Journal of Bioenergetics and Biomembranes, Vol. 22, No. 4, 1990

MINI-REVIEW

Transport Systems Encoded by Bacterial Plasmids

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Received October 23, 1989

Abstract

A variety of bacterial functions are encoded on plasmids, extrachromosomal elements. Examples of plasmid-borne functions are antibiotic production and resistance, degradation of recalcitrant chemicals, virulence factors, and plant symbiotic properties. Several transport systems with diverse functions have recently been found to be carried on plasmids. These systems serve to either accumulate or extrude a compound from a cell. The focus of this review is to present a survey on several of these novel plasmid-borne transport systems emphasizing functions, components, and molecular genetics.

Key Words: Transport; ion pumps; antibiotic resistance; heavy metal resistance; plasmids.

Introduction

Bacterial plasmids are extrachromosomal elements which encode a variety of ancillary physiological functions. These include genes for sexual transmission and for resistance to toxic compounds such as antibiotics and heavy metals. Thus these plasmids can transfer their genes from cell to cell and even from species to species, spreading resistance to chemotherapeutic agents to previously susceptible bacteria. The mechanisms of resistance vary, but a frequently exploited stratagem is transport of the toxic compound out of the cell, reducing the intracellular concentration to sublethal levels. Additionally some plasmid-encoded transport systems are uptake systems which allow for growth on substrates otherwise nonutilizable by the cells. Portions of these topics have been examined recently (Mobley and Summers, 1987; Silver and

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0145-479X/90/0800-0493$06.00/0

Misra, 1988), but the molecular details of several of the systems have been elucidated since then, calling for an update of the subject. This review summarizes the known plasmid-encoded transport systems and explores several in depth.

Arsenic and Antimony Transport

There are two known plasmid-encoded transport systems which function to confer resistance to arsenical and antimonial salts by actively extruding the toxic oxyanions out of the cell. This results in a reduction in the intracellular concentration of the anions. The most thoroughly studied system is one encoded by the conjugative plasmid R773 (Hedges and Baumberg, 1973), which confers resistance to arsenate (As^{V}), arsenite (As^{III}), and antimonite (Sb^{III}) on gram-negative bacteria, in particular *Escherichia coli.* A related resistance determinant is carried by the plasmid pI258, which is expressed in gram-positive bacteia such as *Staphylococcus aureus* (Novik and Roth, 1968).

Silver *et al.* (1981) first demonstrated that either plasmid produced inducible extrusion of arsenate out of cells, of *E. coli* or *S. aureus.* Studies of the energetics of extrusion from intact cells have shown that resistance occurs by an active process (Mobley and Rosen, 1982; Silver and Keach, 1982; Rosen and Borbolla, 1984). Both arsenate and arsenite were extruded unmodified from the cells, indicating that no redox cycle is involved. Conditions were established in which the effect of chemical energy or electrochemical energy were evaluated independently in cells. A strain of *E. coli* lacking a functional H^{+}-translocating ATPase was starved of endogenous energy reserves. These cells were unable to extrude either arsenate or arsenite. When chemical energy was supplied through glucose metabolism, extrusion occurred. Neither inhibitors of respiration nor uncouplers had an effect on extrusion. Conditions were established in which electrochemical energy was produced in the absence of ATP synthesis. Such cells were unable to extrude either arsenical. Thus a proton-motive force was neither necessary nor sufficient for active extrusion of the oxyanions, while chemical energy was sufficient. A temporal relationship between extrusion and ATP synthesis was observed. These results suggested that ATP was the driving force for oxyanion extrusion. Although an anion-translocating ATPase could be postulated, *in vivo* experiments were insufficient to demonstrate the mechanism.

In order to determine the molecular mechanism of anion transport, information is required about the number and properties of the components. The first step in that characterization was the cloning and sequencing of the arsenical resistance (*ars*) operon of plamid R773. The *ars* operon consists of four genes: three structural genes, *arsA*, *arsB*, and *arsC* (Mobley *et al.*, 1983;

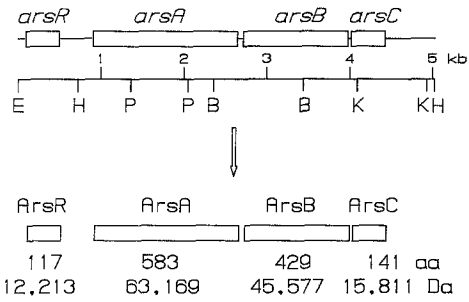

Fig. 1. Physical map of the *ars* operon. Open reading frames in the DNA and their protein products are indicated by boxes, with the size and mass of each protein below. Restriction endonuclease sites: *B*, *BamH*I: *E*, *EcoR*I; *H*, *Hind*III; *P*, *Pst*I; *K*, *Kpn*I.

Chen *et al.*, 1985, 1986) and one regulatory, *arsR* (San Francisco *et al.*, 1990). The genes and polypeptide products of the *ars* operon are summarized in Fig. 1. Resistance to arsenite and antimonite requires only the *arsA* and *arsB* gene products Chen *et al.*, 1985; Rosen and Borbolla, 1984). The *arsC* gene product (141 residues, 15, 811 daltons) is required in conjunction with the *arsA* and *arsB* products to confer arsenate resistance.

The ArsB protein (429 residues, 45, 577 daltons) has been identified as an integral membrane protein localized in the inner membrane of *E, coli* (Chen *et al.*, 1986; San Francisco *et al.*, (1989) and has been postulated to be the anion channel component of the pump. The ArsA protein (583 residues, 63,169 daltons) has been identified (Chen *et al.*, 1985, 1986) and purified to homogeneity from the cytosol of *E. coli* (Rosen *et al.*, 1988; Hsu and Rosen, 1989b). The protein binds ATP and exhibits arsenite (antimonite)-stimulated ATPase activity. When expressed from a strong promoter, the ArsA protein is overproduced to about 10% of total cell protein. Under these conditions, the ArsA protein is a soluble protein located primarily in the cytosol. A small amount of the protein was found to be associated with the inner membrane (Mobley *et al.*, 1983). The ArsB protein is required to anchor the ArsA protein to the inner membrane (Tisa and Rosen, 1990). However, insufficient ArsB protein is produced to anchor all of the overexpressed ArsA protein, explaining the apparent cytosolic location of the ArsA protein. Although the *ars* operon is transcribed as a single mRNA, the transcript is rapidly degraded in the *arsB* region, resulting in the observes differential translation of the *ars* proteins (Owolabi and Rosen, 1990).

Membranes lacking ArsB protein had no bound ArsA protein. Membranes in which the ArsA protein was washed off with urea or membranes from cells expressing the *arsB* gene alone exhibited specific binding of purified ArsA protein. The latter indicates that the ArsB protein is synthesized and inserted into the membrane in the absence of the ArsA

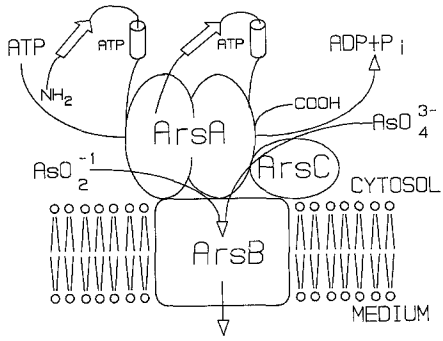

Fig. 2. Model of the arsenical pump. The integral membrane protein ArsB functions as an anion channel. The ArsA protein is an extrinsic membrane protein with oxyanion-stimulated ATPase activity. The complex of the ArsA and ArsB proteins is an ATP-coupled pump for arsenite or antimonite. Interaction of the ArsC protein with the complex allows recognition and extrusion of arsenate.

protein. The membrane-bound ArsA protein exhibited oxyanion-stimulated ATPase activity. The specific activity of the membrane-bound form was as great or greater than the soluble form, while the concentrations of arsenite and antimonite sufficient for half-maximal ATPase activity were of the same order of magnitude. Thus the ArsA and ArsB polypeptides from a membrane complex which is postulated to function as an oxyanion-translocating ATPase (Chen *et al.*, 1986; Tisa and Rosen, 1990). The ArsA protein functions as the catalytic energy-transducing component, while the integral membrane ArsB protein acts as an anion channel and the membrane binding site for the ArsA protein (Fig. 2).

The novel system is unique in being an anion pump. All other ion-translocating ATPase have cations as substrated (Pedersen and Carafoli, 1987). From analysis of primary sequence, the cation pumps all belong to two families of proteins. The oxyanion pump is the first identified member of a third family of ion pump. The only other possible anion-translocating ATPase to be reported is the CFTR protein, the putative product of the recently described CF gene (Riordan *et al.*, 1989). The CFTR protein has two consensus nucleotide binding sequences. These sequences are the same as the two potential nucleotide binding sequences of the ArsA protein, although the two proteins are otherwise unrelated. The CFTR protein belongs to a family of pumps which act on an amazing variety of substrates (Higgins, 1989). Mutation in this gene results in cystic fibrosis. One of the major clinical problems in cystic fibrosis patients is a defect in chloride secretion. This has led to the postulate that the CFTR protein is a chloride-translocating ATPase which catalyzes chloride extrusion from cells.

The availability of purified ArsA protein has facilitated the characterization of this protein (Rosen *et al.*, 1988; Hsu and Rosen, 1989a, b). From an

analysis for the nucleotide sequence, the *arsA* gene is the product of a duplication of a gene ancestral to both the *arsA* and the *nifH* gene of nitrogenase (Chen *et al.*, 1986; Hsu and Rosen, 1989a). Thus the N-terminal half of the ArA protein has similarity to the C-terminal half. Two potential nucleotide binding sequences, one in each half of the protein, are similar to a consensus nucleotide binding sequence (Walker *et al.*, 1982). Ultraviolet photocrosslinking experiments using α-[^{32}P]ATP demonstrate the ability of the ArsA protein to bind ATP in a Mg^{2+}-dependent fashion (Rosen *et al.*, 1988).

The N-terminal ATP binding site was altered by site-directed and bisulfite mutagenesis, changing three residues (Karkaria and Rosen, 1990). Cells bearing the mutated plasmids were sensitive to arsenicals and unable to extrude $^{73}AsO_2^{-1}$. The altered ArsA proteins were produced in normal amounts, but the purified mutant proteins were catalytically inactive and did not bind α-[^{32}P]ATP. These results support the model that the ArsA protein is the catalytic subunit of the oxyanion pump. However, these experiments do not reveal if both potential nucleotide binding sites are functional, and if so, whether catalytic or regulatory.

Hsu and Rosen (1989b) have demonstrated that the presence of ATP and antimony together protects the ArsA protein from tryspin digestion. ATP alone partially protected from proteolysis while antimonite did not. Mg^{2+} was not required for the prophylatic effect of ATP. These results suggest a conformational interaction of oxyanion and the nucleotide binding sites. The results of tryspin protection experiments with the first binding site mutant ArsA proteins were similar to the result with the wild type protein (Karkaria and Rosen, 1990). This implies that the mutant proteins still bind ATP. It is proposed that the first nucleotide binding site recognizes Mg^{2+}-ATP and is a catalytic site. The second putative nucleotide site would not require Mg^{2+}. Mutations in the second ATP binding site are being isolated to determine its function, regulatory of catalytic.

Although the purified protein eludes from molecular sieve columns at a portion corresponding to a monomeric species, several lines of evidence suggest that the ArsA protein is a homodimer when the anionic substrate is bound. First, the enzymatic activity of the ArsA protein is inhibited by the ATP analogue 5'-*p*-fluorosulfonylbenzoyladenosine (FSBA) (C. M. Hsu and B. P. Rosen, unpublished). Inhibition occurs with an FSBA : ArsA stoichiometry of 1 : 1 in the absence of substrates. In the presence of saturating concentrations of antimonite the stoichiometry is 0.5. Second, exposure of the ArsA protein to chemical crosslinking reagents produces dimers. Dimerization is observed only in the presence of antimonite or arsenite. Nucleotides have no effect on crosslinking. The most parsimonious explanation for these results is that there is an equilibrium between monomer and dimer, with

binding of the oxyanionic substrate stabilizing the dimeric species. Since 0.5 mole of FSBA inhibits the avtivity of 1 mole of ArsA, there must be a conformational coupling of the two catalytic subunits. The simplest model for the oxyanion translocating ATPase *in vivo*, therefore, requires two ArsA subunits per pump.

Cadmium Transport

Two different plasmid-encoded cadmium transport systems have been reported, the first resulting from expression of a plasmid in the gram-positive bacterium *Staphylococcus aureus* (Tynecka *et al.*, 1981a, b), and the second carried by a plasmid in the gram-negative bacterium *Alcaligenes eutrophus* (Nies and Silver, 1989; Nies *et al.*, a, b). These transport systems confer resistance to these bacteria by reducing the intracellular concentration of cadmium. Physiological studies have shown than *S. aureus* cells with plasmid pI2538 extrude cadmium by an energy-dependent process (Tynecka *et al.*, 1981a, b). Cadmium efflux was inhibited by uncouplers but was unaffected by agents which affect membrane potential. The cadA resistance of plasmid pI2538 has been cloned and sequenced (Nucifora *et al.*, 1989). From gene distribution analysis the *cadA* resistance determinant was identified as the product of one of two potential open reading frames. From the nucleotide sequence of the most likely reading frame, the *cadA* gene product was predicted to be a polypeptide of 727 amino acyl residues. This predicted protein exhibited significant homology at the amino acid level to bacterial and eukaryotic E_1E_2 cation transporting ATPase. Based on this nucleotide sequence data, the CadA protein is postulated to be a calcium-transporting ATPase which functions to pump cadmium out of the cell using ATP as the energy source. The physiological studies of Tynecka *et al.* (1981a, b) are consistent with this model, even though those investigators originally postulated a Cd^{2+}/H^+ antiporter. The *cadA* gene product has not yet been identified biochemically.

Cadmium resistance in *A. eutrophus* is conferred by a large 238-kilobase plasmid, pD188, which also encodes an active cadmium extrusion system (Nies and Silver, 1989). However, this Cd^{2+} resistance is an inducible energy-dependent cation efflux system conferring resistance also to Zn^{2+} and Co^{2+}. This cadmium–zinc–cobalt (*czc*) operon has been subcloned, and four genes have been identified (*czcA, B, C,* and *D*). Five gene products of the *czc* operon have been identified using a T7 bacteriophage expression system. All five gene products appear to be membrane associated. Three gene products (*czc A, B,* and *C*) are required for cadmium resistance. The CzcA and CzcB polypeptides are required for Zn^{2+} resistance, while CzcA alone is sufficient

for Co^{2+} resistance. The CzcD protein is involved in regulation of the system. The nucleotide sequence of the pMOL30 *czc* genes have been determined (Nies *et al.*, 1989b). However, none of the sequences are similar to those of other transport proteins, including *cadA*, so no inferences about mechanisms can be deduced from analysis of the sequence.

Citrate Transport

The inability to utilize tricarboxylates such as citrate as a sole carbon source has been used as a classical diagnostic test to distinguish *E. coli* from other gram-negative enteric bacteria. Clinical isolates of citrate-positive *E. coli* have been isolated (Ishiguro *et al.*, 1978; Ishiguro and Sato, 1979). The ability of these isolates to grow on citrate was the result of a novel citrate transport system encoded on conjugative plasmids (Ishiguro *et al.*, 1981; Reynolds and Silver, 1983). Transport is partially inducible by citrate and does not require K^+, Na^+, or Mg^{2+}. Reynolds and Silver (1983) demonstrated transport of radiolabeled citrate using both whole cells and right-side-out membrane vesicles. Inhibitor studies show that transport was energy dependent, driven by the membrane potential and not by the pH gradient. Citrate is cotransported with H^+. Minicell analysis has identified a 35-kDa membrane protein from the citrate locus (Hirato *et al.*, 1984). The genes for the citrate transport system have been cloned and sequenced (Ishiguro and Sato, 1985; Sasatsu *et al.*, 1985). Two open reading frames have been identified: *citA* and *citB*. The *citA* gene encodes for the 35-kDa membrane (Hirato *et al.*, 1984; Ishiguro and Sato, 1985), while *citB* gene codes for a 37.5-kDa membrane protein (Ishiguro *et al.*, 1988).

Iron Transport

Bacteria have evolved highly efficient means of accumulating iron, an essential nutrient for growth. It is not surprising that plasmid-encoded iron transport systems exist. The two most extensively studied iron transport systems are located on plasmids involved in bacterial virulence.

The fish pathogen, *Vibro anguillan*, posesses an essential virulence plasmid which encodes for an iron uptake system. The transport system involves a diffusable siderophore and a cell surface receptor (Walters *et al.*, 1983). Six genetic loci have been identified by transpositional mutagenesis (Tolmasky *et al.*, 1988). Five of these loci are involved in biosynthesis of the siderophore, Angrobactin. The receptor region of the iron transport system plasmid pJM1 has been cloned and sequenced (Actis *et al.*, 1988). Five open

reading frames were identified. Two components of the receptor have been identified: an 80-kDa outer membrane protein (called OM2) and a 40-kDa protein (Actis *et al.*, 1985, 1986; Tolmasky *et al.*, 1988). The three other genes appear to be involved in biosynthesis of OM2 (Actis *et al.*, 1986). The 40-kDa protein is predicted to be membrane associated and speculated to play a role with OM2 in transporting iron into the cytoplasm.

Another iron transport system is encoded by the *E. coli* plasmid ColV-k30 (Carbonetti and Williams, 1984). This system also has two components: the hydroxamate siderophore aerobactin (Braun, 1981) and an inducible outer membrane protein which is the receptor for the ferric-areobactin (Binderieff *et al.*, 1982; Grewal *et al.*, 1982). Insertional mutagenesis identified 5.5 kb of plasmid DNA containing five genes involved in iron transport (Carbonetti and Williams, 1984). The gene products of these genes were identified by maxicell and minicell analyses. Four polypeptides (62, 35, 45, and 50 kDa) are required for areobactin biosynthesis. A fifth gene product is a 74-kDa outer membrane protein which function as the receptor.

DNA hybridization studies show how little homology between these two plasmid-encoded iron-transport systems (Walters *et al.*, 1984). There is also little apparent homology between either of these systems and the well characterized chromosomally-encoded iron uptake system.

Mercury Transport

The basic mechanism of plasmid-mediated mercury resistance is via an enzymatic detoxification reaction in which Hg^{2+} is reduced to Hg^0, which is both nontoxic and volatile (Mobley and Summers, 1987). The soluble enzyme Hg^{2+}-reductase catalyzes this reaction. The cytosolic location of mercuric reductase led to the speculation that Hg^{2+} must first be transported into the cell before detoxification could occur (Summers and Sugarman, 1974). Foster *et al.* (1979), using transposon mutagenesis of the *mer* locus, isolated a class of mutants which were supersensitive to mercury ions. While wild type strains failed to show Hg^{2+} uptake, physiological studies of these supersensitive mutants demonstrated Hg^{2+} accumulation (Nakahara *et al.*, 1979). The supersensitivity phenotype was associated with a complete loss of mercuric reductase and the presence of a Hg^{2+} uptake system. The rapid reduction of Hg^{2+} in the wild type strains would indicate that transport is the rate-limiting step in detoxification.

DNA sequence and minicell analyses indicate that the Hg^{2+} transport system is composed of as many as three polypeptides: MerP, MerT, and MerC (Misra *et al.*, 1984; Jackson and Summers, 1982; Barrineau *et al.*, 1984;

NiBhriain and Foster, 1986). The MerP protein is located in the periplasm, while MerT and MerC are inner membrane proteins.

The MerP protein has homology to several periplasmic binding proteins in two region. Residues 1–21 appear to be a signal sequence. Minicell analysis demonstrates a 12-kDa basic polypeptide which was processed from a 13-kDa precursor (Jackson and Summers, 1982). Residues 22–30 are predominantly hydrophobic. The region from residues 31–61 is homologous with the HisJ and ArgT proteins and shows limited homology with the *D*-ribose bondong protein. MerP has been purified and confirmed to be a product of the *merP* gene by amino terminal analysis. MerP acts as a Hg^{2+} periplasm binding protein. Its exact function has not been demonstrated. It could act as a Hg^{2+} sink to prevent damage to the cell membrane or as a true component of the transport system, bearing Hg^{2+} through the periplasmic space and then presenting the ion to the inner membrane MerT protein.

MerT is a 12-kDa membrane protein which shows limited homology to HisM. MerC is a 15-kDa membrane protein with limited homology to HisM, HisQ, and MalF. When MerC and MerT are aligned, they were 45% identical. These results suggest that one of these two genes arose from duplication of the other. The periplasmic binding protein transport systems most likely to utilize ATP as the direct donor of energy. The observed homologies of MerT and MerC with some of the proteins of periplasmic systems might suggest that ATP is the driving force for Hg^{2+} transport. However, neither MerT nor MerC contain the consensus nucleotide binding site found in all periplasmic systems (Higgins *et al.*, 1985, 1986). Thus no mechanism of Hg^{2+} transport can be inferred.

Tetracycline Transport

Bacterial resistance to the antibiotic tetrtacycline can be mediated by three different types of mechanisms: inactivation, ribosome protection, and efflux (Levy, 1984). There are 12 different known genetic classes of these resistances. In the transport-mediated process resistance occurs by transporting the unmodified antibiotic out of the cell using an energy-dependent process. Eight of the 12 known tetracycline resistance classes encode for efflux systems. This section will concentrate on classes A–E, which are the most extensively studied in gram-negative bacteria. These *tet* determinants each encode a single structural protein and a repressor protein. There is 50 to 70% homology at the DNA level among the four determinants whose sequences have been determined (Hillen and Schollmier, 1983; Klock *et al.*, 1985; Nguyen *et al.*, 1983; Unger *et al.*, 1984). In each case the structural gene encodes a 43-kDa integral membrane protein termed the TET protein (Levy

and McMurry, 1974). Because of the similarities of the gene products of each class, it is assumed that the biochemical mechanism will be identical for each determinant. Studies using whole cells and everted membrane vesicles have shown efflux to be an energy-dependent process, and the mechanism of tetracycline transport has been proposed to be a tetracycline/H^+ antiporter (McMurry *et al.*, 1980; Marshall *et al.*, 1986; Kaneko *et al.*, 1985).

From an analysis of the nucleotide sequence, the TET protein is proposed to be composed of two independent domains: TETa and TETb. Both *tetA* and *tetB* mutations map within the same *tet*. Since they complement each other, the tetracycline/proton antiporter is proposed to be composed of multiple copies of the TET protein (Curiale *et al.*, 1984). The results of experiments utilizing *tetA-phoA* gene fusions support the hupothesis that the antiporter is a multimeter of TET polypeptides (Hickman and Levy, 1988). The topology of the integral membrane protein, TETa, has recently been demonstrated by protease susceptibility experiments (Eckert and Beck, 1989). Both the amino terminus and carboxyl terminus of TET were shown to be located in the cytoplasm. The experiments with *phoA* gene fusions support the cytoplasmic location of the carboxyl terminus (Hickman and Levy, 1988).

Other Systems

Several additional plasmid-mediated resistances to toxic compound have been hypothesized to operate by a transport mechanism, but these systems are not as well characterized as the ones described above.

Antiseptics

A number of cationic compounds have antiseptic or disinfectant action in bacteria. These compounds include acriflavin, ethidium bromide, quaternary ammonium compounds, propamidine isethionate, and diamidinodiphenylamine dihydrochloride. Bacterial resistance to these organic cations is carried on plasmids (Jones and Midgley, 1985; Midgley, 1976; Tennent *et al.*, 1985, 1989). Five loci have been identified on plasmids of *Staphylococcus aureus* (Tennent *et al.*, 1989). These loci, termed *qacA–E* (for *q*uaternary *a*mmonium *c*ompounds), fall into two groups. From hybridization studies *qacA* and *qacB* are related and confer resistance to all of the above organics. The other three *qac* loci are primarily resistances to quaternary ammonium compounds and to ethidium bromide.

The 28-kb *S. aureus* plasmid pSK1 carries *qacA* resistance (Tennent *et al.*, 1985). In intact cells ethidium efflux was demonstrated monitoring loss of

ethidium fluorescence or [^{14}C] ethidium form ethidium-loaded cells. The *qacA* gene was cloned from pSKI as a 3.5-kb fragment and inserted into the *E. coli* plasmid pBR322. The resulting 7.7-kb plasmid, pSK449, conferred antiseptic resistance on *E. coli*. From Tn5 insertional mutagenesis the maximum size of the *qacA* gene weas determined to be 1.45 kb, sufficient to encode a protein of about 480 residues. In [^{35}S]methionine-labeled maxicells a membrane polypeptide of approximately 50 kDA was identified as the product of the *qacA* gene. By analogy with TET a proton-coupled antiport mechanism would be a reasonable possibility.

Jones and Midgely(1985) demonstrated that a 1.1-kb fragment from the *S. aureus* plasmid A118 expressed ethidium resistance in *E. coli* when cloned into pUC8. The gene responsible for resistance has been termed *qacE* (Tennent *et al.*, 1989). Expression was not orientation specific; insertion in the opposite orientation in pUC9 gave the same resistance. The insert conferred simultaneous resistance to cetyltriammonium bromide, a quarternary amine. The *qacE* gene encodes an ethidium efflux system as determined by efflux studies using [^{14}C]ethidium fluorescence. The results were complicated, however, by the presence of a somewhat lower activity ethidium efflux system present in the *E. coli* cells without the 1.1-kb insert.

Chromium

Chromate resistance in pseudomonads is plasmid-mediated. The basis of resistance is reduced accumulation of chromate (Bopp *et al.*, 1983; Horitsu *et al.*, 1983; Ohtake *et al.*, 1987). Six times more chromate accumulated in sensitive strains than in resistant strains. Ohtake *et al.*, (1987), using radiolabeled $^{51}CrO_4^{2-}$, demostrated that plasmid pLHBl confers resistance to *P. fluorescens* by reduced $51CrO_4^{2-}$ uptake. Kinetic studies showed that the V_{max} for $^{51}CrO_4^{2-}$ uptake for cells with pLHBl was 2.2 times less than the V_{max} for plasmidless cells. The K_m values for cells with and without the plasmid were similar. Bopp and Ehrlich (1988) observed chromate reduction by both chromate-sensitive and -resistant *P. fluorescens*, indicating that chromate reduction and chromate resistance are unrelated. Active efflux of chromate from resistant cells has not yet been demonstrated. Recently Cervantes *et al.* (1988) reported cloning the chromate resistant determinate.

Chromate resistance in *A. eutrophus* is an inducible plasmid-mediated process encoded by pMOL28 (Mergeay *et al.*, 1985; Nies and Silver, 1989). Although Nies and Silver (1989) were unable to demonstrate efflux of $^{51}CrO_4^{2-}$ because of inability to load cells with radiolabeled chromate at 4°C, they postulated that resistance was based on reduced chromate accumulation. The chromate resistance determinant from plasmid pMOL28 has been cloned (Nies *et al.*, 1989a). DNA hybridization experiments show little homology between the cloned chromate resistance determinant from

pMOL28 and the chromate resistance loci cloned from *Pseudomonas*. The chromate resistance determinant was subcloned into T7 expression vectors in both orientations to allow specific labelling of the polypeptides with [^{35}S]methionine. Three labelled polypeptides were identified when the DNA fragment was in one orientation, and no polypeptides were detected when the insert was cloned in the opposite orientation. The three polypeptides had molecular weights of 31.5, 21, and 14.5 kDa. Cellular fractionation of [^{35}S]methionine labelled cells indicate that the 31.5- and 21-kDa proteins are probably membrane-bound protein, while the 14.5-kDa protein is a cytoplasmic protein.

Copper

Bacterial resistance to cupric ion results both from mutations in chromosomal genes and from plasmid ecoded genes (Trevors, 1987). Decreased accumulation of Cu^{2+} is observed in *E. coli* cells bearing the inducible copper resistance plasmid pRJl004, leading to the postulate that efflux is one component of the resistance mechanism (Rouch *et al.*, 1985). A second *E. coli* plasmid, Rtsl, similarly produced accumulation of Cu^{2+} (Cotter *et al.*, 1987).

Nickel

The chemoautotrophic bacteria *Alcalignes eutrophus* requires Ni^{2+} ions for growth on hydrogen gas. Recently a genetic determinant for a high-affinity Ni^{2+} specific transport system was shown to be localized in the *hox* gene cluster on the pHG megaplasmid in *A. eutrophus* (Eberz *et al.*, 1989).

At high intracellular levels nickel is toxic. Inducible nickel resistance in *A. eutrophicium* was observed to be conferred by plasmid pMOL28, resulting in reduced accumulation of nickel (Siddiqui and Schlegel, 1987). Nickel resistance was demonstrated to occur by an energy-dependent efflux system (Sensfuss and Schlegel, 1988). Plasmid-containing strains actively extruded $^{63}Ni^{2+}$ under areobic conditions, while plasmidless strains did not. No efflux was observed under anoxic conditions for *A. eutrophus*, which is a strict aerobe. Nickel efflux occurred for pMOL28-containing cells after shifting from anaerobic conditions. Siddiqui *et al.* (1989) have recently cloned the nickel resistance genes from pMOL28.

Silver

Plasmid-encoded silver resistance associated with reduced accumulation of silver has been observed in *E. coli* (Starodub and Trevors, 1989). It is not clear whether this reflects a silver efflux system.

Silver-resistant strains of *Klebsiella pneumoniae* have been isolated (Kaur and Vadehra, 1986). Silver accumulation in silver-sensitive and silver-resistant spheroplasts was compared. The susceptible strain accumulated three to four times as much $^{110}Ag^{+}$ than the resistant strain. Although no silver resistance plasmids could be isolated, treatment of resistant cells with either ethidium or acridine orange resulted in loss of resistance (P. Kaur, personal communication). These results are consistent with the resistance being plasmid encoded.

Sucrose

Sucrose is not a normally metabolizable sugar by *E. coli*. Plasmid pUR400 confers on *E. coli* the ability to utilize sucrose as a carbon source through synthesis of a new sucrose transport system (Lengeler *et al.*, 1982, Schmid *et al.*, 1982). The *scr* genes were cloned from pUR400 into pBR328 (Scmid *et al.*, 1988). The *scrA* was sequenced and shown to be an enzyme II^{scr}, that is, a sucrose-specific Enzyme II component of the phosphotransferase system, a PEP-coupled sugar translocation system (Ebner and Lengeler, 1988).

Tellurium

The conjugative plasmid pMER610, from *Alcalignes*, confers resistance to both tellurate and tellurite (Jobling and Ritchie, 1987). The plasmid was isolated after conjugative transfer to *E. coli*. The tellurium resistance operon has been cloned and sequenced (Jobling and Ritchie, 1987, 1988). Nucleotide sequence analysis has identified five open reading frames. Maxicell analysis identified four polypeptides corresponding to four of the opening reading frames. The additinal open reading frame is predicted to code for an unidentified membrane protein. Although transport of tellurium by this system has not been demonstrated, it has been suggested that the as yet unidentified membrane protein may be involved in tellurium extrusion, reducing the intracellular concentration and thus conferring resistance. How the system recognizes both tellurate and tellurite is unknown.

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