH.

Purified ArsH from *S. meliloti* (accession number Q92R45.1) exhibited NADPH-dependent FMN reductase activity, reducing azo dyes and generating hydrogen peroxide but neither reduced or oxidized inorganic arsenicals.⁶² *Synechocystis* ArsH showed quinone reductase activity⁶² and reduced chromate and ferric iron, but no arsenic-associated activity was observed.⁶³

Instead of biotransformation of inorganic arsenicals, the physiological role of *arsH* was recently shown to be oxidation of toxic MAs(III) and other trivalent organoarsenicals to relatively nontoxic pentavalent species.⁶⁴ Rather than being an NADPH-dependent reductase, it is an NADP⁺-dependent oxidase. *P. putida* and *S. meliloti* are both quite

resistant to MAs(III), PhAs(III) and Rox(III). *P. putida* has two *ars* operons, each with an *arsH* gene. Deletion of both *ars* operons in *P. putida* renders the organism sensitive to MAs(III) and PhAs(III), as did deletion of the *ars* operon of *S. meliloti*. Expression of the *arsH* gene in *E. coli* was sufficient to confer resistance to both MAs(III) and aromatic arsenicals, demonstrating that *arsH* alone confers resistance to trivalent organoarsenicals. Expression of *P. putida arsH* in *E. coli* strain AW3110, in which the *ars* operon was deleted,⁶⁵ made the cells resistance to MAs(III), PhAs(III) and Rox(III), showing that *arsH* is sufficient to detoxify the activated trivalent forms of the herbicide MSMA as well as the poultry antimicrobial growth promoter roxarsone. Purified ArsH from either *S. meliloti* or *P. putida* are yellow and show a typical flavoprotein absorption spectrum.^{63,66} *P. putida* oxidized MAs(III), PhAs(III) and Rox(III).⁶⁴ The crystal structure of *S. meliloti* ArsH (Figure 5) was determined at 1.8 Å resolution and is similar to other flavoproteins.⁶² It crystallizes with eight molecules in the asymmetric unit in two identical tetramers, and each tetramer is a functional unit. Thus, ArsH has now been demonstrated to be a trivalent organoarsenical oxidase that confers resistance to organoarsenicals.

ArsP, a MAs(III) efflux permease

A third mechanism for resistance to toxic MAs(III) is catalyzed by the ArsP MAs(III) efflux permease. ArsP from *Campylobacter jejuni* (accession number ACG76368.1) was originally identified as a pentavalent roxarsone resistance,⁶⁶ but it was subsequently demonstrated the substrate of ArsP is actually trivalent organoarsenicals.⁶⁷ *C. jejuni arsP* expressed in *E. coli* conferred resistance to MAs(III) and Rox(III) but not to inorganic As(III) or pentavalent organoarsenicals.⁶⁷ In addition, everted (inside-out) membrane vesicles from those cells accumulated MAs(III) in an energy-dependent manner, consistent with ArsP catalyzing efflux from cells. Transport was selective for MAs(III) over trivalent aromatic arsenicals such as Rox(III), and the vesicles did not transport As(III), MAs(V) or pentavalent roxarsone, showing that ArsP is a MAs(III)-selective efflux permease.

What is the basis of selectivity for MAs(III)? CjArsP has a PXXC₆₅SC₆₇SXXP motif that is conserved in ArsP orthologs. A CXC motif can be predicted to bind MAs(III) with high affinity. Mutation or modification of the two conserved cysteine residues resulted in loss of transport activity, suggesting that they could be part of the MAs(III) binding site. However, in a homology model built on the GlpT structure,⁶⁸ the distance between the sulfur atoms of the two cysteines in transmembrane helix TM2 would be approximately 10.5 Å.⁶⁷ To be capable of binding MAs(III), the thiols would have to be about 2.2 Å from the arsenic atom. This was proposed to occur by bending of TM2 to bring the thiolates into proximity. The bend would be introduced by the two flanking proline residues. We proposed that ArsP has two conformations: 1) an inward-facing conformation in which the two cysteine residues are close enough to each other to bind cytosolic MAs(III), and an outward-facing conformation in which TM2 straightens and releases MAs(III) into the periplasm (Figure 6).⁶⁷ This single-binding site, alternating-access mechanism is similar to that proposed for other permeases such as GlpT.⁶⁸ Thus ArsP is an efflux system that confers resistance to MAs(III).

Summary

While much has been discovered about inorganic arsenic biocycles, knowledge of the parallel cycle of organoarsenical synthesis and degradation lag far behind. The major source of inorganic arsenic is geochemical, but organic arsenicals have been produced biologically for billions of years, with very recent anthropogenic contributions through introduction of organoarsenical herbicides, chemical warfare agents, antimicrobials and industrial contributions. Here we reviewed the mechanisms of organoarsenical biotransformations and their contributions to arsenic biogeocycle.

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Biographical

Barry P. Rosen, Ph.D., is Distinguished University Professor in the Department of Cellular Biology and Pharmacology at Florida International University Herbert Wertheim College of Medicine. He was born in Hartford, Connecticut and received his B.S. from Trinity College and M.S. and Ph.D in Biochemistry from University of Connecticut. He was an NIH postdoctoral fellow at Cornell University. He held faculty positions at University of Maryland School of Medicine and Wayne State University School of Medicine, where he was Distinguished Professor and Chair of the Department of Biochemistry and Molecular Biology for 22 years. Jiaojiao Li, M.D., was born in Yunnan Province, China and received her medical degree in 2005 from Kunming Medical University, Kunming, China. Her enthusiasm for biomedical sciences and desire to become a biomedical scientist motivated her to pursue Ph.D. degree at FIU College of Medicine. Shashank S. Pawitwar, B.S, M.S., was born in Nanded, Maharashtra state, India. He received his BS. In Biotechnology from Swami Ramanand Teerth Marathwada University, Nanded and M.S. in Biotechnology from Amity University, Noida, Uttar Pradesh, India. His goal is to become a research scientist in biotechnology. Both Dr. Li and Mr. Pawitwar are doctoral students in the laboratory of Barry P. Rosen.

References

1. Zhu YG, Yoshinaga M, Zhao FJ, Rosen BP. Earth abides arsenic biotransformations. *Annu Rev Earth and Planet Sci.* 2014; 42:443–467. [PubMed: 26778863]
2. San Francisco MJ, Hope CL, Owolabi JB, Tisa LS, Rosen BP. Identification of the metalloregulatory element of the plasmid-encoded arsenical resistance operon. *Nucleic Acids Res.* 1990; 18:619–624. [PubMed: 2408017]
3. San Francisco MJ, Chen CM, Rosen BP. Identification of the membrane component of the anion pump encoded by the arsenical resistance operon of R-factor R773. *Prog. Clin. Biol. Res.* 1988; 252:311–316. [PubMed: 2450373]

4. Wysocki R, Bobrowicz P, Ulaszewski S. The *Saccharomyces cerevisiae* *ACR3* gene encodes a putative membrane protein involved in arsenite transport. *J. Biol. Chem.* 1997; 272:30061–30066. [PubMed: 9374482]
5. Ghosh M, Shen J, Rosen BP. Pathways of As(III) detoxification in *Saccharomyces cerevisiae*. *Proc Nat. Acad Sci USA.* 1999; 96:5001–5006. [PubMed: 10220408]
6. Mukhopadhyay R, Rosen BP. Arsenate reductases in prokaryotes and eukaryotes. *Environ. Health Perspect.* 2002; 110(Suppl 5):745–748. [PubMed: 12426124]
7. Rosen BP, Weigel U, Karkaria C, Gangola P. Molecular characterization of an anion pump. The *arsA* gene product is an arsenite(antimonate)-stimulated ATPase. *J. Biol. Chem.* 1988; 263:3067–3070. [PubMed: 2449436]
8. Lin YF, Walmsley AR, Rosen BP. An arsenic metallochaperone for an arsenic detoxification pump. *Proc. Natl. Acad. Sci. U. S. A.* 2006; 103:15617–15622. [PubMed: 17030823]
9. van Lis R, Nitschke W, Duval S, Schoepp-Cothenet B. Arsenics as bioenergetic substrates. *Biochim. Biophys. Acta.* 2013; 1827:176–188. [PubMed: 22982475]
10. Thomas, DJ.; Rosen, BP. Encyclopedia of Metalloproteins. Kretsinger, RH.; Uversky, VN.; Permyakov, EA., editors. Springer New York; New York: 2013. p. 138-143.
11. Ye J, Rensing C, Rosen BP, Zhu YG. Arsenic biomethylation by photosynthetic organisms. *Trends Plant Sci.* 2012; 17:155–162. [PubMed: 22257759]
12. Yoshinaga M, Cai Y, Rosen BP. Demethylation of methylarsonic acid by a microbial community. *Environ Microbiol.* 2011; 13:1205–1215. [PubMed: 21272184]
13. Waksman SA. What is an antibiotic or an antibiotic substance? *Mycologia.* 1947; 39:565–569. [PubMed: 20264541]
14. Qin J, Rosen BP, Zhang Y, Wang G, Franke S, Rensing C. Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase. *Proc. Natl. Acad. Sci. U. S. A.* 2006; 103:2075–2080. [PubMed: 16452170]
15. Qin J, Lehr CR, Yuan C, Le XC, McDermott TR, Rosen BP. Biotransformation of arsenic by a Yellowstone thermoacidophilic eukaryotic alga. *Proc. Natl. Acad. Sci. U. S. A.* 2009; 106:5213–5217. [PubMed: 19276121]
16. Chen J, Qin J, Zhu YG, de Lorenzo V, Rosen BP. Engineering the soil bacterium *Pseudomonas putida* for arsenic methylation. *Appl. Environ. Microbiol.* 2013; 79:4493–4495. [PubMed: 23645194]
17. Miyashita S, Fujiwara S, Tsuzuki M, Kaise T. Rapid biotransformation of arsenate into oxo-arsenosugars by a freshwater unicellular green alga, *Chlamydomonas reinhardtii*. *Biosci. Biotechnol. Biochem.* 2011; 75:522–530. [PubMed: 21389618]
18. Yin XX, Chen J, Qin J, Sun GX, Rosen BP, Zhu YG. Biotransformation and volatilization of arsenic by three photosynthetic cyanobacteria. *Plant Physiol.* 2011; 156:1631–1638. [PubMed: 21562336]
19. Thomas DJ, Nava GM, Cai SY, Boyer JL, Hernandez-Zavala A, Gaskins HR. Arsenic (+ 3 oxidation state) methyltransferase and the methylation of arsenicals in the invertebrate chordate *Ciona intestinalis*. *Toxicol. Sci.* 2009; 113:70–76. [PubMed: 19833739]
20. Bentley R, Chasteen TG. Microbial methylation of metalloids: arsenic, asourcetimony, and bismuth. *Microbiol. Mol. Biol. Rev.* 2002; 66:250–271. [PubMed: 12040126]
21. Lomax C, Liu WJ, Wu L, Xue K, Xiong J, Zhou J, McGrath SP, Meharg AA, Miller AJ, Zhao FJ. Methylated arsenic species in plants originate from soil microorganisms. *New Phytol.* 2011; 193:665–672. [PubMed: 22098145]
22. Lin S, Shi Q, Nix FB, Styblo M, Beck MA, Herbin-Davis KM, Hall LL, Simeonsson JB, Thomas DJ. A novel S-adenosyl-L-methionine:arsenic(III) methyltransferase from rat liver cytosol. *J. Biol. Chem.* 2002; 277:10795–10803. [PubMed: 11790780]
23. Hamdi M, Sanchez MA, Beene LC, Liu Q, Landfear SM, Rosen BP, Liu Z. Arsenic transport by zebrafish aquaglyceroporins. *BMC Mol Biol.* 2009; 10:104. [PubMed: 19939263]
24. Challenger F. Biological methylation. *Sci. Prog.* 1947; 35:396–416. [PubMed: 20256237]
25. Yokohira M, Arnold LL, Pennington KL, Suzuki S, Kakiuchi-Kiyota S, Herbin-Davis K, Thomas DJ, Cohen SM. Severe systemic toxicity and urinary bladder cytotoxicity and regenerative

- hyperplasia induced by arsenite in arsenic (+3 oxidation state) methyltransferase knockout mice. A preliminary report. *Toxicol. Appl. Pharmacol.* 2010; 246:1–7. [PubMed: 20423714]
26. Dodmane PR, Arnold LL, Pennington KL, Thomas DJ, Cohen SM. Effect of dietary treatment with dimethylarsinous acid (DMA(III)) on the urinary bladder epithelium of arsenic (+3 oxidation state) methyltransferase (As3mt) knockout and C57BL/6 wild type female mice. *Toxicology.* 2013; 305C:130–135.
 27. Hughes MF, Devesa V, Adair BM, Conklin SD, Creed JT, Styblo M, Kenyon EM, Thomas DJ. Tissue dosimetry, metabolism and excretion of pentavalent and trivalent dimethylated arsenic in mice after oral administration. *Toxicol. Appl. Pharmacol.* 2008; 227:26–35. [PubMed: 18036629]
 28. Del Razo LM, Garcia-Vargas GG, Valenzuela OL, Castellanos EH, Sanchez-Pena LC, Currier JM, Drobna Z, Loomis D, Styblo M. Exposure to arsenic in drinking water is associated with increased prevalence of diabetes: a cross-sectional study in the Zimapan and Lagunera regions in Mexico. *Environ Health.* 2011; 10:73. [PubMed: 21864395]
 29. Le XC, Lu X, Ma M, Cullen WR, Aposhian HV, Zheng B. Speciation of key arsenic metabolic intermediates in human urine. *Anal. Chem.* 2000; 72:5172–5177. [PubMed: 11080860]
 30. Dheeman DS, Packianathan C, Pillai JK, Rosen BP. Pathway of human AS3MT arsenic methylation. *Chem. Res. Toxicol.* 2014; 27:1979–1989. [PubMed: 25325836]
 31. Marapakala K, Packianathan C, Ajees AA, Dheeman DS, Sankaran B, Kandavelu P, Rosen BP. A disulfide-bond cascade mechanism for As(III) S-adenosylmethionine methyltransferase. *Acta Crystallogr. D. Biol. Crystallogr.* 2015; 71:505–515. [PubMed: 25760600]
 32. Abernathy CO, Liu YP, Longfellow D, Aposhian HV, Beck B, Fowler B, Goyer R, Menzer R, Rossman T, Thompson C, Waalkes M. Arsenic: health effects, mechanisms of actions, and research issues. *Environ. Health Perspect.* 1999; 107:593–597. [PubMed: 10379007]
 33. Abernathy CO, Thomas DJ, Calderon RL. Health effects and risk assessment of arsenic. *J. Nutr.* 2003; 133:1536S–1538S. [PubMed: 12730460]
 34. Jomova K, Jenisova Z, Feszterova M, Baros S, Liska J, Hudecova D, Rhodes CJ, Valko M. Arsenic: toxicity, oxidative stress and human disease. *J. Appl. Toxicol.* 2011; 31:95–107. [PubMed: 21321970]
 35. Agusa T, Fujihara J, Takeshita H, Iwata H. Individual variations in inorganic arsenic metabolism associated with AS3MT genetic polymorphisms. *Int J Mol Sci.* 2011; 12:2351–2382. [PubMed: 21731446]
 36. Wood TC, Salavagionne OE, Mukherjee B, Wang L, Klumpp AF, Thomae BA, Eckloff BW, Schaid DJ, Wieben ED, Weinshilboum RM. Human arsenic methyltransferase (AS3MT) pharmacogenetics: gene resequencing and functional genomics studies. *J. Biol. Chem.* 2006; 281:7364–7373. [PubMed: 16407288]
 37. Pierce BL, Kibriya MG, Tong L, Jasmine F, Argos M, Roy S, Paul-Brutus R, Rahaman R, Rakibuz-Zaman M, Parvez F, Ahmed A, Quasem I, Hore SK, Alam S, Islam T, Slavkovich V, Gamble MV, Yunus M, Rahman M, Baron JA, Graziano JH, Ahsan H. Genome-wide association study identifies chromosome 10q24.32 variants associated with arsenic metabolism and toxicity phenotypes in Bangladesh. *PLoS Genet.* 2012; 8:e1002522. [PubMed: 22383894]
 38. Schleich CM, Gattepaille LM, Engstrom K, Vahter M, Jakobsson M, Broberg K. Human adaptation to arsenic-rich environments. *Mol. Biol. Evol.* 2015; 32:1544–1555. [PubMed: 25739736]
 39. Ajees AA, Marapakala K, Packianathan C, Sankaran B, Rosen BP. Structure of an As(III) S-adenosylmethionine methyltransferase: insights into the mechanism of arsenic biotransformation. *Biochemistry.* 2012; 51:5476–5485. [PubMed: 22712827]
 40. Marapakala K, Qin J, Rosen BP. Identification of catalytic residues in the As(III) S-adenosylmethionine methyltransferase. *Biochemistry.* 2012; 51:944–951. [PubMed: 22257120]
 41. Ding L, Saunders RJ, Drobna Z, Walton FS, Xun P, Thomas DJ, Styblo M. Methylation of arsenic by recombinant human wild-type arsenic (+3 oxidation state) methyltransferase and its methionine 287 threonine (M287T) polymorph: Role of glutathione. *Toxicol. Appl. Pharmacol.* 2012; 264:121–130. [PubMed: 22868225]

42. Thomas DJ, Waters SB, Styblo M. Elucidating the pathway for arsenic methylation. *Toxicol. Appl. Pharmacol.* 2004; 198:319–326. [PubMed: 15276411]
43. Waters SB, Devesa V, Del Razo LM, Styblo M, Thomas DJ. Endogenous reductants support the catalytic function of recombinant rat cyt19, a source arsenic methyltransferase. *Chem. Res. Toxicol.* 2004; 17:404–409. [PubMed: 15025511]
44. Yoshinaga M, Rosen BP. A C-As lyase for degradation of environmental organoarsenical herbicides and animal husbandry growth promoters. *Proc. Natl. Acad. Sci. U. S. A.* 2014; 111:7701–7706. [PubMed: 24821808]
45. Garbarino JR, Bednar AJ, Rutherford DW, Beyer RS, Wershaw RL. Environmental fate of roxarsone in poultry litter. I. Degradation of roxarsone during composting. *Environ Sci Technol.* 2003; 37:1509–1514. [PubMed: 12731831]
46. Stolz JF, Perera E, Kilonzo B, Kail B, Crable B, Fisher E, Ranganathan M, Wormer L, Basu P. Biotransformation of 3-nitro-4-hydroxybenzene arsonic acid (roxarsone) and release of inorganic arsenic by *Clostridium* species. *Environ Sci Technol.* 2007; 41:818–823. [PubMed: 17328188]
47. Feng M, Schrlau JE, Snyder R, Snyder GH, Chen M, Cisar JL, Cai Y. Arsenic transport and transformation associated with MSMA application on a golf course green. *J. Agric. Food Chem.* 2005; 53:3556–3562. [PubMed: 15853401]
48. Lehr C, Polishchuk E, Radoja U, Cullen WR. Demethylation of methylarsenic species by *Mycobacterium neoaurum*. *Appl Organomet Chem.* 2003; 17:831–834.
49. Nadar SV, Yoshinaga M, Kandavelu P, Sankaran B, Rosen BP. Crystallization and preliminary X-ray crystallographic studies of the ArsI C-As lyase from *Thermomonospora curvata*. *Acta Crystallogr F Struct Biol Commun.* 2014; 70:761–764. [PubMed: 24915088]
50. Nadar VS, Yoshinaga M, Pawitwar SS, Kandavelu P, Sankaran B, Rosen BP. Structure of the ArsI C-As lyase: Insights into the mechanism of degradation of organoarsenical herbicides and growth promoters. *J. Mol. Biol.* 2016; 428:2462–2473. [PubMed: 27107642]
51. Rutherford DW, Bednar AJ, Garbarino JR, Needham R, Staver KW, Wershaw RL. Environmental fate of roxarsone in poultry litter. Part II. Mobility of arsenic in soils amended with poultry litter. *Environ Sci Technol.* 2003; 37:1515–1520. [PubMed: 12731832]
52. Matteson AR, Gannon TW, Jeffries MD, Haines S, Lewis DF, Polizzotto ML. Arsenic retention in foliage and soil after monosodium methyl arsenate (MSMA) application to turfgrass. *J. Environ. Qual.* 2014; 43:379–388. [PubMed: 25602572]
53. Stellman JM, Stellman SD, Christian R, Weber T, Tomasallo C. The extent and patterns of usage of Agent Orange and other herbicides in Vietnam. *Nature.* 2003; 422:681–687. [PubMed: 12700752]
54. Makris KC, Quazi S, Punamiya P, Sarkar D, Datta R. Fate of arsenic in swine waste from concentrated animal feeding operations. *J Environ Qual.* 2008; 37:1626–1633. [PubMed: 18574196]
55. von Endt DW, Kearney PC, Kafman DD. Degradation of monosodium methanearsonic acid by soil microorganisms. *J. Agric. Food Chem.* 1968; 16:17–20.
56. Chen J, Sun S, Li CZ, Zhu YG, Rosen BP. Biosensor for organoarsenical herbicides and growth promoters. *Environ Sci Technol.* 2014; 48:1141–1147. [PubMed: 24359149]
57. Neyt C, Iriarte M, Thi VH, Cornelis GR. Virulence and arsenic resistance in *Yersinia*. *J. Bacteriol.* 1997; 179:612–619. [PubMed: 9006011]
58. Ryan D, Collieran E. Arsenical resistance in the IncHI2 plasmids. *Plasmid.* 2002; 47:234–240. [PubMed: 12151239]
59. Yang HC, Cheng J, Finan TM, Rosen BP, Bhattacharjee H. Novel pathway for arsenic detoxification in the legume symbiont *Sinorhizobium meliloti*. *J. Bacteriol.* 2005; 187:6991–6997. [PubMed: 16199569]
60. Butcher BG, Deane SM, Rawlings DE. The chromosomal arsenic resistance genes of *Thiobacillus ferrooxidans* have an unusual arrangement and confer increased arsenic and antimony resistance to *Escherichia coli*. *Appl. Environ. Microbiol.* 2000; 66:1826–1833. [PubMed: 10788346]
61. Lopez-Maury L, Florencio FJ, Reyes JC. Arsenic sensing and resistance system in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *J. Bacteriol.* 2003; 185:5363–5371. [PubMed: 12949088]

62. Ye J, Yang HC, Rosen BP, Bhattacharjee H. Crystal structure of the flavoprotein ArsH from *Sinorhizobium meliloti*. FEBS Lett. 2007; 581:3996–4000. [PubMed: 17673204]
63. Xue XM, Yan Y, Xu HJ, Wang N, Zhang X, Ye J. ArsH from *Synechocystis* sp. PCC 6803 reduces chromate and ferric iron. FEMS Microbiol. Lett. 2014; 356:105–112. [PubMed: 24861149]
64. Chen J, Bhattacharjee H, Rosen BP. ArsH is an organoarsenical oxidase that confers resistance to trivalent forms of the herbicide monosodium methylarsenate and the poultry growth promoter roxarsone. Mol. Microbiol. 2015; 96:1042–1052. [PubMed: 25732202]
65. Carlin A, Shi W, Dey S, Rosen BP. The *ars* operon of *Escherichia coli* confers arsenical and antimonial resistance. J. Bacteriol. 1995; 177:981–986. [PubMed: 7860609]
66. Shen Z, Luangtongkum T, Qiang Z, Jeon B, Wang L, Zhang Q. Identification of a novel membrane transporter mediating resistance to organic arsenic in *Campylobacter jejuni*. Antimicrob. Agents Chemother. 2014; 58:2021–2029. [PubMed: 24419344]
67. Chen J, Madegowda M, Bhattacharjee H, Rosen BP. ArsP: a methylarsenite efflux permease. Mol. Microbiol. 2015; 98:625–635. [PubMed: 26234817]
68. Huang Y, Lemieux MJ, Song J, Auer M, Wang DN. Structure and mechanism of the glycerol-3-phosphate transporter from *Escherichia coli*. Science. 2003; 301:616–620. [PubMed: 12893936]

Figure 1. MAs(III): a primordial antibiotic

In communities of soil microbes some bacteria such as *Rhodopseudomonas palustris* carry the *arsM* gene for the As(III) SAM methyltransferase, producing highly toxic MAs(III). This trivalent organoarsenical has antibiotic-like properties. Other soil bacteria carry genes for MAs(III) resistance. Some, such as *Bacillus* MD1, have the *arsI* gene for the ArsI C-As lyase genes that confers resistance to MAs(III) by degrading it into As(III) and formaldehyde. Yet other soil bacteria such as *Pseudomonas putida* have a gene encoding ArsH, a flavoprotein that uses NADP⁺ to oxidize MAs(III) to MAs(V), thus conferring resistance. Finally, other bacteria such as *Campylobacter jejuni*, which inhabits the intestinal track of poultry and other farm animals, carry the *arsP* gene. ArsP is a MAs(III) efflux permease that extrudes trivalent organoarsenicals from cells, conferring resistance. The crystal structures of the relevant enzymes are shown next to their reactions.

Figure 2. Multiple alignment of As(III) SAM methyltransferases

The protein sequences of eukaryotic As(III) SAM methyltransferases were aligned: human AS3MT (accession number AAV68045), rat AS3MT (accession number NP_543166), *Cyanidioschyzon* sp. 5508 CmArsM (accession number ACN39191) and CrArsM from *Chlamydomonas reinhardtii* (accession number XP_001703619). The four conserved cysteine residues are highlighted in yellow background with numbering above according to the human and below the CmArsM sequences. Identical residues are shaded in black, and conservative replacements in grey.

Figure 3. Structure of the CmArsM As(III) SAM methyltransferase

Shown is the structure of CmArsM from *Cyanidioschyzon* sp. 5508 (CmArsM) with the bound aromatic arsenical phenylarsenite (PhAs(III)) at 1.80 Å resolution (PDB ID: 4kw7).³¹ The arsenic atom is coordinated with the thiolates of Cys174 and Cys224. A disulfide bond is visible between residues Cys44 and Cys72. The conserved four cysteine residues are represented in stick form and colored cyan (carbon), blue (nitrogen), red (oxygen) or yellow (sulfur). The dark blue sphere is the arsenic atom.

Figure 4. Structure of the ArsI C-As lyase

The structure of ArsI from *T. curvata* was solved at a final resolution of 1.46 Å (PDB ID: 5C68).⁵⁰ Atoms are colored as in Fig. 1. Fe(II) (orange sphere) is bound to Gln8, His65 and Glu117. The blue spheres are oxygen atoms. The organoarsenic binding site is composed of Cys98 and Cys99, with MAs(III) modeled into the site.

Figure 5. Structure of the ArsH MAs(III) oxidase

The structure of ArsH from *S. meliloti* was determined at 1.8 Å resolution (PDB ID: 2Q62).⁶² The enzyme purifies as a tetramer, which the crystal structure shows as a dimer of dimers. Monomers A, B, C and D are colored yellow, green, red and blue, respectively. Subunits A and B form a dimer, as do monomers C and D. An FMN cofactor (not shown) is bound at each dimer interface. Dimers A-B and C-D form the tetramer. Thus there are two different interfaces between subunits: interfaces between monomers (vertical line) and between dimers (horizontal line).

Figure 6. Proposed catalytic model of the ArsP MAs(III) efflux permease

ArsP employs a binding site that is exposed alternately to the two sides of the membrane.⁶⁷ Helices are represented by cylinders. The positions of Cys65 and Cys67 in TM2 are indicated. **A.** ArsP assumes a conformation in which the two cysteine residues in TM2 are exposed to the cytosol. TM2 is distorted by proline residues on each side of the two cysteine residues to allow the two sulfur atoms to form an S_2 site with MAs(III). **B.** A conformational change reorients the binding site so that it is exposed to the periplasm. TM2 becomes more helical, disrupting the $MAsS_2$ complex and releasing MAs(III) into the periplasm.