Arsenic is a metalloid whose name conjures up images of murder. Nonetheless, certain prokaryotes use arsenic oxyanions for energy generation, either by oxidizing arsenite or by respiring arsenenate. These microbes are phylogenetically diverse and occur in a wide range of habitats. Arsenic cycling may take place in the absence of oxygen and can contribute to organic matter oxidation. In aquifers, these microbial reactions may mobilize arsenic from the solid to the aqueous phase, resulting in contaminated drinking water. Here we review what is known about arsenic-metabolizing bacteria and their potential impact on speciation and mobilization of arsenic in nature.

Despite its low crustal abundance (0.0001%), arsenic is widely distributed in nature and is commonly associated with the ores of metals like copper, lead, and gold (1). Arsenic can exist in four oxidation states: \(\text{As(III)}\), \(\text{As(O)}\), \(\text{As(III)}\), and \(\text{As(V)}\). Native (elemental) arsenic occurs rarely, whereas traces of toxic arsines can be detected in gases emanating from anoxic environments (2). The predominant form of inorganic arsenic in aqueous, aerobic environments is arsenate \([\text{As(V)}]\) as \(\text{H}_3\text{AsO}_4^-\) and \(\text{HAsO}_4^{2-}\), whereas arsenite \([\text{As(III)}]\) as \(\text{H}_2\text{AsO}_3^-\) and \(\text{H}_3\text{AsO}_3\) is more prevalent in anoxic environments. Arsenate is strongly adsorbed to the surface of several common minerals, such as ferricydrate and alumina, a property that constrains its hydrologic mobility. Arsenite adsorbs less strongly and to fewer minerals, which makes it the more mobile oxyanion (3). A number of methylated organoarsenicals (e.g., methylarsonic, methyl-
larsenous, and dimethylarsenic acids) are found in natural waters as breakdown or excretory products from aquatic biota (2, 4), or as urinary excretions of animals, including humans (5). A recent review gives further details on the detection of various organo-arsenicals in nature (6).

Anthropogenic point sources contribute to arsenic found in the environment. These include smelter slag, coal combustion, runoff from mine tailings, hide tanning waste, pigment and storage of chemical weapons (e.g., arsenical arsenide, dimethylarsionate) alone amounted to ~10,000 metric tons per year (7). In a more isolated case, the production and storage of chemical weapons (e.g., phenyl- dichloroarsine, diphenylchloroarsine, diphenylocyanarsine) has resulted in the gross contamination (>900 mg/kg) of several former mili­tary bases in Eastern Europe (8). Arsenic has been replaced in most applications by synthet­ic acids and pesticides, but it is still used in agriculture. Organic arsenicals like roxarsone (4-hydroxy-3-nitrophenyl arsenic acid) act as an intestinal palliative for swine and prevent coccidiosis, and nitrophenyl arsonic acid act as an intestinal system (17). Arsenite is even more broadly toxic because it binds to sulfhydryl groups, impairing the function of many proteins (13). It also affects respiration by binding to the cyto­plasmic thios in pyruvate dehydrogenase and 2-oxo­glutarate dehydrogenase (15). More recently, it has been shown to interact with the glu­cocorticoid receptor (16). Arsenite is un­charged at pH values less than 9.2 and enters the cell via aquaglyceroporins (17).

Several different mechanisms have evolved to rid cells of arsenic. These include methyl­ation, and expulsion involving an As(III)-spe­cific transporter. In higher eukaryotes, glutathione reduces As(V) to As(III), which then accepts a methyl group from S-adenosylmethionine, producing monomethylarsonic acid (MMA) or dimethylarsonic acid (DMA) (15). Fungi produce trimethylarsine (18), whereas bacteria may produce MMA and DMA (19). Such diverse microbes as anaerobic methano­genic Archaea (20) and aerobic Eubacteria (21) can also form methylated arsines. Arsenic may also be converted to arsenobetaine and arsenic-containing sugars, benzoin compounds that are found in high abundance in some marine ani­mals and algae as well as terrestrial plants and animals (2, 6).

The most well studied mechanism of detox­ification and resistance, however, is the ArsC system (17, 22). At least three different but structurally related arsenate reductases have convergently evolved in bacteria and yeast. ArsC, a small-molecular mass protein (13 to 16 kD), mediates the reduction of As(V) to As(III) in the cytoplasm. Although AsIII is more tox­ic, it can be excreted via an As(III)-specific transporter, ArsAB. The ars operon in Escherichia coli has both plasmid and chromosomal loci. The plasmid R733 has four genes—arsA, arsB, arsC, arsD, and arsR—and the chromo­sonal locus has only arsB, arsC, and arsR. A cyanide residue near the N-terminal of ArsC binds the As(V), which is then reduced with electrons donated by the reduced glutathione. The As(III) is then expelled from the cytoplasm through an adenosine 5’-triphosphate (ATP)-dependent arsenite transporter formed by Ar­sAB (17). The ars operon in plasmid pLS28 of Staphylococcus aureus contains only arsB, arsC, and arsR (23, 24). Reduced thioredoxin provides the electrons to reduce As(V), and As(III) is expelled from the cell via an ATP-independent ArsB. Although this process has been studied in detail in E. coli and S. aureus, it is found in many other bacteria and occurs in strict anaerobes like Clostridium (25) and De­salvosibrio (26). Arsenate reduction to As(III) has been noted in several aerobic bacteria iso­lated from As-contaminated soils and mine tail­ings (27, 28), suggesting that As(V) resistance plays an important role in the biogeochemical cycling of this element in nature (29).

**Dissimilatory Arsenate-Reducing Prokaryotes**

Considering the toxicity of arsenic to both prokaryotes and eukaryotes, the discovery that As(V) serves as a “nutrient” to certain anaerobes by functioning as their respiratory oxidant came as a surprise. The reaction is energetically favorable when coupled with the oxidation of organic matter because the As(V)/As(III) oxidation/reduction potential is +135 mV. Two closely related representa­tives of the e-Proteobacteria, Sulfurospirillum arsenophilum and Sulfurospirillum barnesi, were the first microbes reported that could achieve this feat (30–32). Both conserve energy by linking the oxidation of lactate to the reduction of As(V) to As(III) [Gibbs free energy (AG°) = −295 kJ/mol lactate]. At present there are at least 16 species in pure culture, and include representatives from the γ- and e-Proteobacteria, low-GC Gram-positive bacteria, thermophilic Eubacteria, and Crenarchaeota (Fig. 1). We collectively refer to these microbes as dissimilatory ar­senate-reducing prokaryotes (DARPs). They have been isolated from freshwater sedi­ments, estuaries, soda lakes, hot springs, and gold mines [reviewed in (33)]; the gastrointes­tinal tracts of animals (34); and subsurface aquifer materials from Bangladesh (35). They include several extremophiles adapted to high temperature, pH, and/or salinity (36–38). These organisms can use a variety of electron donors including hydrogen, ace­tate, formate, pyruvate, butyrate, citrate, succinate, fumarate, malate, and glucose (39). Recently, some strains have been found to degrade more complex aromatic molecules like benzoate and even toluene (23). Certain species are more sensitive to arsenic than others. Whereas the haloalka­liphile Bacillus selenitireducens grows well at 10 mM As(V), possibly because the product As(III) is charged at high pH and cannot enter the cell, Sulfurospirillum spe­cies grow best at 5 mM. To date, no “ob­ligate” DARPs have been found, because all the strains examined can use other elec­tron acceptors for growth. For example, Desulfothermus auripigmentum (24) and Desulfomicrobium strain Ben-RB (26) also respire sulfate. S. barnesi is the most ver­sitile, because it also respires selenate, nitrate, nitrite, fumarate, Fe(III), thiosulfate, elemental sulfur, dimethylsulfide, and trimethylamine oxide (31, 40). This meta­bolic diversity may be an important ecolog­ical factor, because sulfur, iron, and nitrate

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The putative arsenate reductase from \textit{S. barnesi} is also believed to be oriented in the periplasm, but it consists of a single subunit (48 kD) and has no metal associated with it (43). Enzymological and immunological analyses further indicate notable differences in the enzyme from \textit{S. barnesi} and related \textit{Sulfurospirillum} species (\textit{S. arsenophilum, S. deleyianum}). The ability to respire arsenate does not preclude the presence of a separate, arsenate-resistance system as well. Recently, \textit{Shewanella} strain ANA-3 was found to have both respiratory and detoxifying arsenate reductases (44).

The environmental impact of DARPs has only recently been realized (45–50). Their activity can be readily discerned using incubations of anoxic sediment slurries amended with millimolar (1 to 5) arsenate (46). Most-probable-number determinations of sediments from arsenate-contaminated lakes indicate resident populations of between $10^4$ and $10^5$ cells per gram (48, 51). The process of dissipatory As(V) reduction occurring in near-surface hyporheic zones greatly affects the transport and speciation of arsenic in freshwater streams (52). DARPs can also attack As(V) adsorbed to solid phases like ferricydrate and alumina (45) and reduce the As(V) contained in oxidized minerals like scorodite (24, 47). This latter point contrasts with findings from studies done with nonrespiratory arsenic-reducing bacteria that showed release of adsorbed As(V) as a result of iron reduction (53) or negligible release of As(V) and no dissolution of the mineral substrate (54).

Although considered negligible in most environments, the role of DARPs in the oxidation of autochthonous organic matter can be appreciable in specific cases. In situ measurements of arsenate respiration in Mono Lake, California (a particularly arsenic-rich environment; dissolved inorganic arsenic $= 200 \, \mu M$), made with the ratiometric $^{73}$As(V), revealed that as much as 14% of annual primary productivity was mineralized to CO$_2$ in the anoxic water column by the activity of DARPs (49). In the anoxic water column of Mono Lake, DARPs number between $10^7$ and $10^9$/ml. These numbers appear to be low, probably because the method requires that they achieve growth in the medium provided. Culture-independent polymerase chain reaction (PCR) techniques to enumerate DARPs have not yet emerged, in part because their diverse phylogeny negates the utility of commonly used 16S ribosomal DNA probes and because DARPs isolated thus far are opportunists capable of respiring electron acceptors other than arsenate.

Denatured gradient gel electrophoresis (DGGE) of DNA extracted from anoxic Mono Lake water incubated with 1 mM As(V) resolved bands suggesting that members of the \textit{e-} (Thiomicrospira) and \textit{δ}-Proteobacteria (\textit{Desulfovibrio}) might be contributing to arsenate respiration in these waters (50). In contrast, DGGE resolution of in situ DNA from bottom water indicated that the \textit{Bacillus} and \textit{Clostridia} genera were the dominant population (55). Because the arsenate-respiring \textit{Bacillus arsenoselenatis} and \textit{B. selenitireducens} species were originally isolated from Mono Lake’s bottom sediments (36), they may typify most of the DARPs present in the water column.

**Fig. 1.** Phylogenetic diversity of representative arsenic-metabolizing prokaryotes. Dissimilatory arsenate-respiring prokaryotes (DARPs) are indicated by yellow circles, heterotrophic arsenite oxidizers (HOAs) are indicated by green triangles, and chemoautotrophic arsenite oxidizers (CAOs) are indicated by red squares. In some cases (e.g., \textit{Thermus} sp. strain HR13), the microbe has been found able to both respire As(V) and oxidize As(III).

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**Chemical species:** S(–II), FeOOH, NO$_3^-$

**Gram-positive bacterium:** \textit{Bacillus selenitireducens}

**Initial investigations:** of the As(V) reductase from \textit{C. arsenatis} related metalloprotein superfamily of mononuclear molybdenum enzymes. Initial investigations of the As(V) reductase from the Gram-positive bacterium \textit{B. selenitireducens} revealed similar characteristics. N-terminal sequence data suggest that both subunits contain an iron-sulfur cluster, placing this protein in the di-iron superfamily of di-iron sulfoxide (DMSO) reductase family of sulfur cluster, placing this protein in the di-iron superfamily of di-iron sulfoxide (DMSO) reductase family.
Arsenite-Oxidizing Prokaryotes

The microbiological oxidation of As(III) to As(V) can also impact the mobility and speciation of arsenic in the environment. The process has been known for many years (56), and more than 30 strains representing at least nine genera have been reported to be involved, including α-, β-, and γ-Proteobacteria; Deinococcus (i.e., Thermus); and Crenarchaeota (Fig. 1). Physiologically diverse, they include both heterotrophic arsenite oxidizers (HAOs) and the more recently described chemolithoautotrophic arsenite oxidizers (CAOs). Heterotrophic oxidation of As(III) is viewed primarily as a detoxification reaction that converts As(III) encountered on the cell’s outer membrane into the less toxic form, As(IV), perhaps making it less likely to enter the cell. CAOs couple the oxidation of arsenite (e.g., electron donor) to the reduction of either oxygen or nitrate and use the energy derived to fix CO2 into organic cellular material and achieve growth. In HAOs the oxidation of As(III) is catalyzed by a periplasmic enzyme that is distinct from the dissimilatory arsenate reductase. This monomolecular molybdenum enzyme, belonging to the DMSO reductase family, is structurally similar to the periplasmic nitrate reductase (NapA) from Desulfovibrio desulfuricans (57). It is a heterodimer with a catalytic subunit (85 kD) that contains molybdenum bound to two pterin cofactors and a [3Fe-4S] cluster. The associated subunit (14 kD) presumably functions as an electron shuttle and has a Rieske-type [2Fe-2S] cluster, a feature that is unique among molybdenum enzymes (58). The arsenite oxides of CAOs, however, remain to be fully characterized.

Arsenate oxidation is being studied as the basis for bioremediation of systems where As(III) is a pollutant, because the As(V) can be immobilized onto strong adsorbents (59). Interest in this subject has resulted in the recent isolation of several novel species of both heterotrophic and autotrophic aerobic As(III) oxidizers from arsenic-rich environments (60, 61). Strain NT-26, a fast-growing CAO, is a member of the Rhizobium clade of the α-Proteobacteria and grows either by chemolithoautotrophic As(III) oxidation or as a conventional heterotroph by using organic compounds in lieu of As(III) (62). Gahringer and Banfield (38) isolated a curious thermophilic species of Thermus (strain HR 13) from an arsenic-rich hot spring. Under aerobic conditions it will oxidize As(III) for detoxification purposes without conserving the energy pro-

This nonphotosynthetic bacterium, strain MLHE-1, also grew as an autotroph with sulfide or hydrogen gas in lieu of As(III), and additionally grew as a heterotroph on acetate with oxygen or nitrate as the electron acceptor (65). Curiously, it was unable to grow on or oxidize As(III) under anaerobic conditions. The occurrence of anaerobic arsenite oxidation suggested that there might be a tight coupling between respiratory reduction of As(V) at the expense of electron donors like organic compounds and H2, and its resupply as carried out by microbial As(III) oxidation at the expense of commonly occurring strong oxidants like nitrate, nitrite, or perhaps Fe(III). Such a theoretical coupling is illustrated in Fig. 2 for a stratified system like Mono Lake, in which the abundance of arsenic in the lake is from natural hydrothermal inputs coupled with evaporative concentration. Mono Lake is an “extreme” environment in terms of its high pH (9.8), high salinity (90 g/liter), and high content of other toxic minerals. Recently, nitrate-linked microbial oxidation of arsenite was shown to occur in an arsenic-contaminated freshwater lake (66), and injection of nitrate into a subsurface aquifer resulted in the immobilization of arsenic (67). Thus, this phenomenon appears to be widespread in nature. It remains to be determined what types of microorganisms carry out this reaction in freshwater or marine systems, as compared with those found in soda lakes.

Environmental Impacts of Microbial Arsenic Transformations

The contribution made by microorganisms to the biogeochemistry of arsenic in the environment is extensive and detailed as it involves various oxidation, reduction, methylation, and demethylation reactions of its dominant chemical species. Unlike sulfur, where volatile organic species can play a crucial role in its biogeochemical cycle, it is apparent that natural organoarsenicals do not contribute substantially in this regard. However, from an ecological perspective, we can limit this scope to consider only the flow of energy linked to arsenic metabolism that translates into a capacity to do biological work (i.e., cell growth). We therefore consider the “ecology” of arsenic to be simple in the sense that it is predominantly confined to microbial transformations between its +3 and +5 oxidation states, constrained further by considering only those prokaryotes that conserve the energy associated with these redox reactions to achieve growth. Although energy-yielding biochemical reactions mediating the oxidation or reduction of the 0 or −3 oxidation states of arsenic may be possible, they have not been observed. Regardless of the simplicity of the cycle, understanding the role of microorganisms in the hydrologic mo-
in nature, we can begin to formulate a conceptual model for what might be occurring in the aquifers of Bangladesh. Perhaps the initial process is the oxidation of the original As(III)-containing minerals (e.g., arsenopyrite) during transport and sedimentation by pioneering CAOs and HAOs taking place over recent geologic time periods. This would result in the accumulation of As(V) onto surfaces of oxidized minerals like ferrihydrite. Subsequent human activity in the form of intensive irrigated agriculture, digging of wells, and lowering of groundwater tables would provide oxidants (e.g., oxygen, nitrate) that would further stimulate As(III) oxidation. This would cause a buildup of microbial biomass (and its associated organic matter) and the creation of anoxic conditions. This organic matter, in conjunction with other sources either from decomposing buried peat deposits or from that dissolved in seasonal recharge from agricultural surface waters, would in turn promote the dissipatory reduction of adsorbed As(V) by DARPs and the eventual dissolution of adsorbent minerals like ferrihydrite. The end result of these processes acting in concert over time and accelerated by human activities would be the release of arsenic into the aqueous phase, as illustrated in Fig. 3. Indeed, preliminary evidence suggests the presence of an anaerobic, microbial arsenic cycle in the subsurface aquifers of Bangladesh. Injection of nitrate into the aquifer promoted the rapid removal of As(III) (67), which indicates the presence of a community of microorganisms similar in physiology to MLHE-1. In addition, DARPs have been cultured from As-contaminated Bangladesh aquifer sediments (35).

Future Research Directions

Although there is an immediate research need for a fuller understanding of the role(s) of subsurface microbes in mobilizing arsenic in aquifers, on a more speculative level, it is tempting to contemplate a microbial "biome" supported by arsenic cycling. Indeed, it can be argued that because arsenic is a "chalcophile" (sulfur-loving) element, it should be more abundant in the Earth's interior than in its crust, and possibly more abundant on the surface of less differentiated, volcanically active planetary bodies like Mars and Europa (74). Provided that liquid water was present, and that there were also oxidants available that were stronger than As(V) to recycle As(III) (e.g., nitrate), Mars or Europa could conceivably have evolved primitive microbial ecosystems based in part upon use of arsenic as an energy source (64). Although such speculation on our part certainly borders on the fanciful, it also poses the more relevant question, how did prokaryotes on Earth evolve enzyme systems that are capable of exploiting the energy to be gained by reducing or oxidizing inorganic arsenic? Are these ancient systems dating back to the anoxic Archaean era of some 3.5 billion years past, when noxious substances were abundant on this planet's surface and the ability to exploit them for energy gain may have conferred some selective advantage? Conversely, are they more recent in origin and reflective of the need for an oxidizing atmosphere and strong oxidants to recycle As(III)? Does the wide phylogenetic distribution of DARPs among the prokaryotes (Fig. 1) indicate a long vertical evolution from one original gene, a convergent evolution of several independent genes, or merely a high degree of lateral gene transfer of a useful trait? Future research on the biochemistry of dissimilatory arsenate reductases and their analogous arsenite oxidases, and the genes that encode the proteins of the diverse (and growing) list of microorganisms, may ultimately reveal the answers.

References and Notes
The Biogeochemical Cycles of Trace Metals in the Oceans

F. M. M. Morel* and N. M. Price

Planktonic uptake of some essential metals results in extraordinarily low concentrations in surface seawater. To sequester or take up these micronutrients, various microorganisms apparently release strong complexing agents and catalyze redox reactions that modify the bioavailability of trace metals and promote their rapid cycling in the upper water column. In turn, the low availability of some metals controls the rate of photosynthesis in parts of the oceans and the transformation and uptake of major nutrients such as nitrogen. The extremely low concentrations of several essential metals are both the cause and the result of the ultraefficient uptake systems in the plankton and of widespread replacement of metals by one another for various biochemical functions.

The phytoplankton of the oceans are responsible for about half the photosynthetic fixation of carbon (primary production) on Earth (1). In contrast to most land plants, which grow relatively slowly and contribute only a small percentage of their biomass to the terrestrial food chain on any given day, marine phytoplankton divide every day or every week to keep up with zooplankton grazers. To do this, they must take up from seawater—along with carbon, nitrogen, phosphorus, and silicon (for diatoms)—a suite of essential micronutrients that are present at trace concentrations (<0.1 μM). To make matters worse, these organisms impoverish their own milieu because the elements they require for growth are continuously exported out of the sunlit surface as settling organic biomass. In comparison, terrestrial plants, which can acquire nutrients from soil and recycled litter, have a bountiful life. With regard to essential micronutrients, the ocean, particularly far from land, is the most extreme environment for life on Earth.

How does this system work? How do planktonic organisms acquire micronutrients and control their availability? To what extent does the low availability of these nutrients control the rate of enzymatic reactions, the productivity of the oceans, and the biogeochemical cycles of elements such as carbon and nitrogen? These are questions that oceanographers can now pose as testable hypotheses and can begin to answer.

Low Surface Concentrations of Essential Metals

A dozen or so elements with atomic mass above 50 are known to have a biological role, often as cofactors or part of cofactors in enzymes and as structural elements in proteins. Of those, the trace metals—Mn, Fe, Co, Ni, Cu, Zn, and Cd—have been best studied by oceanographers (2) and are the focus of our discussion. They are present in the plankton biomass at concentrations ranging from about 50 μmol/mol C (1000 μM) for Fe, which is used in a number

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