JOURNAL OF BACTERIOLOGY, Oct. 1999, p. 5891–5897 Vol. 181, No. 19
0021-9193/99/$04.00+0

MINIREVIEW

Families of Soft-Metal-Ion-Transporting ATPases

CHRISTOPHER RENSING, MALLIKA GHOSH, AND BARRY P. ROSEN*

Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, Michigan 48201

Life undoubtedly first arose in waters rich in metal ions. Since most metal ions are toxic in excess, it is reasonable to speculate that metal resistances probably evolved early, predating antibiotic resistances. This minireview focuses on transport ATPases that produce resistance to ions of the metals zinc, cadmium, lead, copper, silver, arsenic, and antimony in prokaryotes. Other aspects of transport and resistance to those metal ions have been reviewed recently (44, 45, 53, 55, 62) and will not be discussed here.

Soft Lewis acids such as the transition metals Cu(I), Ag(I), Zn(II), and Cd(II) and the heavy metal Pb(II) have high polarizing power (a large ratio of ionic charge to the radius of the ion) and typically form strong bonds with soft Lewis bases (30). These include sulfur and nitrogen ligands such as cysteine and histidine residues in proteins. In contrast are the hard Lewis acids of groups 1a and 2a such as Na^+ and Ca^{2+}, which form ionic bonds with hard Lewis bases, for example the oxygen of protein carboxylates. The metalloids As(III) and Sb(III) of group 5a have properties similar to those of soft Lewis acids. For the purposes of this review, alkali and alkaline earth cations are termed hard metals, and transition metals, heavy metals, and metalloids are termed soft metals.

Genes for resistance to inorganic salts of soft metals are found both on plasmids and in chromosomes (44, 53, 55). The physiological role of plasmid-encoded determinants is generally to confer resistance, while the role of chromosomally encoded systems may also include metal ion homeostasis. Copper and zinc ions are required for growth; there must be a fine balance between uptake and efflux to provide metal ion homeostasis. In the case of soft metals that are only toxic, specific uptake systems may not exist. Rather, those ions may be accumulated via other existing transport systems.

ARSENIC AND ANTIMONY

The oxyanions of arsenic and antimony are quite toxic, especially the reduced (trivalent) forms, and enter cells via transporters for other compounds. In *Escherichia coli* and other bacteria, arsenate [As(V)] is taken up by phosphate transport systems such as the ATP-coupled Pst pump (52), a member of the ABC superfamily of transport ATPases (Fig. 1). One route of entry of Sb(III) [and probably As(III)] is via the GlpF polyol transporter (48). Once inside cells, the toxicity of the trivalent metalloids is a double-edged sword. On the one hand, this property made them useful in the development of antimicrobial agents, exemplified by Paul Ehrlich's silver bullet, the organoarsenical Salvarsan. On the other hand, arsenical and antimonial drugs are so toxic to humans that their use was difficult to manage. Paul Ehrlich is not only the father of modern antimicrobial chemotherapy but was also one of the first to comprehend that organisms could become resistant following continued drug use. We now recognize that resistance to arsenicals and antimonials is widespread, occurring in bacteria, fungi, protozoans, and animal cells (44). Bacterial operons encoding resistance to arsenicals and antimonials (*ars* operons) can be found on transmissible plasmids and in chromosomes. These operons usually have either three (*arsRBC*) or five (*arsRDABC*) genes. ArsR is an As(III)/Sb(III)-responsive transcriptional repressor (25, 47, 49). ArsB is a secondary transporter that catalyzes efflux of the arsenite anion from cells (14) (Fig. 1). ArsC is a reductase that catalyzes electron transfer from reduced glutathione and glutaredoxin (in *E. coli*) (18) or thioredoxin (in *Staphylococcus aureus*) (23, 24) to arsenate, reducing it to arsenite, the substrate of the efflux system. Thus, this enzyme extends the spectrum of resistance to include both oxidized and reduced forms of arsenic. While all *ars* operons have those three genes, some operons have two additional genes, *arsDA*. ArsD is a repressor that works independently of ArsR to provide fine tuning of operon expression (9). ArsA is an As(III)/Sb(III)-stimulated ATPase (46) that associates with ArsB to form an extrusion pump that is more efficient than ArsB alone (14) (Fig. 1).

The best-studied ArsAB pump is that encoded by the *ars* operon of *E. coli* plasmid R773 (7). The ArsA subunit has two repeats, A1 and A2, that are clearly the result of an ancestral gene duplication and fusion. Each half contains a consensus nucleotide binding domain (NBD) for ATP. When purified in the absence of ArsB, ArsA is soluble and exhibits an ATPase activity that is allosterically activated by binding of Sb(III) or As(III). Both NBDs are required for enzymatic activity, and it is likely that the two sites must interact to produce a catalytically competent protein (29).

While many enzymes with vicinal cysteines are inhibited by As(III) and Sb(III), ArsA is allosterically activated by those soft metals and is the only known enzyme that requires metalloids for activity (Fig. 2A). The metal binding site is composed of a triad of three cysteine residues, Cys113, Cys172 and Cys422, which form a novel three-coordinate As-S structure (3, 4). This allosteric control ensures that the enzyme will not hydrolyze ATP when the pump is not required. It is unlikely that the As(III) bound at the allosteric site is transported. More likely, the membrane component, ArsB, has a separate binding site for the arsenite oxyanion. Thus, the pump need only be activated by a binding of a single metal ion to allow multiple rounds of transport through the pump.

Recently, ArsA homologues have been identified in eubacteria, archaea, fungi, plants, and animals (44). At this time the physiological functions of these proteins are largely unknown. In addition to the NBDs, there is a highly conserved region

* Corresponding author. Mailing address: Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, MI 48201. Phone: (313) 577-1512. Fax: (313) 577-2765. E-mail: brosen@med.wayne.edu.

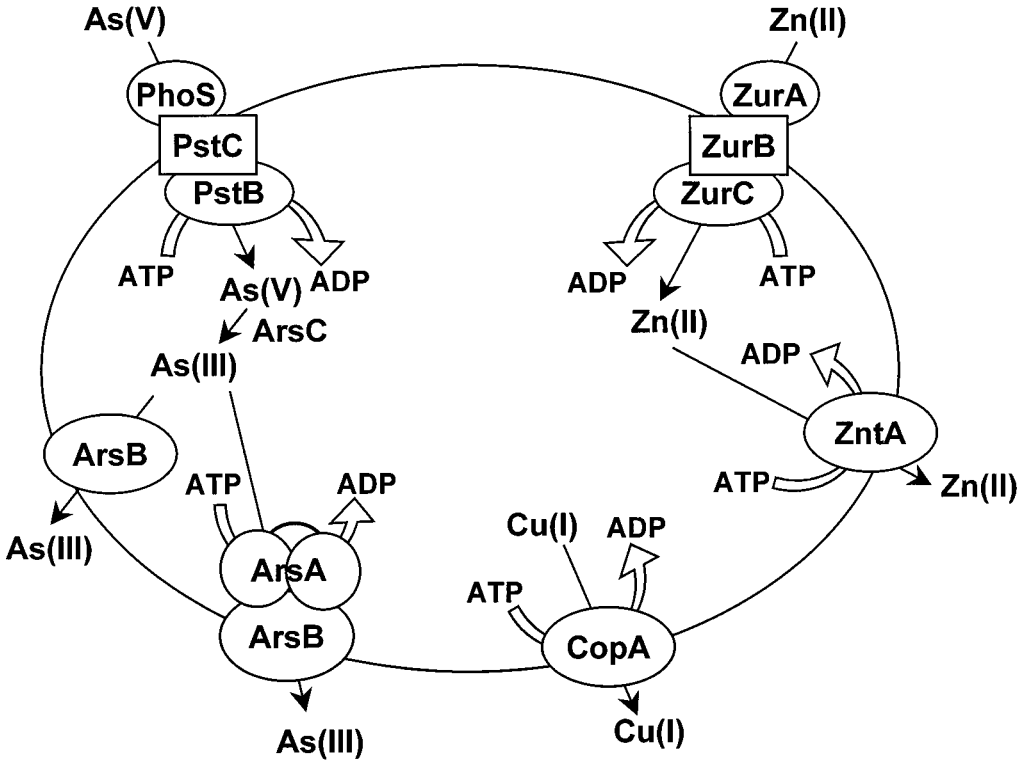

FIG. 1. Soft metal transport ATPases. In *E. coli* there are primary pumps for the uptake and efflux of transition metals, heavy metals, and metalloids. (Left) Arsenate is accumulated by phosphate transporters such the Pst pump (51), a member of the ABC superfamily of transport ATPases. Arsenate is reduced to arsenite via the ArsC arsenate reductase. Arsenite is then extruded from cells by the Ars extrusion system (44). In cells expressing the chromosomal *arsRBC* operon, ArsB is a secondary arsenite carrier protein coupled to the membrane potential that confers low-level resistance to metalloid salts. In contrast, in cells expressing a plasmid-encoded *arsRDABC* operon, the ArsAB complex is an As(III)/Sb(III)-translocating ATPase that confers high level resistance. (Middle) The CopA P-type ATPase confers resistance to copper ion and probably transports the monovalent cation. (Right) ZnuABC is an ABC transport ATPase that catalyzes uptake of Zn(II) (37), and ZntA is a Zn(II)-translocating P-type ATPase (2, 42). It is hypothesized that these two pumps are a homeostatic mechanism that controls the intracellular concentration of Zn(II). In addition, ZntA confers resistance to Pb(II), Cd(II), and excess Zn(II).

termed the DTAP motif (63). In the R773 ArsA the role of the DTAP domain in the A1 half was examined by using the effects of substrates and effectors on intrinsic tryptophan fluorescence. By site-directed mutagenesis two single-tryptophan ArsAs were constructed. One encoded a protein with a single tryptophan residue located at position 141, the N-terminal side of the DTAP domain. The second engineered gene coded for an ArsA with a single tryptophan residue at position 159, the C-terminal side of the DTAP sequence. These two tryptophans served as intrinsic probes of the environment of the DTAP domain. During ATP hydrolysis this domain undergoes significant conformational changes that may be involved in coupling the allosteric site to the catalytic site in the R773 ArsA. Binding of the product, MgADP, but not the substrate, MgATP, produced a decrease and red shift in Trp141 fluorescence, indicative of movement of the N-terminal end of the A1 DTAP domain from a relatively hydrophobic environment into a more hydrophilic one. In contrast, MgATP resulted in a large increase and blue shift in Trp159 fluorescence, which indicates that the C-terminal end of the A1 DTAP domain had moved into a relatively more hydrophobic environment. However, no change in Trp159 fluorescence was observed with MgADP, with nonhydrolyzable ATP analogs, or with MgATP in noncatalytic ArsA enzymes. Thus the N-terminal end of the domain moves in response to ATP hydrolysis, not binding, and the C-terminal end moves as product is formed.

Recently, changes in the intrinsic fluorescence of Trp159 have been used to elucidate a minimum reaction scheme for ArsA catalysis (60) (Fig. 2B). From the results of stopped-flow fluorescence measurements the rates of substrate binding and product release could be determined. In addition, a number of fluorescence changes were observed that suggested that the enzyme underwent several conformational changes during the reaction cycle. Of importance was the fact that a steady-state conformation ($ArsA^7$) builds up following hydrolysis and release of products. The slowest (and hence rate-limiting) step in the overall reaction appears to be the decay of $ArsA^7$ back to the initial state ($ArsA^1$). Although not unique to ArsA, such conformational isomerizations are rarely the rate-limiting step in catalysis. Binding of As(III) or Sb(III) increases the rate of decay of $ArsA^7$; in other words, the acceleration of catalysis produced by the allosteric effector can be accounted for by an increase in the rate of isomerization between two different ArsA conformations. Together, the kinetic, genetic, and biochemical data suggest a model in which ATP hydrolysis at either the A1 or A2 catalytic site in ArsA is slow and limited by a conformational change in each half. Binding of As(III) or Sb(III) to the allosteric site, which is composed of Cys113 and Cys172 in A1 and Cys422 in A2, produces physical movement of the two halves toward each other. At the newly formed interface of A1 and A2, the slow isomerization of the two conformations speeds up, accelerating catalysis (Fig. 2B).

Yet to be answered is the question of how the energy of ATP hydrolysis is coupled to transport through ArsB. As a complex of ArsA and ArsB, the pump can use only chemical energy as an energy source (13). In intact cells electrochemical energy was neither necessary nor sufficient for $^{73}AsO_2^{-1}$ transport. Only ATP could support transport in everted (inside-out)

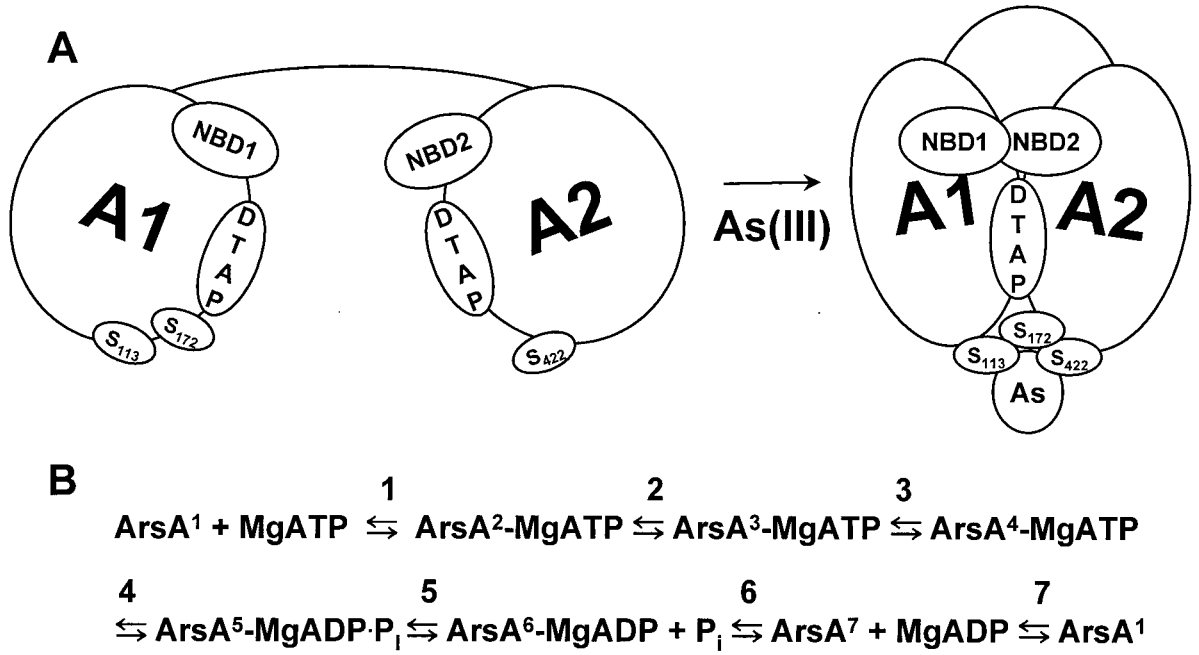

FIG. 2. The ArsA ATPase. (A) Model of ArsA catalysis. In the absence of an allosteric effector, the A1 and A2 halves of ArsA are connected only by a 25-residue flexible linker and have a low basal rate of ATP hydrolysis (46). Binding of either Sb(III) or As(III) to Cys113, Cys172, and Cys422 is proposed to bring together A1 and A2, forming an interface of the two NBDs, promoting hydrolysis of ATP (3, 4). (B) Reaction mechanism of the ArsA ATPase. A minimal multistep kinetic scheme for the reaction catalyzed by ArsA has been proposed (60). In this scheme, ArsA undergoes several conformational changes during binding of substrate (MgATP) and release of products (MgADP and P_i). In the steady state, the $ArsA^7$ conformation of the enzyme builds up, with a slow isomerization back to the $ArsA^1$ form. Since binding of substrate and release of products are all faster than this isomerization, it becomes the rate-limiting step in the reaction in the absence of activator. Binding of As(III) or Sb(III) at the allosteric site increases the rate of $ArsA^7$ conversion to $ArsA^1$, accelerating catalysis.

membrane vesicles. Again, transport in vitro could not be driven by respiratory substrates, and transport was insensitive to uncouplers. Thus, both in vitro and in vivo, electrochemical energy was neither necessary nor sufficient.

As mentioned above, *ars* operons come in two flavors: those with three genes, *arsRBC*, and those with five genes, *arsRD ABC*. How do cells expressing a three-gene operon extrude arsenite without ArsA, the catalytic subunit of the pump? Without ArsA, ArsB can function as a secondary carrier, perhaps as an electrophoretic uniporter coupled to the membrane potential, not ATP (27). The *arsRBC* operons confer lower resistance to metalloids than the five-gene operons, which is consistent with the fact that secondary transporters cannot form concentration gradients of the same magnitude as can primary pumps. It is likely that the form of arsenic recognized by the pump is the arsenite oxyanion. First, there is only a single cysteine residue in ArsB that is not required for activity, so soft metal-thiol chemistry cannot be involved (8). Second, since the membrane potential is the driving force, the most parsimonious interpretation of the data is that an anion is transported from the negative to the positive side of the membrane, that is, out of the cell. This capacity of ArsB to function in a dual mode as a secondary carrier or a subunit of a primary pump is novel. We postulate that ArsB arose first as a low-level mechanism of resistance toward metalloids; under environmental pressure to adapt to higher levels the pump evolved by association with an ATPase ancestor of ArsA (44).

SOFT-METAL-TRANSPORTING P-TYPE ATPases

The superfamily of P-type ATPases is a ubiquitous group of proteins involved in transport of charged substrates across biological membranes (1). The term "P-type" refers to the formation of a phosphoenzyme intermediate in the reaction cycle. P-type ATPases share common features including a conserved aspartate residue that is phosphorylated during the reaction cycle, a conserved proline residue in a membrane domain that probably forms a portion of the ion translocation pathway, and consensus domains for ATP binding and energy transduction. The superfamily of P-type ATPases has five major branches that are distinguished by substrate specificity rather than the relatedness of the organisms in which they are found (1).

One branch contains the phylogenetically related subgroup of P-type ATPases that catalyze transport of transition or heavy-metal ions. They have previously been described as CPx-type ATPases (55) or soft-metal-transporting P-type ATPases (42). These ATPases have eight transmembrane domains (34), in contrast to the hard-metal P-type ATPases, which have 10 transmembrane segments. Other unique features of soft-metal ATPases include a metal binding domain in the cytosolic N-terminal region, a highly conserved CPC motif in the sixth transmembrane domain, the putative translocation domain, and a conserved histidine-proline sequence 34 to 43 residues C terminal to the CPC motif (55).

Soft-metal-transporting P-type ATPases can be further divided into subgroups that contain Cu(I)/Ag(I)-translocating ATPases and Zn(II)/Cd(II)/Pb(II)-translocating ATPases (Fig. 3). The first branch harbors representative members such as CopA from *Enterococcus hirae*, *Helicobacter pylori*, and *E. coli*. The second branch includes ZntA from *E. coli* and CadA from *S. aureus* plasmid pI258. As discussed below, members of the first branch most likely transport monovalent soft-metal cations, while members of the second branch are divalent soft-metal-cation pumps. This is similar to the two branches of the hard-metal-ion P-type ATPases, where members of one branch pump monovalent alkali cations such as H^+, K^+, and Na^+ and

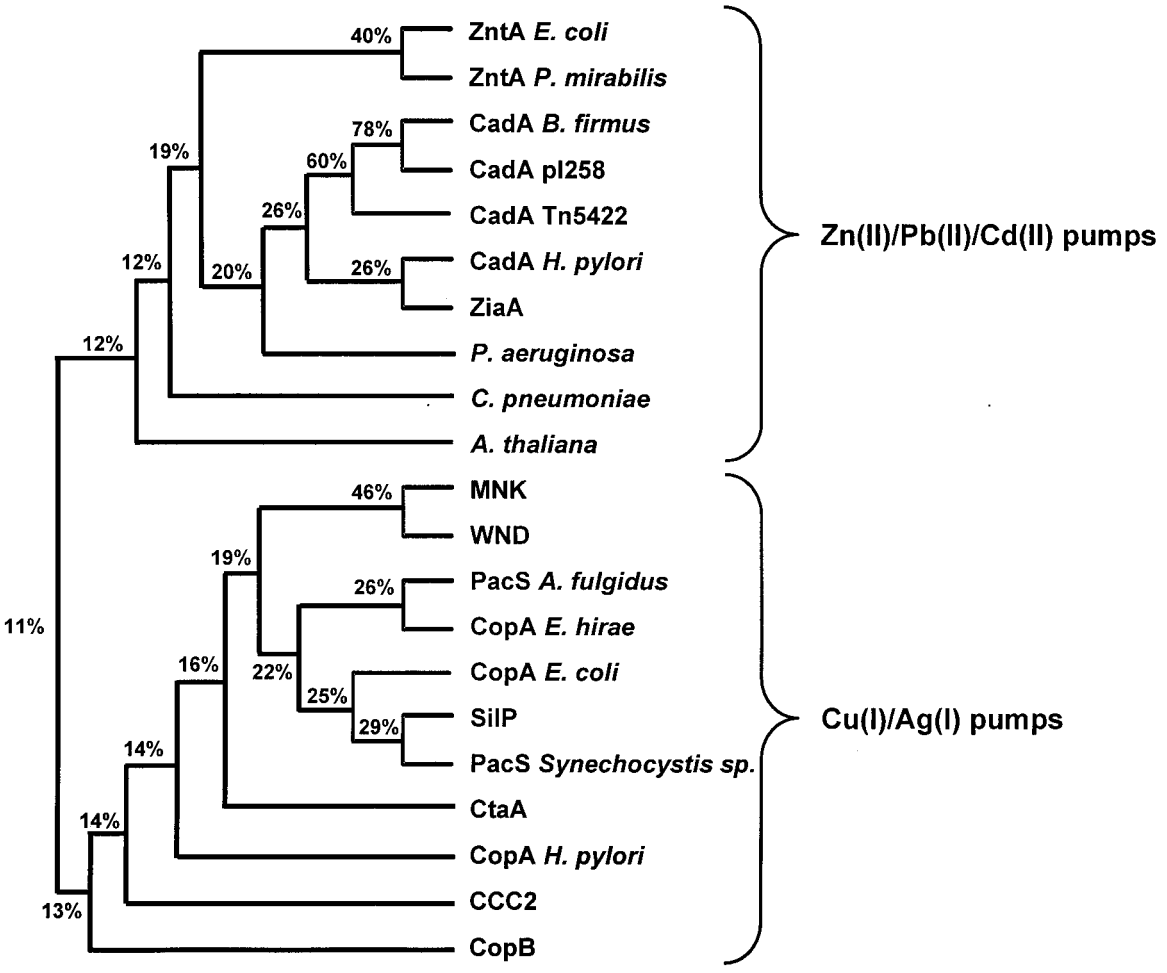

FIG. 3. The family of soft-metal-ion-translocating P-type ATPases. This family is growing rapidly, so only representative ones are shown. GenBank accession numbers are given in parentheses. The dendograms were made using the CLUSTAL4 algorithm (22) with DNASIS software from Hitachi Software Engineering Co., Ltd. The calculated matching percentages are indicated at each branch point. Zn(II)/Pb(II)/Cd(II) pumps: ZntA, *E. coli* (P37617); ZntA, *P. mirabilis* (CAA04762); CadA, *Bacillus firmus* (AAA22858); CadA, pI258 (AAB59154); CadA, Tn*5422* (L28104); CadA, *H. pylori* (AAA93043); ZiaA, *Synechocystis* sp. strain PCC 6803 (Q59998); *P. aeruginosa* reading frame (unfinished genome); *Chlamydia pneumoniae* reading frame (AAD19006); *A. thaliana* reading frame (CAB16773). Cu(I)/Ag(I) pumps: MNK, human Menkes disease-related protein (Q04656); WND, human Wilson disease-related protein (U03464); PacS, *Archaeoglobus fulgidus* (AE0010711); CopA, *E. hirae* copper uptake pump (P32113); CopA, *E. coli* (AAB02268); SilP, *S. typhimurium* Ag(I) pump (AAD11750); PacS, *Synechocystis* sp. strain PCC 6803 (P73241); CtaA, *Synechococcus* sp. strain PCC 7942 (P37385); CopA, *H. pylori* (AAB05475); CCC2, *S. cerevisiae* vacuolar copper pump (L363171); CopB *E. hirae* copper efflux pump (P05425).

members of the other branch transport divalent alkaline earth cations such as Ca^{2+} and Mg^{2+}.

COPPER AND SILVER

Copper is an essential metal ion for most organisms, required for enzymes such as cytochrome oxidase and superoxide dismutase. However, cells need to keep the intracellular concentration of free copper ion low, since the free metal ion can facilitate oxidative damage through generation of free radicals. In contrast to copper, silver is not required for growth and is only detrimental to cells. In bacteria, energy-dependent copper uptake by a specific copper transporter has not been directly demonstrated.

The best-studied copper transport systems are the copper ATPases of *E. hirae*, CopA and CopB (36). CopB is a Cu(I)/Ag(I) efflux pump, while CopA is thought to be involved in copper uptake. Cells with a disruption of the *copA* gene are copper requiring and more silver resistant than the wild type, while disruption of *copB* rendered the cells hypersensitive to copper salts. Structurally, CopB differs from CopA and its homologues primarily in the N-terminal metal binding domain. CopA and homologues have one or more N-terminal CXXC metal binding motifs. In contrast, CopB has a histidine-rich repeat at the N terminus. In vitro, CopB has been shown to catalyze accumulation of either ^{110}Ag or ^{64}Cu in membrane vesicles prepared from *E. hirae* (54). These vesicles are presumably everted (inside-out), so uptake corresponds to extrusion from intact cells.

A close homologue of CopA is CtaA from *Synechococcus* strain 7942; disruption of the *ctaA* gene rendered the cells more resistant to copper (38). Other CopA homologues include PacS from *Synechococcus* strain 7942 and CopA from *H. pylori*. Disruption of *pacS* rendered the cells more sensitive to copper and silver salts. PacS is apparently localized in the thylakoid membrane and is specifically induced by copper and silver (26). Disruption of *H. pylori copA* also resulted in copper sensitivity (33). Another CopA homologue, SilP, was isolated from a plasmid of a pathogenic *Salmonella* strain (20). SilP, which has a unique histidine-rich N terminus, is part of a silver

resistance determinant and has been suggested to be a specific Ag(I)-translocating ATPase.

E. coli has only two genes for soft-metal P-type ATPases, one of which is *zntA* (2, 42), which encodes a Zn(II)/Cd(II)/Pb(II)-translocating ATPase and is described in more detail below. The other is a CopA homologue, *f834* (GenBank accession no. AE0001541) (Fig. 3). Disruption of *E. coli copA* renders the cells more sensitive to copper ions, indicating that it is a copper-translocating ATPase that probably catalyzes copper extrusion (42a) (Fig. 1), as opposed to the homologous *E. hirae* CopA, which is thought to transport copper into the cytosol. Consistent with its role as a copper efflux pump, the copper-sensitive phenotype of a *copA*-disrupted *E. coli* strain could be complemented by *copB* from *E. hirae* (42a).

Eukaryotes possess similar copper ATPases, examples of which are CCC2 in *Saccharomyces cerevisiae* (16) and ATP7A (59) and ATP7B (6) in humans. These proteins have been shown to be involved in intracellular copper trafficking in all organisms examined, and putative homologues have been found in most sequenced genomes but are notably absent from some of the smaller ones such as the *Mycoplasma genitalium* genome.

What is the ionic species transported by copper-translocating ATPases? Although it is difficult to verify physiologically, the substrate of copper efflux P-type ATPases is probably Cu(I). Cells are usually presented with Cu(II) because Cu(I) rapidly oxidizes in air. However, the cytosol of cells is highly reducing, with high concentrations of reduced glutathione or other thiols, indicating that copper should be in the form of Cu(I) intracellularly. The results of in vitro copper or silver transport experiments indicate that the transported species is likely Cu(I) or Ag(I). Both ATP7A and *E. hirae* CopB were shown to transport copper only in the presence of dithiothreitol, consistent with transport of Cu(I) (58). Additionally, Lutsenko and coworkers cloned and expressed the N-terminal cysteine-rich sequences from ATP7A and ATP7B and demonstrated that the peptides bound 5 to 6 nmol of copper per nmol of protein (32). The copper released from polypeptides reacted with bicinchoninic acid, which reacts with Cu(I) but not Cu(II).

By multidimensional nuclear magnetic resonance the solution structures of a 72-residue polypeptide corresponding to the fourth metal binding domain of ATP7A was recently determined (17). In the absence of metal, the region containing the putative metal binding sequence GMTCXXC was disordered. Binding of Ag(I) induced order to that domain. Ag(I) was bound in a linear bicoordinate manner to the two cysteine residues, again suggesting that both Cu(I) and Ag(I) may be substrates of copper pumps. On the other hand, the N-terminal domain from ATP7B was expressed and purified as a glutathione *S*-transferase chimera (15). The chimeric protein bound to metal chelate columns charged with various transition metals. The order of binding affinity was Cu(II) >> Zn(II) > Ni(II) > Co(II), with no binding to Fe(II) or Fe(III). Cu(I) binding was also detected by neutron activation. Thus, it is possible that these proteins recognize both oxidized and reduced forms of copper ion. In contrast to the copper efflux ATPases, copper uptake systems such as *E. hirae* CopA would be exposed primarily to extracellular Cu(II), which is probably reduced extracellularly prior to transport (61).

Since free copper produces oxidative damage, eukaryotes have evolved intracellular chaperones that reduce free intracellular copper to undetectable concentrations (12, 19, 40). These proteins bind copper tightly and deliver the ion to specific target enzymes. Three eukaryotic copper chaperones were identified in *S. cerevisiae*, but functional homologues have since been identified in many eukaryotic species, including humans. In yeast, Lys7p delivers copper to cytosolic superoxide dismutase 1 (Sod1p) (11); Cox17p directs copper to the mitochondria for activation of cytochrome oxidase (19); and Atx1p specifically carries copper to the secretory pathway for incorporation into surface or extracellular copper enzymes (39). Although homologues of these copper chaperones have not been identified in prokaryotes, they likely possess other metal chaperones. For example, CopZ of *E. hirae* appears to function as a copper chaperone, delivering Cu(I) to a copper-responsive repressor, CopY, that controls *cop* expression (10). CopZ is homologous to MerP, a periplasmic mercury binding protein that is probably an Hg(II) chaperone for the MerT transporter (21). A common motif in all of these chaperones is a CXXC motif reminiscent of the N-terminal repeat of soft-metal-transporting P-type ATPases.

ZINC, CADMIUM, AND LEAD

Zinc is essential for all organisms, in part because it plays a critical role in the catalytic activity and/or structural stability of many enzymes. As an essential but potentially toxic ion, zinc homeostasis is crucial for all organisms. In prokaryotes homeostasis is maintained by regulating the uptake and the efflux of zinc. In *E. coli* zinc deficiency induces expression of a specific zinc uptake system, ZnuACB, which is an ABC transporter for zinc uptake (37) (Fig. 1). ZnuA is a periplasmic binding protein. ZnuB is the membrane sector or the pump, and ZnuC is the ATPase catalytic subunit. Genes encoding a ZnuABC homologue have also been identified in *Haemophilus influenzae* (31). Under conditions of zinc sufficiency, expression of the pump is repressed by the Fur homologue Zur, which presumably binds to the bidirectional promoter region of *znuA* and *znuCB*. In addition to repressing zinc uptake, growth of *E. coli* in high concentrations of zinc results in zinc efflux by induction of ZntA, a zinc-transporting P-type ATPase (2, 42) (Fig. 1). ZntA is activated by ZntR, a MerR homologue that is an activator of *zntA* (5). Disruption of *zntA* results in sensitivity to Zn(II), Cd(II), and Pb(II). The strain did not exhibit increased sensitivity to any other metals ions, including copper and silver, suggesting that this P-type ATPase is a specific Zn(II)/Cd(II)/Pb(II) pump. ZntA has been shown to catalyze ATP-coupled accumulation of ^{65}Zn(II) and ^{109}Cd(II) in everted (inside-out) membrane vesicles of *E. coli*, where accumulation in everted membrane vesicles is equivalent to efflux from intact cells (42). Vesicles prepared from the *zntA*-disrupted strain did not accumulate ^{65}Zn(II), and the transport defect could be complemented by expression of *zntA* on a plasmid. The unavailability of a Pb(II) radioisotope prevents direct demonstration that ZntA transports Pb(II). However, ATP-dependent Zn(II) transport by ZntA was inhibited to the same degree by either Pb(II) or Cd(II), and the latter is a demonstrated pump substrate (43).

ZntA homologues are widespread in gram-negative bacteria, including *Salmonella typhimurium*, *Vibrio cholerae*, *Yersinia pestis*, *Deinococcus radiodurans*, and *Proteus mirabilis*. A mutation in the *P. mirabilis* gene produces a swarming defect (28). The relationship between zinc and swarming is unclear but suggests a possible role of Zn(II) in pathogenesis. The *E. coli* and *P. mirabilis* ZntA proteins exhibit 61% identity and 69% overall similarity. The *zntA* gene from *P. mirabilis* complemented the Zn(II)/Cd(II)-sensitive phenotype of the *E. coli zntA*-disrupted strain. Everted membrane vesicles from the complemented strain catalyzed ATP-dependent ^{65}Zn(II) transport (41).

Another homologue of ZntA is CadA from *S. aureus* plasmid pI258 (35). The *cad* operon of pI258 has two genes, *cadC*, which encodes a transcriptional repressor, and *cadA*, which

encodes CadA, a P-type ATPase that is about 30% identical to ZntA. CadA has been shown to catalyze ATP-dependent $^{109}Cd(II)$ uptake in everted membrane vesicles from *B. subtilis* (57). CadA homologues have been identified in plasmids and chromosomes from a number of gram-positive organisms, in *Synechocystis* sp. strain PCC 6803 (1), and in the incomplete genome sequences from *Arabidopsis thaliana* and *Pseudomonas aeruginosa*. Recently, the CadA homologue from *Synechocystis* sp. strain PCC 6803 has been named ZiaA and shown to confer Zn(II) and Cd(II) tolerance due to reduced accumulation (56). The pI258 *cadA* gene also complemented the Zn(II)/Cd(II)/Pb(II)-sensitive phenotypes of an *E. coli zntA*-disrupted strain (41). While the *E. coli zntA* gene is positively regulated by ZntR, *cadA* and *ziaA* are negatively regulated by the CadC (25) and ZiaR (56) repressors, respectively, both of which are members of the ArsR family of metalloregulatory proteins (50). CadC responds to Zn(II), Cd(II), and Pb(II), the same metal ion specificity as the pumps themselves (43).

SUMMARY AND PERSPECTIVES

Within the last 5 years, much progress has been made in understanding the mechanism of action and evolution of soft-metal ATPases. The evolutionary pressure to flourish despite the geological omnipresence of metals has ensured that gene families for soft-metal resistances are not confined to a single species or even a single kingdom. Many questions remain to be answered, and we look forward to an accelerated acquisition of knowledge in the near future.

One question is the function of ArsA homologues. Clearly in bacteria the *arsA* gene encodes the catalytic subunit of the ArsAB pump. It is not known whether the eukaryotic and archeal *arsA* homologues are orthologues or encode proteins with unrelated functions. Eukaryotes have resistance mechanisms for arsenicals. In both *Leishmania* and Chinese hamster cells we have shown that resistance is related to active extrusion of arsenite (44). But at this time the genes or gene products that produce the resistance have not been identified.

A second question is the distribution of chaperones for soft metals in bacteria. While homologues of copper chaperones such as Atx1p and Lys7p are widely distributed in eukaryotes (12), they do not appear to exist in bacteria. CopZ serves a chaperone function in *E. hirae* (10), but homologues with copper chaperone activity have not been found in other bacteria. Additionally, it is not clear whether cells require chaperones for non-redox-active metals such as Zn(II), Cd(II), and Pb(II).

A third question is the distribution of ZntA homologues in nature. The related Cu(I)-translocating P-type ATPases are found in most organisms. In humans these pumps are required for copper homeostasis, and mutations in either the ATP7A or ATP7B gene produce inborn errors of copper metabolism, resulting in Menkes or Wilson disease, respectively. Even though zinc is not as toxic as copper, in part because it is not a redox-active metal, it is still toxic in excess, and mechanisms to prevent overaccumulation must be present. To date a growing number of *zntA* and *cadA* genes for zinc-translocating P-type ATPases have been identified. Genes for putative ATP-dependent zinc pumps are being discovered in other kingdoms, for example in the genome of the archeaon *Methanobacterium thermoautotrophicum* (GenBank accession no. 2621474) and in the genome of the plant *A. thaliana* (GenBank accession no. 4210504). Within the next 5 years the sequencing of the human genome—and many others—will be completed. At that point the phylogenic distribution of zinc pumps and their involvement in human health and disease will be better understood.

ACKNOWLEDGMENTS

Research in our laboratory is funded by U.S. Public Health Service grants GM55425 and GM52216 to B.P.R and National Research Service Award GM18973 to C.R.

REFERENCES

1. **Axelsen, K. B., and M. G. Palmgren.** 1998. Evolution of substrate specificities in the P-type ATPase superfamily. J. Mol. Evol. **46:**84–101.
2. **Beard, S. J., R. Hashim, J. Membrillo-Hernandez, M. N. Hughes, and R. K. Poole.** 1997. Zinc(II) tolerance in *Escherichia coli* K-12: evidence that the *zntA* gene (*o732*) encodes a cation transport ATPase. Mol. Microbiol. **25:** 883–891.
3. **Bhattacharjee, H., J. Li, M. Y. Ksenzenko, and B. P. Rosen.** 1995. Role of cysteinyl residues in metalloactivation of the oxyanion-translocating ArsA ATPase. J. Biol. Chem. **270:**11245–11250.
4. **Bhattacharjee, H., and B. P. Rosen.** 1996. Spatial proximity of Cys113, Cys172, and Cys422 in the metalloactivation domain of the ArsA ATPase. J. Biol. Chem. **271:**24465–24470.
5. **Brocklehurst, K. R., J. L. Hobman, B. Lawley, L. Blank, S. J. Marshall, N. L. Brown, and A. P. Morby.** 1999. ZntR is a Zn(II)-responsive MerR-like transcriptional regulator of *zntA* in *Escherichia coli*. Mol. Microbiol. **31:** 893–902.
6. **Bull, P. C., G. R. Thomas, J. M. Rommens, J. R. Forbes, and D. W. Cox.** 1993. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. Nat. Genet. **5:**327–337.
7. **Chen, C. M., T. K. Misra, S. Silver, and B. P. Rosen.** 1986. Nucleotide sequence of the structural genes for an anion pump. The plasmid-encoded arsenical resistance operon. J. Biol. Chem. **261:**15030–15038.
8. **Chen, Y., S. Dey, and B. P. Rosen.** 1996. Soft metal thiol chemistry is not involved in the transport of arsenite by the Ars pump. J. Bacteriol. **178:** 911–913.
9. **Chen, Y., and B. P. Rosen.** 1997. Metalloregulatory properties of the ArsD repressor. J. Biol. Chem. **272:**14257–14262.
10. **Cobine, P., W. A. Wickramasinghe, M. D. Harrison, T. Weber, M. Solioz, and C. T. Dameron.** 1999. The *Enterococcus hirae* copper chaperone CopZ delivers copper(I) to the CopY repressor. FEBS Lett. **445:**27–30.
11. **Culotta, V. C., L. W. Klomp, J. Strain, R. L. Casareno, B. Krems, and J. D. Gitlin.** 1997. The copper chaperone for superoxide dismutase. J. Biol. Chem. **272:**23469–23472.
12. **Culotta, V. C., S. J. Lin, P. Schmidt, L. W. Klomp, R. L. Casareno, and J. Gitlin.** 1999. Intracellular pathways of copper trafficking in yeast and humans. Adv. Exp. Med. Biol. **448:**247–254.
13. **Dey, S., D. Dou, and B. P. Rosen.** 1994. ATP-dependent arsenite transport in everted membrane vesicles of *Escherichia coli*. J. Biol. Chem. **269:**25442–25446.
14. **Dey, S., and B. P. Rosen.** 1995. Dual mode of energy coupling by the oxyanion-translocating ArsB protein. J. Bacteriol. **177:**385–389.
15. **DiDonato, M., S. Narindrasorasak, J. R. Forbes, D. W. Cox, and B. Sarkar.** 1997. Expression, purification, and metal binding properties of the N-terminal domain from the Wilson disease putative copper-transporting ATPase (ATP7B). J. Biol. Chem. **272:**33279–33282.
16. **Fu, D., T. J. Beeler, and T. M. Dunn.** 1995. Sequence, mapping and disruption of CCC2, a gene that cross-complements the Ca(2+)-sensitive phenotype of *csg1* mutants and encodes a P-type ATPase belonging to the Cu(2+)-ATPase subfamily. Yeast **11:**283–292.
17. **Gitschier, J., B. Moffat, D. Reilly, W. I. Wood, and W. J. Fairbrother.** 1998. Solution structure of the fourth metal-binding domain from the Menkes copper-transporting ATPase. Nat. Struct. Biol. **5:**47–54.
18. **Gladysheva, T. B., K. L. Oden, and B. P. Rosen.** 1994. Properties of the arsenate reductase of plasmid R773. Biochemistry **33:**7288–7293.
19. **Glerum, D. M., A. Shtanko, and A. Tzagoloff.** 1996. SCO1 and SCO2 act as high copy suppressors of a mitochondrial copper recruitment defect in Saccharomyces cerevisiae. J. Biol. Chem. **271:**20531–20535.
20. **Gupta, A., K. Matsui, J. F. Lo, and S. Silver.** 1999. Molecular basis for resistance to silver cations in *Salmonella*. Nat. Med. **5:**183–188.
21. **Hamlett, N. V., E. C. Landale, B. H. Davis, and A. O. Summers.** 1992. Roles of the Tn21 *merT*, *merP*, and *merC* gene products in mercury resistance and mercury binding. J. Bacteriol. **174:**6377–6385.
22. **Higgins, D. G., and P. M. Sharp.** 1988. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. Gene **73:**237–244.
23. **Ji, G., E. A. Garber, L. G. Armes, C. M. Chen, J. A. Fuchs, and S. Silver.** 1994. Arsenate reductase of *Staphylococcus aureus* plasmid pI258. Biochemistry **33:**7294–7299.
24. **Ji, G., and S. Silver.** 1992. Reduction of arsenate to arsenite by the ArsC protein of the arsenic resistance operon of *Staphylococcus aureus* plasmid pI258. Proc. Natl. Acad. Sci. USA **89:**9474–9478.
25. **Ji, G., and S. Silver.** 1992. Regulation and expression of the arsenic resistance operon from *Staphylococcus aureus* plasmid pI258. J. Bacteriol. **174:** 3684–3694.
26. **Kanamaru, K., S. Kashiwagi, and T. Mizuno.** 1994. A copper-transporting P-type ATPase found in the thylakoid membrane of the cyanobacterium

Synechococcus species PCC7942. Mol. Microbiol. **13:**369–377.
27. **Kuroda, M., S. Dey, O. I. Sanders, and B. P. Rosen.** 1997. Alternate energy coupling of ArsB, the membrane subunit of the Ars anion-translocating ATPase. J. Biol. Chem. **272:**326–331.
28. **Lai, H. C., D. Gygi, G. M. Fraser, and C. Hughes.** 1998. A swarming-defective mutant of *Proteus mirabilis* lacking a putative cation-transporting membrane P-type ATPase. Microbiology **144:**1957–1961.
29. **Li, J., S. Liu, and B. P. Rosen.** 1996. Interaction of ATP binding sites in the ArsA ATPase, the catalytic subunit of the Ars pump. J. Biol. Chem. **271:** 25247–25252.
30. **Lippard, S. J., and J. M. Berg.** 1994. Principles of bioinorganic chemistry. University Science Books, Mill Valley, Calif.
31. **Lu, D., B. Boyd, and C. A. Lingwood.** 1997. Identification of the key protein for zinc uptake in *Haemophilus influenzae*. J. Biol. Chem. **272:**29033–29038.
32. **Lutsenko, S., K. Petrukhin, M. J. Cooper, C. T. Gilliam, and J. H. Kaplan.** 1997. N-terminal domains of human copper-transporting adenosine triphosphatases (the Wilson's and Menkes disease proteins) bind copper selectively *in vivo* and *in vitro* with stoichiometry of one copper per metal-binding repeat. J. Biol. Chem. **272:**18939–18944.
33. **Melchers, K., L. Herrmann, F. Mauch, D. Bayle, D. Heuermann, T. Weitzenegger, A. Schuhmacher, G. Sachs, R. Haas, G. Bode, K. Bensch, and K. P. Schafer.** 1998. Properties and function of the P type ion pumps cloned from *Helicobacter pylori*. Acta. Physiol. Scand. Suppl. **643:**123–135.
34. **Melchers, K., T. Weitzenegger, A. Buhmann, W. Steinhilber, G. Sachs, and K. P. Schafer.** 1996. Cloning and membrane topology of a P type ATPase from *Helicobacter pylori*. J. Biol. Chem. **271:**446–457.
35. **Nucifora, G., L. Chu, T. K. Misra, and S. Silver.** 1989. Cadmium resistance from *Staphylococcus aureus* plasmid pI258 *cadA* gene results from a cadmium-efflux ATPase. Proc. Natl. Acad. Sci. USA **86:**3544–3548.
36. **Odermatt, A., H. Suter, R. Krapf, and M. Solioz.** 1993. Primary structure of two P-type ATPases involved in copper homeostasis in *Enterococcus hirae*. J. Biol. Chem. **268:**12775–12779.
37. **Patzer, S. I., and K. Hantke.** 1998. The ZnuABC high-affinity zinc uptake system and its regulator Zur in *Escherichia coli*. Mol. Microbiol. **28:**1199–1210.
38. **Phung, L. T., G. Ajlani, and R. Haselkorn.** 1994. P-type ATPase from the cyanobacterium *Synechococcus* 7942 related to the human Menkes and Wilson disease gene products. Proc. Natl. Acad. Sci. USA **91:**9651–9654.
39. **Pufahl, R. A., C. P. Singer, K. L. Peariso, S. J. Lin, P. J. Schmidt, C. J. Fahrni, V. Cizewski Culotta, J. E. Penner-Hahn, and T. V. O'Halloran.** 1997. Metal ion chaperone function of the soluble Cu(I) receptor Atx1. Science **278:**853–856.
40. **Rae, T. D., P. J. Schmidt, R. A. Pufahl, V. C. Culotta, and T. V. O'Halloran.** 1999. Undetectable intracellular free copper: the requirement of a copper chaperone for superoxide dismutase. Science **284:**805–808.
41. **Rensing, C., B. Mitra, and B. P. Rosen.** 1998. A Zn(II)-translocating P-type ATPase from *Proteus mirabilis*. Biochem. Cell Biol. **76:**787–790.
42. **Rensing, C., B. Mitra, and B. P. Rosen.** 1997. The *zntA* gene of *Escherichia coli* encodes a Zn(II)-translocating P-type ATPase. Proc. Natl. Acad. Sci. USA **94:**14326–14331.
42a. **Rensing, C., B. Mitra, and B. P. Rosen.** Unpublished results.
43. **Rensing, C., Y. Sun, B. Mitra, and B. P. Rosen.** 1998. Pb(II)-translocating P-type ATPases. J. Biol. Chem. **273:**32614–32617.
44. **Rosen, B. P.** 1999. Families of arsenic transporters. Trends Microbiol. **7:** 207–212.
45. **Rosen, B. P.** 1998. The role of efflux in bacterial resistance to soft metals and metalloids, p. in press. *In* S. J. Higgins and D. P. Ballou (ed.), Metalloproteins, vol. 34. Portland Press, Ltd., London.
46. **Rosen, B. P., U. Weigel, C. Karkaria, and P. Gangola.** 1988. Molecular characterization of an anion pump. The *arsA* gene product is an arsenite-(antimonate)-stimulated ATPase. J. Biol. Chem. **263:**3067–3070.
47. **Rosenstein, R., A. Peschel, B. Wieland, and F. Götz.** 1992. Expression and regulation of the antimonite, arsenite, and arsenate resistance operon of *Staphylococcus xylosus* plasmid pSX267. J. Bacteriol. **174:**3676–3683.
48. **Sanders, O. I., C. Rensing, M. Kuroda, B. Mitra, and B. P. Rosen.** 1997. Antimonite is accumulated by the glycerol facilitator GlpF in *Escherichia coli*. J. Bacteriol. **179:**3365–3367.
49. **San Francisco, M. J., C. L. Hope, J. B. Owolabi, L. S. Tisa, and B. P. Rosen.** 1990. Identification of the metalloregulatory element of the plasmid-encoded arsenical resistance operon. Nucleic Acids Res. **18:**619–624.
50. **Shi, W., J. Wu, and B. P. Rosen.** 1994. Identification of a putative metal binding site in a new family of metalloregulatory proteins. J. Biol. Chem. **269:** 19826–19829.
51. **Silver, S., K. Budd, K. M. Leahy, W. V. Shaw, D. Hammond, R. P. Novick, G. R. Willsky, M. H. Malamy, and H. Rosenberg.** 1981. Inducible plasmid-determined resistance to arsenate, arsenite, and antimony(III) in *Escherichia coli* and *Staphylococcus aureus*. J. Bacteriol. **146:**983–996.
52. **Silver, S., and D. Keach.** 1982. Energy-dependent arsenate efflux: the mechanism of plasmid-mediated resistance. Proc. Natl. Acad. Sci. USA **79:**6114–6118.
53. **Silver, S., and L. T. Phung.** 1996. Bacterial heavy metal resistance: new surprises. Annu. Rev. Microbiol. **50:**753–789.
54. **Solioz, M., and A. Odermatt.** 1995. Copper and silver transport by CopB-ATPase in membrane vesicles of *Enterococcus hirae*. J. Biol. Chem. **270:** 9217–9221.
55. **Solioz, M., and C. Vulpe.** 1996. CPx-type ATPases: a class of P-type ATPases that pump heavy metals. Trends Biochem. Sci. **21:**237–241.
56. **Thelwell, C., N. J. Robinson, and J. S. Turner-Cavet.** 1998. An SmtB-like repressor from *Synechocystis* PCC 6803 regulates a zinc exporter. Proc. Natl. Acad. Sci. USA **95:**10728–10733.
57. **Tsai, K. J., K. P. Yoon, and A. R. Lynn.** 1992. ATP-dependent cadmium transport by the *cadA* cadmium resistance determinant in everted membrane vesicles of *Bacillus subtilis*. J. Bacteriol. **174:**116–121.
58. **Voskoboinik, I., H. Brooks, S. Smith, P. Shen, and J. Camakaris.** 1998. ATP-dependent copper transport by the Menkes protein in membrane vesicles isolated from cultured Chinese hamster ovary cells. FEBS Lett. **435:** 178–182.
59. **Vulpe, C., B. Levinson, S. Whitney, S. Packman, and J. Gitschier.** 1993. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. Nat. Genet. **3:**7–13.
60. **Walmsley, A. R., T. Zhou, M. I. Borges-Walmsley, and B. P. Rosen.** 1999. The ATPase mechanism of ArsA, the catalytic subunit of the arsenite pump. J. Biol. Chem. **274:**16153–16161.
61. **Wunderlie-Ye, H., and M. Solioz.** 1999. Effects of promoter mutations on the in vivo regulation of the *cop* operon of *Enterococcus hirae* by copper (I) and copper (II). Biochem. Biophys. Res. Commun. **259:**443–449.
62. **Xu, C., T. Zhou, M. Kuroda, and B. P. Rosen.** 1998. Metalloid resistance mechanisms in prokaryotes. J. Biochem. **123:**16–23.
63. **Zhou, T., and B. P. Rosen.** 1997. Tryptophan fluorescence reports nucleotide-induced conformational changes in a domain of the ArsA ATPase. J. Biol. Chem. **272:**19731–19737.