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Mini Review

The Bacterial View of the Periodic Table: Specific Functions for All Elements

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Bacterial chromosomes have genes for transport of inorganic nutrient cations (such as NH_4^+, K^+, Mg^{2+}, Co^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+} and other trace cations) and oxyanions (such as PO_4^{3-} and SO_4^{2-} and less abundant oxyanions). Together these account for sometimes several hundred genes in many bacteria. Bacterial plasmids encode resistance systems for toxic metal and metalloid ions including Ag^+, AsO_2^-, AsO_4^{3-}, Cd^{2+}, Co^{2+}, CrO_4^{2-}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, Sb^{3+}, TeO_3^{2-}, Tl^+, and Zn^{2+}. Most resistance systems function by energy-dependent efflux of toxic ions. A few involve enzymatic (mostly redox) transformants. Some of the efflux resistance systems are ATPases and others are chemiosmotic ion/proton exchangers. Mercury resistance is due to enzymatic detoxification with organomercurial lyase (cutting the C-Hg bond of compounds such as methylmercury and phenylmercury) and mercuric reductase ($Hg^{2+} \rightarrow Hg^0$). The Cd^{2+}-resistance cation P-type ATPases of Gram-positive bacteria drives Cd^{2+} (and Zn^{2+}) efflux from resistant cells. The genes defective in the human hereditary diseases of copper metabolism, Menkes syndrome and Wilson's disease, encode Cu-specific P-type ATPases that are similar to bacterial Cd^{2+} ATPases. The arsenic resistance system transports arsenite [As(III)], alternatively with the ArsB protein functioning as a chemiosmotic efflux transporter or with two proteins, ArsB and ArsA, functioning as an ATPase transporter. The third protein of the arsenic resistance system is an enzyme that reduces intracellular arsenate [As(V)] to arsenite [As(III)], the substrate of the efflux system. In Gram negative cells, a three polypeptide complex functions as a chemiosmotic cation/proton exchanger to efflux Cd^{2+}, Zn^{2+}, and Co^{2+}. This pump consists of an inner membrane (CzcA), an outer membrane (CzcC) and a membrane-spanning (CzcB) protein that function together.

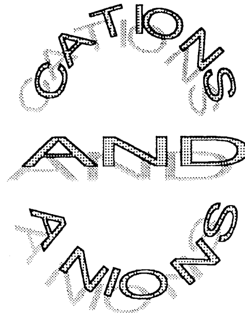
Key words: Bacterial plasmids, Toxic metal resistance, Mercury, Cadmium, Arsenic

Strategies for Metal Handling by Microorganisms

This review describes the general mechanisms by which microorganisms transport metals and other nutrients, with emphasis on the strategies they have evolved to deal with otherwise toxic concentrations of metals. The underlying theme is that for each and every inorganic cation or anion that is encountered in normal environments, there are corresponding genes and proteins that govern movements[53,57]. For required nutrients there are highly specific membrane transport systems that concentrate needed nutrients from dilute media. Once inside the cell, some inorganic nutrients are sequestered (for example the protein metallothionein binds intracellular cadmium, copper and zinc) or enzymatically incorporated into specific proteins (for example using the enzyme ferrochelatase, which places cationic iron into heme groups).

Excess ions are either stored (for example iron in ferritin) or excreted (again by highly specific membrane transporters).

Cations of related elements often are associated in pairs, with one needed for intracellular nutrition and the other not used intracellularly (but sometimes coupled for co-transport or cellular signaling, and needing a highly specific efflux mechanism). For example, K and Na both serve biological functions. However, K^+ (with several parallel uptake transporter pathways) is essential for intracellular nutrition whereas Na^+ is not. There are no intracellular Na^+-specific enzymes and sodium functions basically outside of microbial cells and for many bacteria (*E. coli* is a well-known example) Na^+ is not needed at all for growth or survival. There are a few "normally" proton-driven membrane transport pumps that are in a few rare bacteria Na^+ gradient driven instead. A similar situation occurs with Mg and Ca. Mg^{2+} functions in many intracellular roles and must be transported inward and carefully regulated; Ca^{2+} is not needed within the cell and is maintained at low intracellular levels by efflux transport pathways. Ca^{2+} and Na^+ are indeed frequently used for biological processes, such as signal transduction or as co-transport substrates, but these are secondary processes, not common to all cells. Ca^{2+} and Na^+ frequently function extracellularly rather than intracellularly in biological processes. This is true of animal cells as well as free-living bacterial cells, surviving in basically distilled water or in



The Periodic Table of Nutrient Cation and Oxyanion Transport Systems

1) K^+. Four separate systems in *E. coli*. Three chemiosmotic and one ATPase.

2) Mg^{2+}. Three separate systems in *S. typhimurium*. One chemiosmotic and two ATPase.

3) Fe^{3+}. At least five separate systems in *E. coli*. Specificities for different siderophores.

4) Mn^{2+}. Found in Gram positive and Gram negative bacteria. Chemiosmotic and ATPases

5) Zn^{2+}. Newly reported ATPase in *E. coli*.

6) Ni^{2+}. ABC ATPase in *E. coli*.

7) PO_4^{3-}. Separate Pit (chemiosmotic) and Pst (ABC ATPase) systems in *E. coli* and *Bacillus*.

8) SO_4^{2-}. Five component ABC-ATPase in *S. typhimurium*.

Fig. 1. Nutrient cation and anion transport systems

hypertonic saline environments.

For some higher atomic number elements of the Period Table (the lanthanides and actinides, including uranium and trans-uranium elements), there appear to be no specific genes or proteins for metal ion resistances. Presumedly, these were not encountered early in life on earth in natural environments at levels that were toxic. Similarly, there are no known genes or proteins specific for Al^{3+}. Although Al is the second most abundant element in the Earth's crust (after Si), it occurs in extremely low concentrations in most solutions near the Earth's surface. This, combined with the low toxicity of aluminum cations means that specific biological roles for Al^{3+} would have low survival value and furthermore that resistance systems for Al^{3+} are not needed. There also are no resistance genes for halides, although halides are abundant in the environment and toxic in higher concentrations. This is somewhat of a surprise.

Bacteria have genes (and proteins) specific for transport of all needed nutrients and for resistances to the toxic ions of most heavy metal elements. Required

Heavy Metals

PLASMID HEAVY METAL RESISTANCE SYSTEMS

1. Hg^{2+}. *mer.* Hg^{2+} and organomercurials are enzymatically detoxified.

2. AsO_4^{3-}, AsO_2^{-}, SbO^{+}. *ars.* Arsenate is enzymatically reduced to arsenite by ArsC Arsenite and antimonite are "pumped" out by the membrane protein ArsB chemiosmotically0 or with the additional ArsA protein as an ATPase.

3. Cd^{2+}. *cadA.* Cd^{2+} (and Zn^{2+}) are pumped from bacteria by a P-type ATPase.

4. Cd^{2+}, Zn^{2+}, Co^{2+}, and Ni^{2+}. *czc.* Cd^{2+}, Zn^{2+}, Co^{2+}, and Ni^{2+} are pumped from Gram negative bacteria by a three polypeptide membrane complex that functions as a divalent cation/2 H^{+} antiporter.

5. Ag^{+}. *sil.* Ag^{+} resistance results from pumping from bacteria by three polypeptide chemiosmotic exchanger plus a P-type ATPase.

6. Cu^{2+}. *cop.* Plasmid Cu^{2+} resistance results from a four polypeptide complex, consisting of an inner membrane protein, an outer membrane protein, and two periplasmic copper-binding proteins. In addition, chromosomally-encoded P-type ATPases provide partial resistance by effluxing Cu^{2+} or Cu^{+}.

7. CrO_4^{2-}. *chr.* Chromate resistance results from a single membrane polypeptide that causes reduced net cellular uptake.

8. TeO_3^{2-}. *tel.* Tellurite resistance results from any of several genetically-unrelated plasmid systems. Reduction to metallic Te^{0} frequently does not seem to be involved.

9. Pb^{2+}. *pum.* Lead resistance appears to be due to an efflux ATPase in Gram negative and accumulation of intracellular $Pb_3(PO_4)_2$ in Gram positive bacteria.

Fig. 2. Plasmid toxic metal resistance systems and mechanisms modified from[54]

inorganic nutrients include the cations NH_4^+, K^+, Mg^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Zn^{2+} and other trace cations[53] and the oxyanions PO_4^{3-}, SO_4^{2-} and less abundant anions. Fig. 1 presents a current summary of inorganic nutrient transport systems and Fig. 2 a listing of inorganic ion resistance systems and their biochemical mechanisms. Toxic inorganics with genetically-defined resistances include Ag^+, AsO_2^-, AsO_4^{3-}, Cd^{2+}, Co^{2+}, CrO_4^{2-}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, Sb^{3+}, TeO_3^{2-}, Tl^+ and Zn^{2+}.

Mechanisms of Metal Resistance

Toxic metal resistance systems probably arose shortly after life started, in an already metal-abundant world. As with nutrient organic compounds, the environment provided strong selection pressures for the transport and accumulation of needed inorganic nutrients and for the removal or detoxification of abundant toxic cations and oxyanions. The recent activities of humans create locally polluted environments, which indeed exert a high selection pressure for metal resistance on microbial populations. Highly specific systems have evolved in response to these pressures[54,57]. However, there is nothing new about toxic heavy metal resistance determinants, as microbes have been exposed to these cations and oxyanions periodically since the origin of cellular life.

Plasmid and chromosomal genes

Most frequently, toxic metal resistance systems are found on plasmids, small circular DNA molecules that can readily move from one cell to another, when there is a need for their functions. Plasmid-based resistance facilitates transfer of toxic metal resistance from cell to cell over a short time scale (weeks or months) that would not allow new invention by evolution of such resistance mechanisms. In other organisms, however, resistances to toxic metals are coded for by chromosomal genes, which may indicate that most bacteria experience selection for toxic metal resistances frequently in natural environments. Some cations such as Co^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} are essential nutrients at low levels but toxic at higher levels.

For these cations, separate transport systems for uptake are coded for by chromosomal genes, and additional genes (either on the chromosomes or on plasmids) efflux the same cations when present at toxic levels. The paired uptake and efflux systems are carefully regulated, assuring homeostasis and maintenance of stable intracellular cation levels, more or less independent of external instabilities[53,54,55,57].

Three generalizations about metal resistance mechanisms may be made: (i) Plasmid-determined metal resistance systems are very specific, as much so as those for antibiotic resistances, or sugar or amino acid metabolism. There is no general mechanism for resistance to all heavy metal ions. (ii) Metal-ion resistance systems have been found with every bacterial group tested, from *Escherichia coli* to *Streptomyces*. These mechanisms have not been found in eukaryotic microbes (which appear to have different mechanisms) or Archaea, for which both thermophiles and halophiles live in metal-rich environments but for which such mechanisms as we study in bacteria have never been reported. (iii) The mechanisms of resistance are most often efflux "pumping" (removing toxic ions that had entered the cell by means of transport systems designed for uptake of nutrient cations or oxyanions) and less often enzymatic detoxification (generally redox chemistry) converting a more toxic to a less toxic or less available metal-ion species.

Efflux pumps

It would seem easier to keep toxic ions out (by evolving highly specific uptake transport systems), rather than to expend ATP bringing in toxic ions and then more energy pumping them out. Apparently, the design penalties in terms of chemical specificities and rates for having uptake pumps more specific is greater than the genetic cost of having plasmid genes (in part of the overall population) that can spread when needed. The efflux pumps that are the protein basis of plasmid resistance systems can be either ATPases or membrane potential driven. Transport ATPases are enzymes that use the chemical energy from cleavage of the high-energy phospho-ester bond of adenosine triphosphate (ATP) to drive the formation of concentration gradients (a potential gradient)[29]. Membrane

potential-driven pumps couple a membrane potential gradient (which often is about 200 mV, internal negative, across most bacterial membranes) to establish a concentration gradient. Alternatively, "primary gradients" of protons (H^+) or Na^+ [high outside/low inside] drive the formation of secondary gradients of nutrients (for example K^+) or toxic cations (for example Cd^{2+} in some bacteria) by "co-transport" either in the same direction or opposite direction. Other examples of ATPase pumps include the Cu^{2+} efflux ATPases of many bacteria and the arsenite-ATPase of Gram negative bacteria. Other examples of secondary gradient-driven pumps are the arsenite efflux systems of the chromosomes of *Bacillus* and *E. coli* and of plasmids in Gram positive bacteria.

The mechanisms are not precisely the same in all bacterial types: while the mercury-resistance and arsenic-resistance systems are highly homologous in all bacteria studied, cadmium resistance involves ATPases in Gram positive bacteria and unrelated chemiosmotic proton/cation exchangers in Gram negative bacteria. These divalent cation systems appear to be of independent evolutionary origin and never to have shared a common ancestral system. There is also a well-described bacterial metallothionein, found so far only in cyanobacteria, and conferring resistances to Cd^{2+} and Zn^{2+} [67]. Metallothionein protects the cellular interior by sequestration—specific binding of toxic cations within the cell but so tightly that harm is avoided.

The mechanisms of bacterial plasmid-determined resistances to many toxic inorganic cations and anions have been studied by my own and other laboratories. Because of limitations of space and the need for a global and general picture, I will limit references frequently only to review articles from our laboratory[27,53,54,55,57]. These reviews have more specific references to the contributions of a large group of investigators.

Archaea

No one has reported resistance mechanisms for Archaea such as described in this report for bacteria. Whether this reflects a difference in biology or a lack of research effort is unclear. Archaea grow in environments with high levels of toxic heavy metal ions and therefore are expected to have the same or alternative mechanisms of metal resistances. A step in this direction was the publication of the entire 1.66 million nucleotide DNA sequence of the methanogen *Methanococcus jannaschii*[6,13]. This sequence, unlike those of bacteria that are now being released almost monthly, contains only two sequenced potential genes that are reported as homologous to those we earlier reported from bacteria. These encode homologs for ArsA (the ATPase subunit of the arsenite effluxing membrane transport system) and ChrA (the membrane protein required for chromate resistance). Why there are so few such recognizable genes in a deep-sea vent-dwelling organism (expected to have been exposed to a range of toxic heavy metals), compared with the larger number for the human pathogen *Haemophilus influenzae* is unclear.

Metal Resistance in Bacteria: Case Studies

Mercury

Mercury in the environment

Mercury is an important metal contaminant in "natural environments" such as volcanos and vents in the bottom of the sea. There is therefore a natural mercury geocycle (Fig. 3) with microbial activity important at many levels. Bacteria oxidize Hg(0) that is deposited in rain world-wide to Hg(II), which is taken up and absorbed more effectively by living organisms. Microbial oxidation of Hg(0) is carried out by the widely-found enzyme catalase. Bacteria also reduce Hg(II) found in waters and soil to Hg(0), which is volatile in stirred environments such as sewage systems or from the surfaces of leafs. Microbes also methylate inorganic Hg(II) to methylmercury, which is both more toxic and "bio-accumulated." It is methylmercury of biological origin that is found in fish and other seafood and that represents a major human toxicity problem.

However, methylmercury levels are high in tuna (for example) which live in the cleanest water in the middle of the sea (and therefore it is presumedly "natural" methylmercury in tuna) and in fish from Minamata Bay (the site of a major mercury pollution problem) in Kyushu Japan[58] or from the goldmining

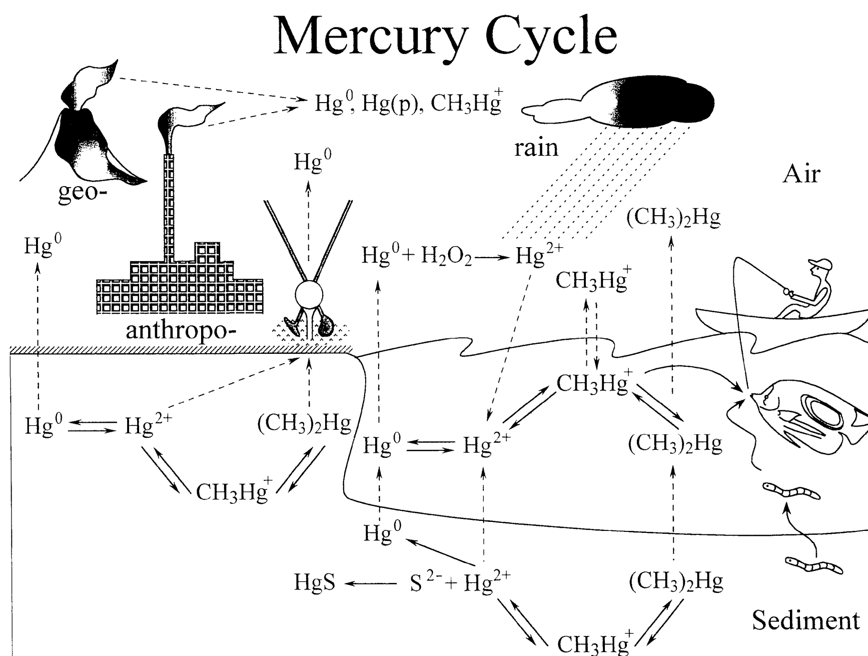


Fig. 3. The mercury geocycle and global biocycle, showing both microbial activities and geophysical and industrial release of mercury compounds

region of Brazil (where liquid mercury is thought to be used by more than a hundred-thousand miners panning for gold). Again the conclusion is that toxic mercury levels have existed from the origin of life, although it is clear that human activities have significantly impacted on local and global levels of mercury (and other metals). Finally, an enzyme organomercurial lyase which is found in our mercury resistance systems (see below) cleaves the mercury-carbon bond in methylmercury (and also phenylmercury), completing the cycle and yielding Hg(II) (Fig. 3).

Mercury resistance in bacteria

Closely related systems for resistance to inorganic mercury have been found on plasmids of all bacteria tested. For one example, in the collection of some 800 antibiotic-resistance plasmids from various Gram negative bacteria, 25% carried mercury resistance[52]. In most cases, the number of genes (approximately six) and the functions of the genes are the same for all bacterial types[57,59]. Almost all *mer* systems start with a regulatory gene, *merR*, whose product is a unique positively-acting activator protein that in the presence of Hg^{2+} twists and bends the operator DNA region, allowing RNA polymerase to synthesize messenger RNA[63].

In the mercury resistance systems of Gram negative bacteria, the regulatory gene is transcribed separately from the remaining genes. This allows tighter control of the *mer* operon than is possible with Gram positive bacteria, where the regulatory gene is the first gene on the multi-gene *mer* operon, so that the gene that regulates and the genes for enzymes that bind and change the chemical form of mercury are always transcribed together[27,59]. Following the regulatory gene, there are one to three genes in the operon whose products are involved in transport of toxic Hg^{2+} across the cell membrane to the intracellular detoxifying enzyme, which is called mercuric reductase. In some *mer* operons, the gene for mercuric reductase is followed immediately by a small gene which encodes the enzyme organomercurial lyase. This lyase breaks the carbon-mercury bond in highly toxic organomercurials such as phenyl- and methyl-mercury.

The structure of the *Bacillus* mercuric reductase was solved by X-ray diffraction[19,51]. The structure is very similar to that of glutathione reductase from mammalian sources, and is a homo-dimer, with each subunit containing a highly conserved active site with two critical cysteine residues, a bound FAD per subunit, and an NADPH-binding site for electron transfer from NADPH to FAD to the substrate Hg^{2+}. The active site includes the redox-active disulfide region on one subunit and the substrate-binding site at the C-terminal (including conserved vicinal Cys residues) of the other subunit[51]. The point here is that mechanistic studies have progressed and we anticipate that these studies at the atomic level will affect use of this system in "bioremediation" and understanding of functioning of the global mercury cycle.

Two lessons concerning mercury resistance genes in the environment come from the experiences with mercury resistant *Bacillus* isolates from the methylmercury polluted area at Minamata Bay Japan[36,58] and the quantitating of mercuric resistance genes from Gram negative bacteria from North American mercury polluted sites[49]. The conclusions from both studies are (i) the genetic determinants that have been extensively studied in the laboratory are representative of what is out there in the environment and (ii) what occurs in the environment is sophisticated, so that a thorough understanding from laboratory studies is essential to understand the distribution of heavy metal resistance genes and the chemical transformations in real environments.

Arsenic

Arsenic in the environment

Water supplies in many areas of many counties are extensively polluted or threatened by high concentrations of arsenic, sometimes from natural sources and sometimes from the activities of humans. Most recently, there were reports of arsenic in drinking water in India and USA that reaches levels that cause human disease, sometimes cancers. In Taiwan and China, high arsenic in drinking water causes a condition called "black foot" where tissues die and gangrene sets in leading to the loss of toes and feet. Since microbial activities can in some cases mobilize otherwise mineral-bound arsenic or alternatively immobilize otherwise water-soluble arsenic, the transformations of arsenic by bacteria is important in geocycles.

In mining, especially during bioleaching of gold from arsenopyrite ores[48], soluble arsenic (mostly arsenate) can build up in large bodies of mine waste water to levels of 0.4 M (30 g/l). This clearly provides a localized environment where microbial activities will either help or harm. Another example, just North of Boston, industrial activity produced a large amount of arsenic waste, which was immobilized in buried waste materials but has recently been releasing (apparently through microbial activities[1])troublesome amounts of arsenic into local recreational waters. The overall message is as above: it is not simply chemistry that determines the speciation of arsenic in the environment. Microbial activities play major roles. There are many less familiar and less studied environments where arsenic is significant: Many sea foods such as shrimp contain high levels of natural organoarsenicals, synthesized by living organisms. There is a marine arsenic geocycle of synthesis and degradation of natural organoarsenicals. I am told that all chicken sold in the United States has been fed industrial-made organoarsenicals as a growth stimulant. What happens to the waste arsenic is not known.

As resistance

Understanding of bacterial arsenic resistance systems is less complete and more recent than that of mercury resistance. Fundamentally the same genes (and encoded biochemical mechanism) are found on plasmids in Gram negative and Gram positive bacteria[7,8,54,57]. Closely similar gene clusters have been found to determine normal background arsenic resistance both in *Bacillus* and in *E. coli*[54,57,60] and as yet uncharacterized systems have different bases for arsenic resistance in environmentally important bacteria[1,2,33]. Therefore what we think today may be only the first half of a larger picture of microbial arsenic transformations.

To start, the same cluster of genes, the *ars* operon, confers resistances to As(III), As(V) and Sb(III). However, the number of genes can vary somewhat and the details of their functions can differ. Seven *ars*

operons have been sequenced[54,57]. There are two extra genes, *arsA* and *arsD*, on the plasmids of Gram negative bacteria that are missing from arsenic resistance systems of plasmids of Gram positive bacteria[59] and the chromosomal systems of both *E. coli*[7,18,60] and *Bacillus subtilis*[57,64]. The ArsD protein is a secondary regulator of *ars* operon transcription[12,69], so its presence or absence might have little effect on resistance. Its existence in two different plasmids, however, indicates a role in the environment under conditions more subtile than we have come to understand.

The ArsA protein is a membrane-associated ATPase[16,28] attached to the ArsB inner-membrane protein[65,70] and energizing the arsenite efflux pump by ATP hydrolysis[16,57]. It is ArsA that makes this an ATPase pump and in the absence of ArsA, the remaining ArsB protein functions as a membrane potential-driven secondary pump, providing a lower level of resistance[3,16]. Such alternative energy coupling is unique among known bacterial uptake or efflux transport systems. To date, all other systems that have been studied are either chemiosmotic or ATP-driven transporters. The arsenite pump is the only one that can be converted from one mode of energy coupling to the other by addition of or removal of genes, which happens in natural systems[30,57] and can be reconstructed also in laboratory studies[16].

A gene product common to all *ars* operons is ArsC, the reductase enzyme that reduces less toxic arsenate [As(V)] to more toxic arsenite [As(III)][21,24,26]. It is only As(III) and not As(V) that is pumped out from the cells by the ArsB transport protein[3,16]. It seems illogical from an environmental biology or chemistry point of view to convert a less toxic compound to a more toxic form, but ArsC activity is closely coupled with efflux from the cells so that intracellular arsenite never accumulates[26].

Arsenate reductases from plasmids of Gram negative bacteria and Gram positive bacteria both reduce arsenate and both confer arsenate resistance[21,24,25,26]. However, their in vitro measured properties are very different and their energy coupling is different. Arsenate reductase of Gram positives derives reducing power from a small protein called thioredoxin[24,26], which is used in many processes of central metabolism of bacteria and higher organisms. In contrast arsenate reductase of Gram negative bacteria uses glutaredoxin[21], which is a related but different protein. The small coupling proteins are not exchangable.

In addition to plasmid arsenic resistance that is well understood and for which clusters of genes have been isolated and sequenced, there are bacterial arsenic metabolism systems that involve oxidation of arsenite to arsenate[2], reduction of arsenate to arsenite as part of an oxyanion-coupled anaerobic respiration[1,33] or the coupled cleavage of carbon-arsenic bonds with oxidation to arsenate[47]. These systems appear to be of major environmental concern in arsenic-containing settings, but they have not been approached by molecular genetics as yet.

Copper

We do not know how copper in natural environments effect or select for resistant bacteria. What has been studied to date are one set of related systems for plasmid resistance in Gram negative bacteria that have been selected by agricultural use of copper salts, both as sprays for tomato plants and walnuts, and as growth-stimulating food additives for pigs, and another totally different system found on the chromosomes of many bacteria, both Gram positive and Gram negative, and apparently representing a low level normal homeostatic mechanism for regulating and maintaining the intracellular copper needed for nutrition in the presence of varying high and low levels of copper in the environment.

Strong copper resistance has been described with plasmids in Gram negative bacteria from agricultural sources[4,14,15]. There are two regulatory genes, called *pcoR* and *pcoS* in *E. coli* and the five structural genes are *pcoABCDE*[4,5] (Fig. 4). For *Pseudomonas*, the comparable genes are called *copR, copS* and *copABCD* and there is no equivalent to *pcoE*[14,15]. The Cop/PcoR and Cop/PcoS proteins were the first example among the metal resistance systems of transcriptional regulation by a "two component" regulatory system. (A second and third example will be described below.) The sensor protein PcoS is found in the membrane and probably can be labeled with radio-

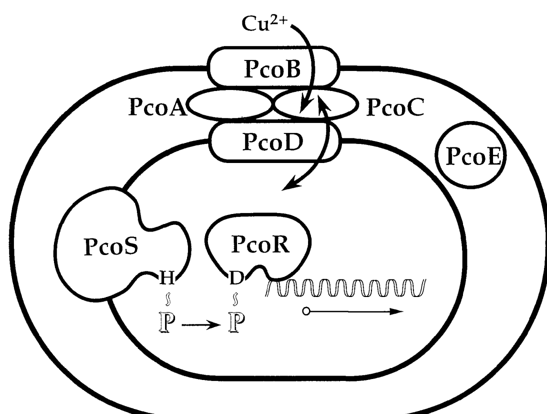


Fig. 4. Plasmid copper resistance. The proposed locations and functions of the *pco* gene products from *E. coli* (see[4,5] for specifics[5]).

active ^{32}P-phosphate at a specific conserved histidine residue from radioactive ATP. The DNA-binding responder protein PcoR is thought to have the phosphate transferred from PcoS to a specific conserved aspartate amino acid residue to form ^{32}P-labeled Pco R[4,5,14].

The four structural proteins determining copper resistance have been characterized in *Pseudomonas* and are the inner membrane protein CopD, the outer membrane protein CopB, and two periplasmic proteins CopA and CopC[10,11,15] (Fig. 4). CopA and CopC are blue copper-containing proteins. It is thought that storage of excess copper in the periplasmic space between the outer and inner cell membranes protects the cell from toxic copper. How CopD and CopB are involved in movement of copper across the membranes is not understood. A major problem in understanding of this system is that colonies of the copper resistant *Pseudomonas* turn blue when grown in high copper-containing media, while those of other bacteria turn brown, and show no sign of periplasmic copper storage. Furthermore, there is preliminary evidence for copper efflux (not uptake) associated with the *E. coli* copper resistance system[4,5]. How the same genes can lead to different overall processes is unclear. However, the *E. coli* plasmid system[4,5] includes an additional gene *pcoE*, the product of which is a periplasmic copper binding protein, and that is highly produced[50].

Models for these gene functions have changed from year to year. However, all models of copper homeostasis and resistance involve the control of cellular uptake and efflux of copper by chromosomal genes, as well as plasmid systems for additional resistance in high copper environments[4,14,15].

Chromosomal copper resistance in *Enterococcus hirae* is entirely different from that reported above and is indeed the best understood copper transport and resistance system[42,43,44,61,62]. Two genes, *copA* and *copB*, that determine respectively uptake and efflux P-type ATPases, are found in a single operon[42]. The system is regulated in response to both copper-starvation (when the CopA uptake ATPase is needed) and copper-excess (when the CopB efflux ATPase is needed)[44]. *Enterococcus* CopA and CopB have the same names but different structures and functions from the *Pseudomonas* plasmid genes for copper resistance[14]. *E. hirae* mutants lacking the CopA uptake ATPase become somewhat copper-resistant and require higher levels of medium copper for growth. Bacterial mutants lacking the CopB efflux ATPase become copper-hypersensitive.

The CopB copper efflux ATPase of *E. hirae* is unusual among bacterial cation efflux ATPases in that actual subcellular ATPase, ATP-labeling and transport data are available. Solioz and Odermatt[61] isolated inside-out, subcellular, membrane vesicles from *E. hirae* cells and the vesicles required ATP in order to accumulate $^{64}Cu^{+}$ and $^{110m}Ag^{+}$. The in vitro substrate for CopB is thought to be Cu^{+} rather than Cu^{2+}. Whether copper is taken up initially as Cu^{2+} and subsequently reduced to Cu^{+} or whether copper is reduced at the cell surface (before or concomitant with transport) is not established.

Cadmium

The Cd^{2+} efflux ATPase is found in Gram positive bacteria from diverse sources, including soil bacilli and clinical *Listeria*[56,57,59]. The protein structure as diagramed (Fig. 5A) is typical of P-type ATPases. It is presented here for environmental scientists as a strong example of the claim that understanding only comes from the details. As in chemical sciences, to

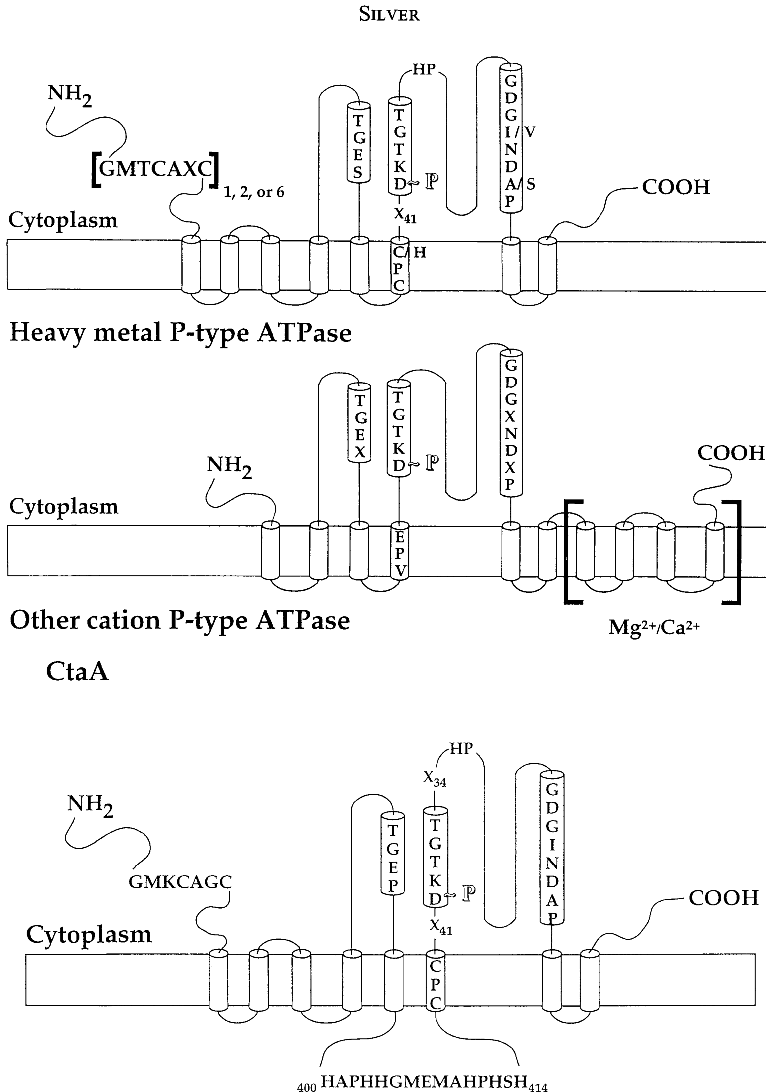


Fig. 5. Heavy-metal cation (e.g. Cd^{2+}) (Part A) and nutrient cation (e.g. Ca^{2+} and Mg^{2+} (Part B) P-type ATPases. The predicted motifs (cation-binding, phosphatase, membrane channel, and aspartyl kinase) regions are shown. Modified after Solioz and Vulpe[62] and earlier models

skim the surface often means missing the point. The Cd^{2+} efflux ATPase starts with a metal-binding motif, including a pair of adjoining cysteine amino acids with metal-binding thiol groups. Six metal-binding motifs occur in the human copper transporting efflux ATPases that are defective in the hereditary diseases Menkes and Wilson's[56,59]. The remarkable similarity between these ATPases of animals, plants and bacteria is a major recent finding. There follows a membrane ATPase region closely homologous to other P-type ATPases. This includes the eight predicted membrane spanning regions shown in Fig. 5A for the heavy metal translocating ATPases[62], the sixth of which is thought to be involved in the cation translocation pathway. It includes a conserved proline residue (as shown) between cysteines that are found in the Cd^{2+} ATPases and related proteins.

Melchers et al.[34] recently provided detailed data us-

ing protein fusions supporting the eight segment model for the first presumed Ni^{2+} effluxing ATPase, from *Helicobacter pylori*. Whereas the bacterial potassium ATPase KdpB had been modeled as having six membrane spanning segments in the comparable region, the Mg^{2+} ATPases and (postulated) Ca^{2+} ATPases have an addition four segments toward the carboxyl end for a total of 10 as shown in Fig. 5B. Therefore, we conclude that P-type ATPases differ in membrane topology and length, depending more on cation specificity than on the difference between uptake and efflux directions.

Two intracellular domains shown in the model and common to P-type ATPases are the aspartyl kinase domain (including the site of aspartyl-phosphorylation) and the phosphatase domain, involved in removing the phosphate from the aspartate residue during the reaction and transport cycle. The name for this class of ATPases, "P-type", is used since they are the only transport ATPases that have a covalent phospho-protein intermediate.

Large plasmids of the soil chemilithotrophic autotroph *Alcaligenes* have numerous heavy metal resistance determinants, including mercury and chromate resistance, and three related ones for divalent cations, called *czc* (for Cd^{2+}, Zn^{2+} and Co^{2+} resistances), *ncc* (for Ni^{2+}, Cd^{2+} and Co^{2+} resistances), and *cnr* (for Co^{2+} and Ni^{2+} resistances)][17,41,57]. These closely related systems contain basically the same three structural proteins. Indeed, mutations of the Cnr system give additional Zn^{2+} resistance, again showing that the two systems are fundamentally the same. Czc is an efflux pump that functions as a chemiosmotic divalent cation/proton antiporter[39,40]. The proteins involved have become the paradigm for a new family of three-component chemiosmotic exporters[17,41], which we call CBA systems for the order of transcription of the genes and to contrast them with the ABC ("ATP-binding cassette" multicomponent ATPases)[53]. CzcC is thought to be an outer membrane protein. CzcB appears to be a "membrane fusion protein" that bridges the inner and outer cell membrane of Gram negative bacteria[17,27]. And CzcA is the central inner membrane transport protein of over 1000 amino acids in length. Several additional regulatory genes are involved, but there is incomplete understanding of their number or functions. Two of these, *czcR* and *czcS* encode a second pair of cation-sensing sensor kinase (CzcS) and transphosphorylated responder (CzcR) proteins, homologous to PcoS and PcroR for plasmid copper resistance.

Chromate

Chromate resistance and chromate reduction both occur, but resistance to chromate governed by bacterial plasmids appears to have nothing to do with chromate reduction. Furthermore, it is not clear whether chromate reduction ability that has been found with several bacterial isolates confers resistance to CrO_4^{2-} [46]. Plasmid-determined chromate resistance results from reduced uptake of CrO_4^{2-} by the resistant cells[45]. The DNA sequences of the *P. aeruginosa* and *A. eutrophus* chromate resistance systems share homologous *chrA* genes, which encode membrane proteins[9,38]. A third *chrA* gene was found on a plasmid of a cyanobacterium[37], so we anticipate more examples of chromate resistance operons will be found as new total genomes are sequenced[6,60].

Tellurite

There are well-studied and indeed several sequenced determinants of plasmid-governed tellurite resistance, but in each case we do not understand the mechanism of tellurite resistance[27,68]. Tellurite resistance does not appear to involve reduction to black metallic tellurium—which indeed occurs, especially if resistance allows cell growth. As the British philosopher Lugwig Wittgenstein suggested, if you do not know anything then be quiet. It is enough to say that microbial tellurite resistance and metabolism exist, but are inadequately understood..

Silver

We have started working on the genetic basis of plasmid-determined silver resistance, beginning with a plasmid from *Salmonella* and Fig. 6 represents a summary of current understanding and our first presentation of as yet unpublished results. It requires 14 kb of DNA to encode the 9 genes apparently involved in bacterial silver resistance and the functions

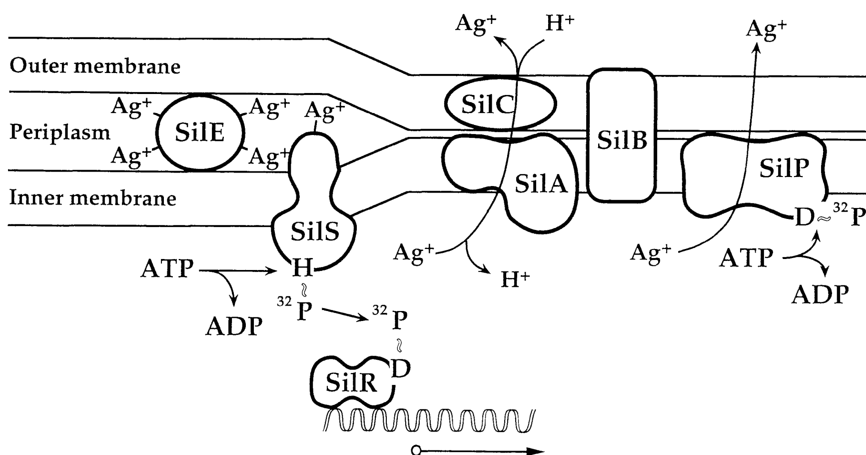


Fig. 6. Model for proteins and functions of the new plasmid silver resistance determinant (from A. Gupta, et al., submitted)

of 7 of these are diagramed in Fig. 6. Two of these, *silS* and *silR* encode another pair of cation-sensing sensor kinase (SilS) and transphosphorylated responder (SilR) proteins, homologous to PcoS/PcroR for copper resistance and CzcS/CzcR for cadmium, zinc and cobalt resistances (Figure 6). As with the *pco* system, *silRS* is followed by *silE*, the determinant of a periplasmic Ag^{+} -binding protein (direct data are available), homologous to PcoE.

However, the remainder of the silver resistance determinant is transcribed in the opposite direction (unlike the situation in *pco*)[5] and encodes both a three component CBA system (Fig. 6), weakly homologous to CzcCBA and a P-type ATPase that is generally speaking in the family of heavy metal responding enzymes diagramed in Fig. 5A, except for the absence of a CX2C metal-binding motif. In the same position, a series of histidine residues may be functioning to recognize and bind Ag^{+}. After cloning and sequencing the first such silver resistance determinant, gene-specific DNA probes were used to show that similar (but not identical) systems occur in a wide range of enteric bacteria from clinical sources, including burn patients, where silver salts were used as antiseptics, and metallic silver catheters(A. Gupta et al., submitted).

Whereas, silver resistance is initially being studied in clinical isolates, as had been the case for mercury and arsenic resistances in earlier years, we anticipate that this resistance system will be important for developing metal-resistant microbes for the mining industry, which is frequently becoming an applied microbiology subject[48]. We expect that the next few years will allow us to solve the molecular genetic and biochemical basis for highly specific Ag^{+} resistance in some detail, as well as to find the comparable systems in other groups of bacteria. It already is clear, however, that reduction of Ag^{+} to metallic Ag(0) is not involved.

Other toxic metals

In addition to the specific resistances discussed above, several additional resistances are listed in the Figure 2. These have been studied still less and are material for future research. Lead (Pb^{2+}) resistance on plasmids appears to have a different basis in soil *Alcaligenes*, where still another cation-specific P-type ATPase has been found (D. van der Lelie, personal communication) and in *Staphylococcus*, where a process involving precipitation in intracellular lead-phosphate granules[31] has been proposed. A plasmid resistance to tributyltin (used as anti-fouling compounds for ship hulls) has been reported[35].

Bacterial metallothionein

Bacterial metallothioneins, functionally homologous to the small (approximately 60 amino acids long), thiol-rich (perhaps 20 of those 60 amino acids are cysteines) mammalian metal binding proteins, have rarely been reported and has been studied in detail only for the cyanobacterial genus *Synechococcus*[22,67]. The 58 amino acid polypeptide product of the *smtA* gene contains nine cysteine residues, which are clustered in groups of 4 and 5 respectively, as are the cysteines in animal metallothioneins. Metallothionein cysteines are clustered in two domains that bind divalent cations independently. The synthesis of metallothionein is regulated at three levels[67]. Firstly, the SmtB repressor protein binds divalent cations and dissociates from the target DNA[66]. Secondly, there is gene amplification so that tandem multiple copies of the metallothionein locus are produced in metal-stressed cells[22]; and thirdly, a specific deletion between repeated sequences on the DNA removes most of the *smtB* gene[23] for the repressor protein.

Final Comment

This summary of how microbial cells "cope" with the inorganic cations and anions from elements of the Periodic Table is not directly microbial ecology. The approach here has been from the gene to the physiological process. Such an approach reflects the success of microbial genetics in our lifetimes, where a genetic approach is usually the most powerful. A quite different approach to understanding the biology of inorganic elements is being called "bioinorganic chemistry"[20,32] and is generally based more on chemical principles, minimizing the powers of microbiology and genetics. Obviously, the middle ground is what is sought by all concerned—that is fundamental understanding behind problems of universal and practical importance.

Acknowledgments and Apology

The references below are highly selected and frequently to review articles written by myself and many colleagues and friends (frequently listed as authors or co-authors below) over 30 years. This is a limitation necessitated by space and by the needs of the intended audience only for a general view, and not for a highly technical listing of where facts were first reported. Apologies are given here for this limitation.

References

1) Ahmann D., A.L. Robert, L.R. Krumholz and F.M.M. Morel. 1994. Microbe grows by reducing arsenic. Nature 371: 750.
2) Anderson G.L., J. Williams and R. Hille. 1992. The purification and characterization of arsenite oxidase from *Alcaligenes faecalis*, a molybdenum-containing hydroxylase. J. Biol Chem 267: 23674–23682.
3) Broer S., G. Ji, A. Broer and S. Silver. 1993. Arsenic efflux governed by the arsenic resistance determinant of *Staphylococcus aureus* plasmid pI258. J. Bacteriol 175: 3480–3485.
4) Brown N.L., B.T.O. Lee and S. Silver. 1994. Bacterial transport of and resistance to copper. In: Metal Ions in Biological Systems (Sigel H. and A. Sigel, eds) vol 30, pp 405–434, Marcel Dekker, N.Y.
5) Brown N.L., S.R. Barrett, J. Camakaris, B.T.O. Lee and D.A. Rouch. 1995. Molecular genetics and transport analysis of the copper-resistance determinant (*pco*) from *Escherichia coli* plasmid pRJ1004. Mol Microbiol 17: 1153–1166.
6) Bult C., O. White, G.J. Olsen, L. Zhou, R.D. Fleischmann, G.G. Sutton, J.A. Blake, L.M. FitzGerald, R.A. Clayton, J.D. Gocayne, A.R. Kerlavage, B.A. Dougherty, J.-F. Tomb, M.D. Adams, C.I. Reich, R. Overbeek, E.F. Kirkness, K.G. Weinstock, J.M. Merrick, A. Glodek, J.L. Scott, N.S.M. Geohagen, J. Weidman, J.L. Fuhrmann, D. Nguyen, T.R. Utterback, J.M. Kelley, J.D. Peterson, P.W. Sadow, M.C. Hanna, M.D. Cotton, K.M. Roberts, M.A. Hurst, B.P. Kaine, M. Borodovsky, H.-P. Klenk, C.M. Fraser, H.O. Smith C.R. Woese, and J.C. Venter. 1996. Complete genome sequence of the methanogenic Archaeon, *Methanococcus jannaschii*. Science 273: 1058–1073.
7) Carlin A., W. Shi, S. Dey and B.P. Rosen. 1995. The *ars* operon of *Escherichia coli* confers arsenical and antimonial resistance. J Bacteriol 177: 981–986.
8) Cervantes C., G. Ji, J.L. Ramrez and S. Silver. 1994. Resistance to arsenic compounds in microorganisms. FEMS Microbiol Rev 15: 355–367.
9) Cervantes C., H. Ohtake, L. Chu, T.K. Misra and S. Silver. 1990. Cloning, nucleotide sequence and ex-

pression of the chromate resistance determinant of *Pseudomonas aeruginosa* plasmid pUM505. J. Bacteriol 172: 287–291.

10) Cha J.-S. and D.A. Cooksey. 1991. Copper resistance in *Pseudomonas syringae* mediated by periplasmic and outer membrane proteins. Proc Natl Acad Sci USA 88: 8915–8919.
11) Cha J.-S. and D.A. Cooksey. 1993. Copper hypersensitivity and uptake in *Pseudomonas syringae* containing cloned components of the copper resistance operon. Appl Environ Microbiol 59: 1671–1674.
12) Chen Y. and B.P. Rosen. 1997. Metalloregulatory properties of the ArsD repressor. J. Biol Chem 272: 14257–14262.
13) Clayton R.A., O. White, K.A. Ketchum and J.C. Venter. 1997. The first genome from the third domain of life. Nature 387: 459–462.
14) Cooksey D.A. 1993. Copper uptake and resistance in bacteria. Mol Microbiol 7: 1–5.
15) Cooksey D.A. 1994. Molecular mechanisms of copper resistance and accumulation in bacteria. FEMS Microbiol Rev 14: 381–386.
16) Dey S. and B.P. Rosen. 1995. Dual mode of energy coupling by the oxyanion-translocating ArsB protein. J. Bacteriol 177: 385–389.
17) Diels L., Q. Dong, D. van der Lelie, W. Baeyens and M. Mergeay. 1995. The *czc* operon of *Alcaligenes eutrophus* CH34: from resistance mechanism to the removal of heavy metals. J. Industr Microbiol 14: 142–153.
18) Diorio C., J. Cai, J. Marmor, R. Shinder and M.S. DuBow. 1995 An *Escherichia coli* chromosomal *ars* operon homolog is functional in arsenic detoxification and is conserved in Gram-negative bacteria. J Bacteriol 177: 2050–2056.
19) Distefano M.D., MJ Moore and C.T. Walsh. 1990. Active site of mercuric reductase resides at the subunit interface and requires Cys135 and Cys140 from one subunit and Cys558 and Cys559 from the adjacent subunit: evidence from in vivo and in vitro heterodimer formation. Biochemistry 29: 2703–2713.
20) Frausto da Silva J.J.R. and R.J.P. Williams. 1991. The Biological Chemistry of the Elements. The Inorganic Chemistry of Life. Clarendon Press, Oxford, 561 pp.
21) Gladysheva T.B., K.L. Oden and B.P. Rosen. 1994. Properties of the arsenate reductase of plasmid R773. Biochemistry 33: 7288–7293.
22) Gupta A., B.A. Whitton, A.P. Morby, J.W. Huckle and N.J. Robinson. 1992. Amplification and rearrangement of a prokaryotic metallothionein locus *smt* in *Synechococcus* PCC 6301 selected for tolerance to cadmium. Proc Roy Soc Lond B248: 273–281.
23) Gupta A., A.P. Morby, J.S. Turner, B.A. Whitton and N.J. Robinson. 1993. Deletion within the metallothionein locus of cadmium-tolerant *Synechococcus* PCC 6301 involving a highly iterated palindrome (HIP1). Mol Microbiol 7: 189–195.
24) 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.
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) 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: 7974–7978.
27) Ji G. and S. Silver. 1995. Plasmid resistance mechanisms for heavy metals of environmental concern. J Industr Microbiol 14: 61–75.
28) Kaur P. and B.P. Rosen. 1993. Complementation between nucleotide binding domains in an anion-translocating ATPase. J. Bacteriol 175: 351–357.
29) Koonin E.V. 1993. A superfamily of ATPases with diverse functions containing either classical or deviant ATP-binding motif. J. Mol Biol 229: 1165–1174.
30) 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.
31) Levinson H.S., I. Mahler, P. Blackwelder and T. Hood. 1996. Lead resistance and sensitivity in *Staphylococcus aureus*. FEMS Microbiol Letters 145: 421–425.
32) Lippard S.J. and J.M. Berg. 1994. Principles of Bioinorganic Chemistry. University Science Books, Mill Valley, CA.
33) Macy J.M., K. Nunan, K.D. Hagen, D.R. Dixon, P.J. Harbour, M. Cahill and L.I. Sly. 1996. *Chrysiogenes arsenatis* gen. nov., sp. nov., a new arsenate-respiring bacterium isolated from gold mine wastewater. Intern J System Bacteriol 46: 1153–1157. .
34) Melchers K., T. Weitzenegger, A. Buhmann, W. Steinhilber, G. Sachs and K.P. Schfer. 1996. Cloning and membrane topology of a P type ATPase from *Helicobacter pylori*. J. Biol Chem 271: 446–457.
35) Miller C.E., S. Wuertz, J.J. Cooney and R.M. Pfister. 1995. Plasmids in tributyltin-resistant bacteria from fresh and estuarine waters. J. Industr Microbiol 14: 337–342.
36) Nakamura K. and S. Silver. 1994. Molecular analysis of mercury-resistant *Bacillus* isolates from sediment of Minamata Bay, Japan. Appl Environ Mi-

crobiol 60:4596–4599.

37) Nicholson M.L. and D.E. Laudenbach. 1995. Genes encoded on a cyanobacterial plasmid are transcriptionally regulated by sulfur availability and CysR. J Bacteriol 177: 2143–2150.

38) Nies A., D.H. Nies and S. Silver. 1990. Nucleotide sequence and expression of a plasmid-encoded chromate resistance determinant from *Alcaligenes eutrophus*. J. Biol Chem 265: 5648–5653.

39) Nies D.H. 1995. The cobalt, zinc, and cadmium efflux system CzcABC from *Alcaligenes eutrophus* functions as a cation-proton antiporter in *Escherichia coli*. J. Bacteriol 177: 2707–2712.

40) Nies D.H., A. Nies, L. Chu and S. Silver. 1989. Expression and nucleotide sequence of a plasmid-determined divalent cation efflux system from *Alcaligenes eutrophus*. Proc Natl Acad Sci USA 86: 7351–7355.

41) Nies D.H. and S. Silver. 1995. Ion efflux systems involved in bacterial metal resistances. J. Industr Microbiol 14: 186–199.

42) 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.

43) Odermatt A., H. Suter, R. Krapf and M. Solioz. 1994. Induction of the putative copper ATPases, CopA and CopB, of *Enterococcus hirae* by Ag^{+} and Cu^{2+}, and Ag^{+} extrusion by CopB. Biochem Biophys Res Commun 202: 44–48.

44) Odermatt A. and M. Solioz. 1995. Two trans-acting metalloregulatory proteins controlling expression of the copper-ATPases of *Enterococcus hirae*. J. Biol Chem 270: 4349–4354.

45) Ohtake H., C. Cervantes and S. Silver. 1987. Decreased chromate uptake in *Pseudomonas fluorescens* carrying a chromate resistance plasmid. J. Bacteriol 169: 3853–3856.

46) Ohtake H. and S. Silver. 1994. Bacterial reduction of toxic chromate. In: Biological Degradation and Bioremediation of Toxic Chemicals (Chaudhry GR ed), pp. 403–415. Chapman and Hall, London.

47) Quinn J.P. and G. McMullan. 1995. Carbon-arsenic bond cleavage by a newly isolated Gram-negative bacterium, strain ASV2. Microbiology 141: 721–727.

48) Rawlings D.E. and S. Silver. 1995. Mining with microbes. Nature Bio/Technology 13: 773–778.

49) Rochelle P.A., M.K. Wetherbee and B.H. Olson. 1991. Distribution of DNA sequences encoding narrow- and broad-spectrum mercury resistance. Appl Environ Microbiol 57: 1581–1589.

50) Rouch D.A. and N.L. Brown. 1997. Copper-inducible transcriptional regulation from two promoters in the *Escherichia coli* copper resistance determinant *pco*. Microbiology 143: 1191–1202.

51) Schiering N., W. Kabsch, M.J. Moore, M.D. Distefano, C.T. Walsh and E.F. Pai. 1991. Structure of the detoxification catalyst mercuric ion reductase from *Bacillus* sp. strain RC607. Nature 352: 168–171.

52) Schottel J., A. Mandal, D. Clark, S. Silver and R.W. Hedges. 1974. Volatilisation of mercury and organomercurials determined by inducible R-factor systems in enteric bacteria. Nature 251: 335–337.

53) Silver S. 1996. Transport of inorganic cations. In: *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology, Second Edition (Neidhardt FC et al, eds), pp.1091–1102, ASM Press, Washington, DC.

54) Silver S. 1996. Bacterial resistances to toxic metal ions. Gene 179: 9–19.

55) Silver S. and G. Ji. 1994. Newer systems for bacterial resistances to toxic heavy metals. Environ Health Perspect 102 (Suppl 3): 107–113.

56) Silver S., G. Nucifora and L.T. Phung. 1993. Human Menkes X chromosome disease and the staphylococcal cadmium resistance ATPase: a remarkable similarity in protein sequences. Mol Microbiol 10: 7–12.

57) Silver S. and L.T. Phung. 1996. Bacterial heavy metal resistance: new surprises. Annu Rev Microbiol 50: 753–789.

58) S. Silver, G. Endo, and K. Nakamura. 1994. Mercury in the environment and laboratory. J Japan Soc Water Environ 17, 235–243. .

59) Silver S. and M. Walderhaug. 1995. Bacterial plasmid-mediated resistances to mercury, cadmium and copper. In: Toxicology of Metals. Biochemical Aspects (Goyer RA and Cherian MG, ed), pp. 435–458. Springer-Verlag, Berlin.

60) Sofia H.J., V. Burland, D.L. Daniels, G. Plunkett III and F.R. Blattner. 1994. Analysis of the *Escherichia coli* genome. V. DNA sequence of the region from 76.0 to 81.5 minutes. Nucleic Acids Res 22: 2576–2586.

61) 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.

62) 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.

63) Summers A.O. 1992 Untwist and shout: a heavy metal-responsive transcriptional regulator. J. Bacteriol 174: 3097–3101.

64) Takemaru K.-I., M. Mizuno, T. Sato, M. Takeuchi and Y. Kobyashi. 1995. Complete nucleotide sequence of a skin element excised by DNA rearrangement during sporulation in *Bacillus subtilis*. Microbiology 141: 323–327.

65) Tisa L.S. and B.P. Rosen. 1990. Molecular charac-

terization of an anion pump: the ArsB protein is the membrane anchor for the ArsA protein. J. Biol Chem 265: 190–194 .

66) Turner J.S., P.D. Glands, A.C.R. Sampson and N.J. Robinson. 1996. Zn^{2+} -sensing by the cyanobacterial metallothionein repressor SmtB: different motifs mediate metal-induced protein-DNA dissociation. Nucleic Acids Res 24: 3714–3721.

67) Turner J.S. and N.J. Robinson. 1995. Cyanobacterial metallothioneins: biochemistry and molecular genetics. J. Industr Microbiol 14: 119–125.

68) Walter E.G. and D.E. Taylor. 1992. Plasmid-mediated resistance to tellurite: expressed and cryptic. Plasmid 27: 52–64.

69) Wu J. and B.P. Rosen. 1993. Metalloregulated expression of the *ars* operon. J. Biol Chem 268: 52–58.

70) Wu J., L.S. Tisa and B.P. Rosen. 1992. Membrane topology of the ArsB protein, the membrane subunit of an anion-translocating ATPase. J. Biol Chem 267: 12570–12576.