Arsenic is classified as a carcinogen by the U.S. Environmental Protection Agency (U.S. EPA) (Smith et al. 1992). Exposure to arsenic in drinking water is associated with increased risk of skin, kidney, lung, and bladder cancers. Chronic effects of arsenic in the water supply include skin hyperpigmentation and keratoses of the hands and feet that frequently progress to skin cancers. In about 10% of these cases, exposure is associated with a very high incidence of lung, bladder, and other cancers. According to the National Resources Defense Council (NRDC), millions of Americans are consuming tap water every day that poses unacceptable cancer risks (NRDC 2001). Over 56 million Americans in the 25 reporting states consumed water from systems containing arsenic at levels presenting a potentially fatal cancer risk. In 1976 the U.S. EPA set the maximum contamination level (MCL) for arsenic at 50 µg/L. The National Research Council, the operating arm of the U.S. National Academy of Sciences, has recommended an MCL of 10 µg/L (U.S. EPA 2001), which will be put in place in the near future.

Arsenic enters the water supply primarily from geochemical sources (USGS 1998a). In Michigan, arsenic leaches into water from the Marshall Sandstone and Coldwater Shale (Detroit Free Press 1999; USGS 1998b). In regions of Oakland County the concentration of arsenic in water supplies greatly exceeds the current U.S. EPA MCL of 50 µg/L (Detroit Free Press 1999). Based on geology and limited sampling, 20–30% of water supply wells in Michigan have concentrations exceeding 10 µg/L.

Although in general arsenic enters the biosphere by leaching from geologic formations, anthropomorphic sources include arsenical-containing fungicides, pesticides, and herbicides. For example, in Novi, Michigan, 95 acres of land proposed for development are contaminated with arsenic from insecticides dating from when the land was used for orchards. In Michigan, chronic effects of arsenic include neurological effects similar to those of multiple sclerosis (Detroit Free Press 1997). It is likely that over a period of decades some of these effects will progress to various cancers (Detroit Free Press 1999). Thus, study of the mechanisms of arsenic detoxification in humans and other organisms is of considerable importance.

**Convergent Evolution of Arsenate Reductases**

Pathways for arsenic detoxification exist in all organisms examined, including bacteria and yeast (Bhattacharjee et al. 1999) (Figure 1). An initial step is biotransformation of arsenate ([As(V)] to arsenite ([As(III)] (Figure 1). Although As(V) can be reduced to As(III) nonenzymatically, this process is too slow to be physiologically significant, and organisms use enzymes that catalyze As(V) reduction (Bhattacharjee et al. 1999). These thiol-linked reductases are required to confer resistance to As(V) in both prokaryotes (Gladysheva et al. 1994; Ji et al. 1994) and eukaryotes

Figure 1. Pathways of arsenic detoxification in prokaryotes and eukaryotes. *E. coli*, a typical prokaryote, accumulates As(V) by phosphate transport systems such as the Pst ABC ATPase. As(V) is reduced to As(III) by ArsC, a Grx–GSH-linked enzyme. The resulting As(III) is pumped out of the cells by the ArsA/ArsB ATPase. The eukaryote *S. cerevisiae* uses a similar cycle of As(V) uptake, reduction, and extrusion from the cytosol, but the proteins that catalyze these reactions are the products of independent evolution from the bacteria. Phosphate transporters such as Pho84p accumulate As(V). Acr2p, a Grx–GSH-linked enzyme, reduces As(V) to As(III). As(III) can be extruded from the cells by the Acr3p carrier. Alternatively, As(III) can be conjugated with GSH to form As(GS)3. The Ycf1p ABC ATPase removes As(III) from the cytosol by pumping As(GS)3 into the vacuole.

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These enzymes arose independently at least three times by convergent evolution (Figure 2). One family includes the *Staphylococcus aureus* plasmid pI258-encoded *arsC* gene product (Ji et al. 1994). This ArsC enzyme is homologous to low molecular weight protein phosphotyrosine phosphatases (lmwPTPases) (Bennett et al. 2001). A second family includes the *Escherichia coli* plasmid R773 ArsC arsenate reductase (Rosen 1999). The third family includes the only known euukaryotic arsenate reductase, Acr2p from *Saccharomyces cerevisiae* (Bobrowicz et al. 1997; Mukhopadhyay and Rosen 1998). These enzymes are involved in As(V) detoxification have apparently evolved independently at least three times. One family is typified by the *E. coli* plasmid R773 ArsC that uses Grx and GSH as reductants. A second family represented by the *S. aureus* plasmid pI258 ArsC uses thioredoxin (Trx) as a reductant and is related to a family of lmwPTPases. Acr2p is the only euukaryotic arsenate reductase so far identified. It belongs to the superfamily of PTases that includes the Cdc25a cell-cycle phosphatase.
We have recently reported the X-ray crystal structures of ArsC at 1.65 Å (Martin et al. 2001). The active site can be seen to consist of the catalytic residue Cys12 and several arginine residues, including Arg60 and Arg94 (Figure 4). In addition to the native structure, structures of ArsC in complex with As(V) or As(III) were obtained. One structure is that of the covalent Cys12–S–As(V) adduct, which has a tetrahedral geometry with a sulfur-arsenic distance of 2.18 Å. The other structure is that of the Cys–S–As(OH)–SG complex. An ArsC reaction scheme with the As(V) and As(III) diolate could be a novel thiarsahydroxy crystallographic data suggest that the intermediate is shown in Figure 3.

ArsC: A Prokaryotic Arsenate Reductase

The R773 ArsC purified from E. coli requires reduced glutathione (GSH) and the small thiol transfer protein glutaredoxin (Grx) for arsenate reductase activity (Gladysheva et al. 1994). ArsC has a single catalytic cysteine residue, Cys12 (Liu et al. 1995), that forms a covalent thiolate–As(V) intermediate (Martin et al. 2001). Grx is required to reduce the enzyme-bound ES–As(V) intermediate to an ES–As(III) intermediate (Gladysheva et al. 1994; Shi et al. 1999). Members of the Grx family have two ways in which they can participate in thiol reduction reactions. First, they can catalyze reduction of intramolecular disulfide bonds (Bushweller et al. 1992). Because ArsC has only a single required cysteine residue, this seemed an unlikely mode of action of Grx. Second, Grx can participate in reduction of mixed disulfides between protein thiols and glutathione. The two modes can be differentiated by the use of appropriate Grx mutants. There are three glutaredoxins in E. coli, Grx1, Grx2, and Grx3 (Åslund et al. 1994), each of which can function in ArsC catalysis (Shi et al. 1999). Each has the consensus sequence Cys-Pro-Tyr-Cys. Mutants lacking the N-terminal cysteine are unable to participate in either mode of reduction. In contrast the C-terminal cysteine mutants function in reduction of mixed protein-glutathione disulfides but not reduction of intramolecular protein disulfide bonds (Bushweller et al. 1992). N-terminal cysteine mutants of Grx could not participate in ArsC-catalyzed As(V) reduction (Shi et al. 1999). On the other hand, Grx mutants lacking the C-terminal cysteine still supported ArsC activity. These results demonstrate that ArsC does not form an intramolecular disulfide during the catalytic cycle and are consistent with the existence of the mixed disulfide intermediate ArsC–S–SG. However, as described below, crystallographic data suggest that the intermediate could be a novel thioarsahydroxy ArsC–S–As(OH)–SG complex. An ArsC reaction scheme with the As(V) and As(III) intermediates is shown in Figure 3.

Acr2p: A Eukaryotic Arsenate Reductase

Although it is likely that enzymes that catalyze As(V) reduction are ubiquitous, the only eukaryotic arsenate reductase identified is the 130-residue Acr2p protein from S. cerevisiae (Bobrowicz et al. 1997; Mukhopadhyay and Rosen 1998). The ACR2 gene is required for As(V) resistance (Bobrowicz et al.; Mukhopadhyay and Rosen 1998). Acr2p is apparently unrelated to bacterial arsenate reductases. On the other hand, it is a member of the superfamily of PTPases such as the human cell-cycle dual-specific phosphatase Cdc25A (Gladysheva et al. 1994; Martin et al. 1998; DeMel et al. 1999; Fauman et al. 1998). The consensus Cys(X)5R motif of A2p is likely to be part of the active site as well: either Cys76 or Arg82 mutations result in loss of As(V) resistance in vivo and As(V) reduction in vitro (Mukhopadhyay and Rosen 2001). These results suggest that Cys76 is the equivalent of Cys12 in ArsC and may form As(V) and As(III) intermediates. In support of this hypothesis, in vitro Acr2p exhibits the same Grx and GSH requirement as ArsC. All Grx proteins examined supported Acr2p activity, including a S. cerevisiae Grx and all three E. coli Grx proteins. Just as in the case of ArsC, the Grx C-terminal cysteine mutants functioned in As(V) reduction, indicating that Acr2p could have a catalytic cycle with an Acr2p–S–As(III)–SG intermediate (Mukhopadhyay et al. 2000).

Where are the mammalian arsenate reductases? Reductase activity has recently been reported in human liver (Radabaugh and Apoisan 2000), but it is not known whether the enzyme that catalyzes this activity is related to Acr2p or either of the bacterial enzymes. The S. aureus plasmid p258 ArsC and the yeast Acr2p enzymes are both homologues of protein phosphotyrosine phosphatases (although to different families of phosphatases) (Bennett et al. 2001; Fauman et al. 1998). Acr2p is also homologous to rhodanases, which are thiosulfate sulfutransferases (Hofmann et al. 1998). We suggest that all arsenate reductases, whether eukaryotic or prokaryotic, share a common evolutionary lineage with phosphatases. Analysis of the phosphatase-like proteins identified in eukaryotic genomes may reveal the identity of mammalian arsenate reductase.
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