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Understanding cellular responses to toxic agents: a model for mechanism-choice in bacterial metal resistance

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SUMMARY

Bacterial resistances to metals are heterogeneous in both their genetic and biochemical bases. Metal resistance may be chromosomally-, plasmid- or transposon-encoded, and one or more genes may be involved; at the biochemical level at least six different mechanisms are responsible for resistance. Various types of resistance mechanisms can occur singly or in combination and for a particular metal different mechanisms of resistance can occur in the same species. To understand better the diverse responses of bacteria to metal ion challenge we have constructed a qualitative model for the selection of metal resistance in bacteria. How a bacterium becomes resistant to a particular metal depends on the number and location of cellular components sensitive to the specific metal ion. Other important selective factors include the nature of the uptake systems for the metal, the role and interactions of the metal in the normal metabolism of the cell and the availability of plasmid (or transposon) encoded resistance mechanisms. The selection model presented is based on the interaction of these factors and allows predictions to be made about the evolution of metal resistance in bacterial populations. It also allows prediction of the genetic basis and of mechanisms of resistance which are in substantial agreement with those in well-documented populations. The interaction of, and selection for resistance to, toxic substances in addition to metals, such as antibiotics and toxic analogues, involve similar principles to those concerning metals. Potentially, models for selection of resistance to any substance can be derived using this approach.

INTRODUCTION

The pervasive nature of metals in the environment has resulted in the widespread appearance of metal resistance in microorganisms. Strong selection for metal resistance is exemplified by metal ion release from natural mercury deposits [28].

Microbial metal resistances are heterogeneous in both their genetic and biochemical bases and may be chromosomally-, plasmid- or transposon-encoded with one or more genes being involved. At the biochemical level microorganisms demonstrate a diversity in the types of resistance mechanisms they have evolved, which includes six different fundamental types. These different mechanisms may occur singly or in various combinations to produce resistance.

The genesis of a given metal resistance mechanism is primarily dependent on the interactions of the metal with the cell. Metal toxicity studies provide a basis for modelling such interactions with microorganisms, by defining the nature of the damage caused by metals to cellular materials and the site(s) of their action. This together with other relevant information can be used to model the evolution of resistance in microorganisms to both metals that are required physiologically and metals which are not. In addition, toxicity studies can be used to model the metabolism of physiologically important metals, such as copper and zinc, in bacterial and other organisms [48,49]. Analysis of metal toxicity is useful since metal resistance and metabolism involve protection of the cellular sites susceptible to metal damage. Using these principles we have synthesised a predictive model for the development of resistance mechanisms to a range of metals, outlined in Fig. 1.

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The evolution of metal resistance in bacteria: a selection model

A cell acquires metal resistance by preventing the access of metals to sensitive cellular components or altering them to reduce their sensitivity. A number of factors will influence the means and degree of protection. These include: 1) the number and nature of cellular uptake systems for the metal; 2) the role and interactions of the metal in normal metabolism; and 3) the availability of preformed resistance gene cassettes for metal control which are often carried on plasmids or transposons.

The five mechanisms generally proposed for heavy metal resistance in bacteria and other microorganisms, illustrated in Table 1, are: 1) exclusion of the metal by a permeability barrier; 2) exclusion by active export of metal from the cell; 3) intracellular physical sequestration of the metal by binding proteins to prevent it from damaging metal-sensitive cell materials; 4) extracellular sequestration; and 5) detoxification where the metal is chemically modified to render it less active.

In addition to the five general resistance mechanisms, the specific reduction in metal sensitivity of cellular targets for metal damage provides a sixth mechanism of resistance. The specific maintenance of a metal-sensitive cell component may be achieved in four ways, namely: 1) by mutations altering

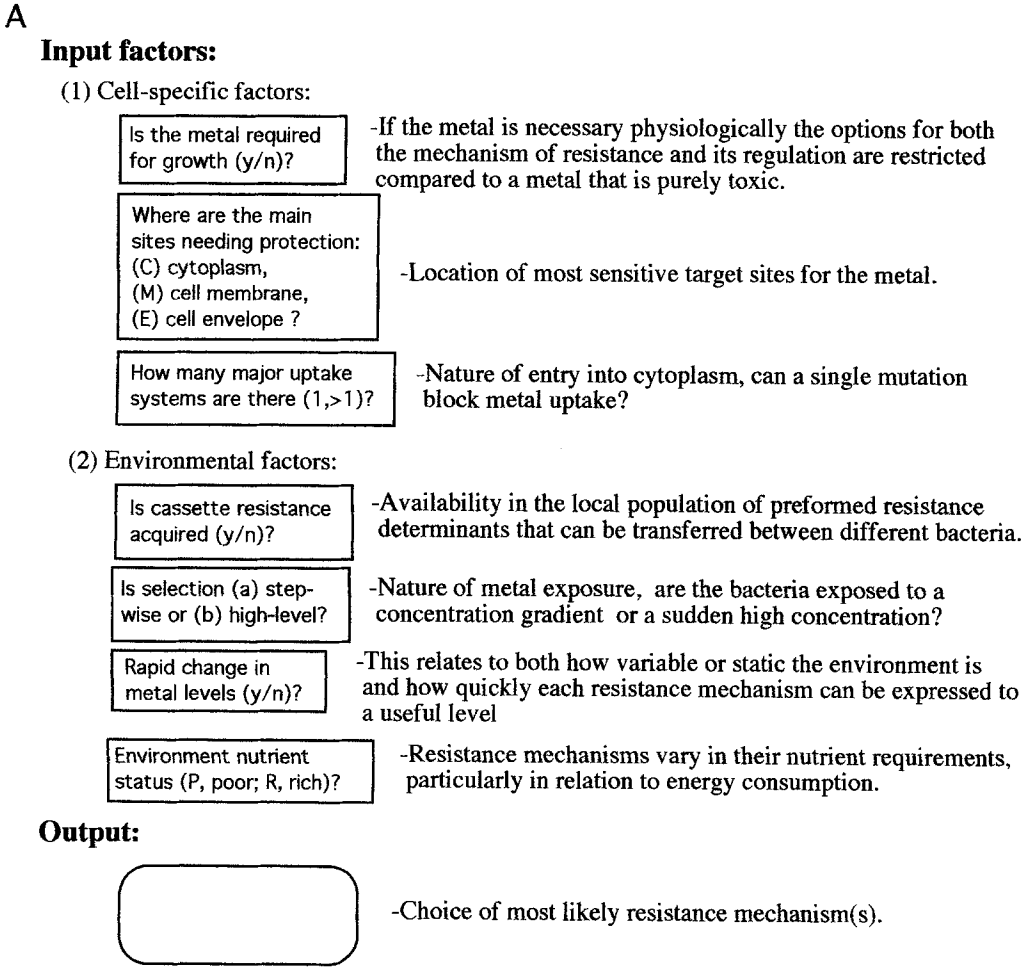

Fig. 1. Outline of the selection model for mechanism-choice in metal resistance. (A) Guide to the model components.

the component to decrease its sensitivity, without unduly affecting its normal role; 2) by increasing the amount of the affected cell component, if inactivation is not total; 3) by repair of the component, in general only feasible for DNA; and 4) by bypassing it, either through utilising a plasmid-encoded metal-resistant form of the component to bypass the metal-sensitive chromosomal component, analogous to the common mechanism of trimethoprim resistance [1], or through increasing activity in an alternative (shunt) pathway that is relatively metal-resistant. The six resistant mechanisms may occur singly or in various combinations.

Metal-sensitive cellular components and resistance

In examining selection for metal resistance it is useful to delineate the nature of the damage caused by metals to the cell. Major cellular targets for metal toxicity, for a range of metal ions, are summarised in Table 2. All types of cell components are susceptible to metal-induced damage.

Mercury can cause breakdown of the essential barrier function of cell membranes [44]. Transition metals like iron and copper, acting as redox reagents, may cause membrane destruction by catalysing the radical peroxidation of lipids. Lipid peroxidation involves the direct reaction of oxygen and the polyunsaturated component of membrane lipids to form free radical intermediates. These radicals react readily with adjacent cell components, resulting in damage to these materials. Iron and copper may catalyse radical forming reactions in the cytoplasm, resulting in the formation of the highly toxic hydroxyl radical [3,19]. The highly reactive hydroxl radical will react with whatever biological molecule is in the immediate vicinity.

Metals ions can decrease or increase enzyme activity, or alter enzyme specificity by inducing conformational changes in enzymes, by locking enzymes in specific conformations; or by forming stable bonds with active and other essential sites in enzymes and transport systems, thereby preventing their function [29,51].

Metal ions can directly damage DNA structure, for example by producing strand crosslinks [63]. Many metals may affect the information content of DNA indirectly by reducing the fidelity of DNA synthesis [7].

In general, the toxicity of metals is due to their ability to form stable bonds with vital cell components, such as the sulphydryl groups in the active sites of some enzymes. The affinity of metal ions toward essential chemical groups in the cell is described according to their covalent and ionic binding properties [14,39]. Metal ions with strong covalent binding character, such as Hg(II), Ag(I) and Cd(II), form strong bonds

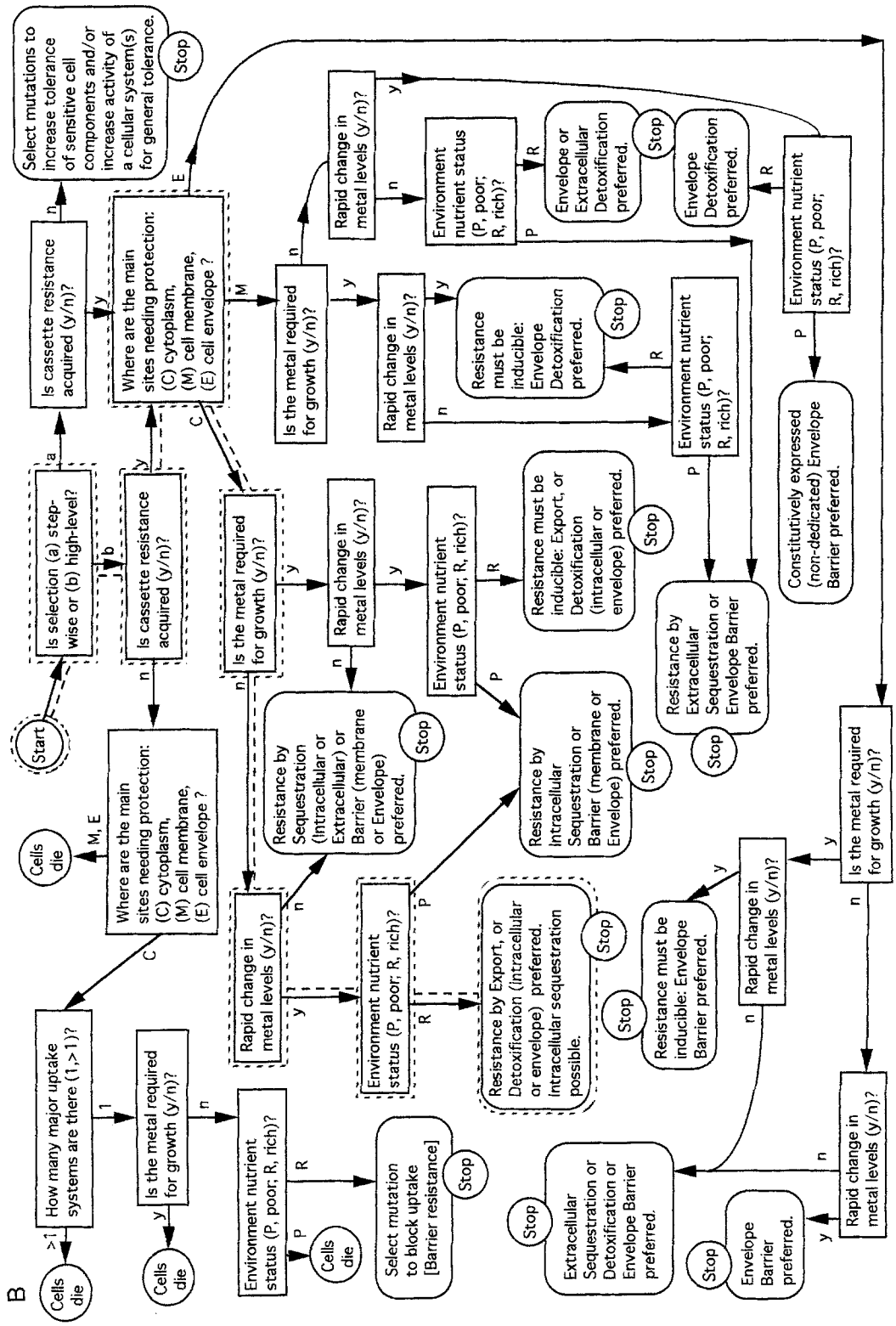

Fig. 1 continued. (B) The model as a decision-making flow diagram. 'Start' toward the middle of the top side marks entry into the model. For a particular case, a single line is taken through the model by following the arrows through successive rectangular decision-making boxes, for each taking the appropriate branch, normally until a round-cornered conclusion box is reached, with formal ending of the modelling path marked by a termination circle. For example, the boxes surrounded by broken lines indicate the decision path for the arsenite and cadmium resistance cases discussed in the text.

TABLE 1

Basic types of resistance mechanisms

Type of mechanism	Properties			Examples	
	Cell regions protected	Optimum conditions	Suitable for metals required for growth?	Metal	Genetic basis
(1) Intracellular sequestration	cytoplasm	active growth	+	Cd(II), Zn(II)	chromosome [47]
(2) Export	cytoplasm	active growth	+	Cd(II); AsO_2^-, AsO_4^{3-}	cassette [25,41]; cassette [53]^
(3) Permeability barrier:					
(a) cytoplasmic membrane	cytoplasm	(a) and (b); high nutrient levels	+/−*	AsO_4^{3-}	chromosome [62]
(b) envelope	whole cell		+/−*	Cu(II)	chromosome [33]
(4) Extracellular sequestration	whole cell	static environment	+/−*	Pb(II); Cu(II); Cd(II), La(III), UO_2(II)	chromosome [12, 16,34]
(5) Extracellular detoxification ~	whole cell	dependent on mechanism details	+/−	Hg(II)	chromosome [43]

* In general these mechanisms are only suitable for low-level resistance to trace metals, as resistance is traded off against nutrient access.
^ AsO_4^{3-} reduced to AsO_2^- before export.
~ While Tn*510* encoded MerA-dependent Hg(II) reduction is the best studied detoxification system [9,55], its complexity means it is not included here as a basic mechanism, but is discussed in the review section.

with easily polarised electronegative ligands such as sulphydryl groups. Metal ions with more ionic binding character, such as Fe(III), Co(III) and Mn(III), form weak bonds with such ligands, but form strong bonds with weakly polarizable electronegative ligands, for example, oxygen in hydroxyl and carboxyl groups. In contrast, the toxicity of arsenate (AsO_4^{3-}) is in part due to its ability to form unstable linkages where it substitutes for phosphate (PO_4^{3-}) in phosphorylation reactions [38].

Although a wide range of cellular components are potential targets for metal-induced damage, a subset of these components are necessary for essential cell functions, such as DNA for replication. Cell death will result from metal-induced inactivation of one or more of these necessary components. These metal-sensitive components may be assumed to have a range of metal sensitivities so that as the concentration of the particular metal rises, functions are inactivated as a critical concentration is reached. Therefore, depending on the concentration of the metal, the cell must have some means of protection for one or more target sites to survive. The greater the concentration of metal the greater the number of essential components that require protection.

If only one cellular site requires protection, resistance may be achieved by a single mutation, which causes reversal or replacement of its metal-decreased activity, leading to an increase in cell growth-rate. The selective metal concentration however may be elevated to a level such that a number of components require protection. If this is so, alteration of target sensitivity by one mutation cannot be expected to produce resistance, since the sensitivity of a number of cell components must change at the same time.

The location of metal-sensitive essential cell components is also important in determining which options will produce resistance. If sensitive components are located in the cytoplasm, then changes in cell envelope permeability may be used to protect them. When a cell is exposed to a metal the first sites of interaction are at the cell envelope [44]. The bacterial cytoplasmic membrane, and to a lesser extent the outer membrane in Gram-negative bacteria, are a major barrier to the entry of hydrophilic substances, including metal ions, into the interior of the cell. In Gram-negative bacteria, like *Escherichia coli*, the outer membrane contains protein channels called porins, that allow low molecular weight substances such as metal ions to diffuse across the membrane into the periplasmic space [40]. In *E. coli* B production of the major porin can be prevented by mutations in a single gene resulting in increased metal resistance [33]. The outer envelope can also act as a limited (i.e. saturable) trap for heavy metals by non-specifically binding them, therefore contributing to the natural metal tolerance of cells [8].

TABLE 2

Metal toxicity by cellular location

Metal ion	Hg(II)	Ag(I)	Cu(I,II)	Cd(II)	Pb(II)	Zn(II)	Fe(II,III)	Al(III)	VO_4^{3-}	AsO_4^{3-} AsO_2^-	TeO_2^-
Chemical Class[a]	B	B	B, Bord.	Bord.	Bord.	Bord.	Bord.	A	Bord.	Bord.	Bord.
Preferred ligand types[a]	S/N	S/N	S/N,S/N/O	S/N/O	S/N/O	S/N/O	S/N/O	O	basic	basic	basic
Cellular targets											
Outer envelope:	−	−	−	−	−	−	−	−	−	−	−
Cytoplasmic membrane[b]:											
Lipids (redox damage[c])	−	−	+,−	−	−	−	+,−	−	−	−	−
Sulphydryl groups	+	+	+/−	−	−	−	−,−	−	−	−	−
Cytoplasm:											
Sulphydryl groups[d],	+++	+++	+++/++	++	++	+	+,−	−	−	+	−
e.g. respiratory chain	+	+	+,−?	+	+	−	−	−	−	+	−
DNA/RNA	+	+	+,+	+	+	+	+,+	−	−	−	−
Analogue toxicity	−	−	−	−	−	−	−	+	+	+	−
Redox damage[c]	−	−	+,−	−	−	−	+,−	−	−	−	+

[a] Chemical classification according to [39,61]: classes are A, B, borderline, with class B being the most toxic.
[b] For bacteria with a periplasm, this can be included in this section for protection analysis.
[c] Formally only Cu(I) and Fe(II) of the valence pairs are essential for redox (free radical) damage.
[d] Semi-quantitative measurement of binding to sulphydryl groups given here, as this relates to the degree of toxicity. Detailed principles of metal-cell component interaction are discussed in [14,22].
Metal specific references: [44] Hg/Cu, [59] Hg/Cd/Pb, [24] Hg, [56] Ag, [11,27] Cd, [42] Pb, [21,36] Zn, [6] Cu, [3,19] Cu/Fe, [5] Fe, [35] Al, [46] V, [20,26,38] As, [32] Te.

Metal-sensitive biologically essential components which require protection may be located in the periplasm or inner membrane and be accessible to metal ions from the outward face. To achieve resistance in this case, the resistance mechanism must act externally to these sites, either by acting as a cell envelope barrier or a detoxification system. Resistance could not be produced by an inner membrane efflux system since this would only affect cytoplasmic metal levels and not the metal levels in the cell envelope. Extracellular sequestration could also produce resistance provided that sufficient levels of the sequestering substance could be produced. This would generally only be feasible in a relatively static environment, where the cell is not exposed to a continuously renewed metal-load. This mechanism, however, may be advantageous in low nutrient-status environments.

Basic cell tolerance to toxic metals

In contrast to the discussion of target sites for metal-induced damage in the cell, it is pertinent to consider the properties of a normal cell that give it a base level of tolerance. Any auxilliary resistance mechanism adds to base-level tolerance and may also interact with components of it. Three examples of tolerance components are glutathione, metallothionein, and DNA repair systems. Reduced glutathione is the major low molecular weight sulphydryl compound in most cells, and so might be expected to contribute to metal tolerance, particularly for class B ions (see Table 2). Studies with mutants in gluthatione metabolism support this hypothesis, by demonstrating that the absence of reduced glutathione lowers Cu tolerance in *E. coli* K-12 by 6-fold (D.A. Rouch and N.L. Brown, unpublished). The study of cells biochemically depleted of glutathione has also indicated its importance in metal tolerance [15]. While metal-binding may be the sole protective property of glutathione for non-redox metals like cadmium, another activity, that of forming a reducing buffer, may help in containing potential damage from free radicals catalysed by transition metal ions, such as copper and iron. In this activity the glutathione buffering system acts with a group of enzymes dedicated to free radical control, which include catalase and superoxide dismutase [57]. *Synechococcus* species harbour a cytoplasmic metal sequestrating protein, the SmtA metallothionein, that contributes to their tolerance for cadmium and zinc [58]. DNA is a target for metal damage and the resistance of a cell can be enhanced by maintenance of DNA integrity upon exposure to metal by DNA repair systems. Cadmium-induced DNA damage in *E. coli* B is repaired in cells accommodating to cadmium stress [37], and an error-free DNA repair system, encoded along with *pco* copper resistance on plasmid pRJ1004, enhances the level of copper tolerance in *E. coli* K-12 [48].

Metal uptake systems and resistance

For cells in which highly metal-sensitive essential cell components are located in the cytoplasm, the number of uptake systems for the entry of metal into the cell will influence the choice of resistance mechanism. The lipid component of bio-

logical membranes is highly impermeable to hydrophilic ions such as metal cations [2]. Metals therefore will generally pass across bacterial membranes through the less resistant sites in the membranes, namely the transport system(s) designated for transport of hydrophilic substrates into the cell. This occurs in the case of arsenate and of cobalt which enter *E. coli* cells mainly by way of the *Pit* phosphate transport system and the *mgt* magnesium transport system, respectively. In these cases metal resistance can occur by a single mutation that inactivates the transport system responsible for uptake of the metal [54]. For selection of such a mechanism to occur, the advantage to the cell of resistance would have to be greater than deleterious affects on the cell resulting from loss of a transport system. Alternatively, a metal may be able to penetrate the cytoplasmic membrane via a number of different transport systems, protection of cytoplasmic targets then cannot occur by a single mutation. This situation may occur particularly for non-physiological metal ions. It is likely that these non-physiological cations will commonly have low affinities for uptake since specific transport systems for these metals are not required by cells [52]. Low affinity cadmium uptake has been observed for a number of *Pseudomonas* spp. (S. Koroneos and B.T.O. Lee, unpublished; L. Eager, pers. comm.). High-level resistance to non-physiological metals such as cadmium and lead therefore may not generally be possible through a single mutation blocking uptake, since accumulation may occur via a number of non-metal (e.g. non-lead) specific (low affinity) uptake systems. Similarly, hydrophobic (lipid-soluble) metal compounds, for example $HgCl_2$, mass pass through the lipid component of membranes directly, making selection for uptake mutants unlikely.

The metal as a biological requirement or not

A number of heavy metals are necessary to maintain essential metabolic activities of bacterial cells. Copper, iron and zinc are required by most bacterial species, and molybdenum, cobalt, nickel and tungsten in some species [14,22]. In general these metals will be less toxic to the cell compared to metals with no positive metabolic roles, as cells will have appropriate mechanisms that will cope with small fluctuations in local concentrations. For resistance to metals which are physiologically required, survival is optimised by cooperation between the resistance mechanism and the normal cellular metal metabolism, allowing the cell to accumulate sufficient metal for the maintenance of metal-dependent activities whilst responding to supra-optimal metal concentrations. These resistance mechanisms should be carefully controlled, i.e. be inducible since, constitutive expression may starve cells of the metal when it is present at low concentrations. In contrast over-expression of resistance is not generally deleterious in the case of metals which are not physiologically necessary.

For metals that are required physiologically, barrier type resistance mechanisms, such as those involving polysaccharide capsules, may not be suitable if rapid change of expression is necessary for adequate metal accumulation by the cell. The time necessary for constructing and dismantling a large extracellular structure like a capsule means that expression of resistance could not be induced or repressed rapidly.

Factors affecting the regulation of resistance

How the expression of resistance mechanism is regulated is primarily dependent on: 1) Is there a physiological requirement for the metal? 2) What is the metabolic load imposed by expression of the resistance? 3) How rapidly can the mechanism become effective? and 4) Does the mechanism specify any other functions within the cell?

If the metal is required physiologically there is likely to be selection for inducible control (possibly linked to the expression of the normal homeostatic system) in order to maintain metal homeostasis, as discussed above, and the degree of expression will mirror the external metal concentration (Fig. 2) [30,50]. This is because over-expression as well as under-expression of resistance can be deleterious to the cell.

Sequestration mechanisms acting alone are inherently inefficient as sequestering agents easily become saturated, so that high levels of expression may be necessary to achieve a reasonable degree of resistance. In contrast, enzymic type mechanisms, detoxification and efflux systems, are reusable, therefore producing effective resistance at low levels of expression.

Some kinds of extracellular barriers may have other intrinsic functions apart from providing protection against metals and they will be regulated accordingly. For example, an extracellular capsule, which sequesters metal ions, may primarily be involved in protecting a pathogen against host defence systems and therefore be constitutively expressed rather than be inducible by metal ions, the pathogenicity requirement taking precedence.

The efficiency of enzymic mechanisms means that relatively little expression of the encoding genes is necessary for an adequate level of resistance, so they may be selected where rapid changes in expression of resistance are desirable. These systems can require highly sensitive switch-type control, so that the resistance mechanism can be maximally functional before the metal substantially damages the cell (see Fig. 2). In

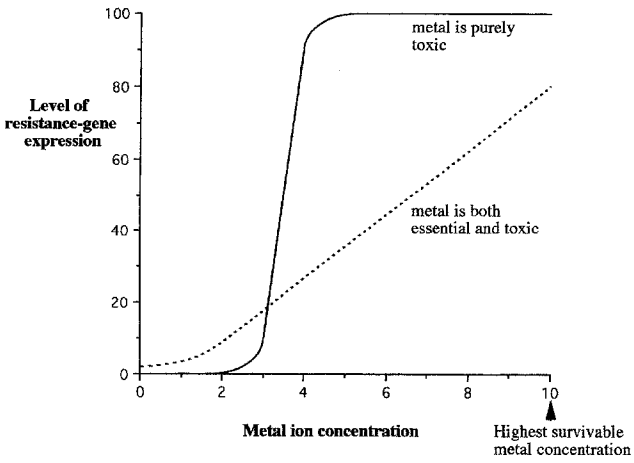

Fig. 2. An idealised representation of expression of resistance vs metal concentration for a growth-required metal, such as copper and a purely toxic metal, such as mercury: axes are shown with arbitrary units. Based on a diagram from [30], with permission. Expression of resistance genes can be monitored by fusing them with reporter genes: see [50] for examples of observed responses.

contrast, extracellular sequestration mechanisms, most suitable in relatively static micro-environments, may need little control in these environments as the rapid induction of such mechanisms would be unimportant.

Further factors that may affect regulation in practice, are the compatability of control systems between species, and the pattern of exposure to the metal. A mechanism may well function in a different species upon horizontal transfer, but some aspect of the existing control system, such as the gene promoter or regulator, may not function very efficiently. This can result in mutation to constitutive expression, such as observed with transferable tellurite resistance [60].

Gene-cassette- versus chromosome-mutation-determined resistance

The genetic basis of resistance to a metal in resistant bacteria will be determined by the availability in the local population of a preformed gene-cassette(s) that specifies a dedicated mechanism of resistance. These are thought to have been adapted to give effective resistance by previous long-term evolutionary selection, and can be borne on chromosomes, plasmids or transposons. The last two can promote transfer of the linked resistance cassette between bacteria. Cassette-mediated resistance may give higher levels of resistance than available chromosomal mutations may allow. Therefore, cassette-mediated resistances, when available, may be preferentially selected over chromosome-mutation derived resistance.

Selection for resistance in a population where appropriate cassette-borne mechanisms are rare or non-existent may favour chromosomal mutation and stepwise selection for resistance, which may be capable of producing certain levels of resistance through multiple mutations. These can include mutations that cause a specific reduction in the metal sensitivity of metal targets, the sixth mechanism of resistance (see e.g. [45]). Also, the activity of a general tolerance system may be increased through stepwise selection of multiple mutations, such as with the *smt* locus of *Synechococcus* PCC 6301, within which the *smtA* gene encodes a metallothionein that sequesters cadmium and zinc. In this case stepwise selection with cadmium resulted in both gene amplification and gene deletion; the *smtA* structural gene was found to be amplified, while the *smtB* gene, which specifies a repressor of *smtA* expression, suffered an internal deletion [17,18]. The cumulative effect of these mutations was to markedly increase cadmium tolerance in the *Synechococcus* cells. Stepwise or growth rate selection for resistance may occur naturally in environments where metals gradually accumulate, creating metal concentration gradients, such as in lakes and coastal marine sediments.

In a bacterial community exposed to a heavy metal for the first time the frequency of plasmids determining resistance will mainly depend on the size of a community and on previous selection for other characteristics encoded by cassette-bearing transposon or plasmids. The larger a population the greater the probability of a relevant cassette-bearing genetic element being present. Previous selection for transposons or plasmids via other characteristics such as antibiotic resistance, carbon source utilisation, and colicin or enterotoxin production, may introduce any metal resistance genes carried on these plasmids into the population.

Plasmids or transposons bearing metal resistance determinants may be proficient at horizontal as well as vertical transmission, and thus be capable of spreading through bacterial populations at faster rates than chromosomal mutation-derived resistances, which in the main are limited to vertical transmission only. Multiple metal resistances can accumulate on plasmids [55], which allows resistance to a number of metals to simultaneously spread through a population. The mobility of resistances in populations will be enhanced if they are determined by transposons which are contained on plasmids, as mercury resistance can be [55]; these may readily transfer between bacteria.

A selection model for metal resistance

The effects of the factors described above on the choice of mechanism used to achieve metal resistance in bacterial populations were synthesised into the algorithm that forms the selection model outlined in Fig. 1. Some important corresponding properties of the various resistance mechanisms are summarised in Table 1. In the algorithm decisions are made concerning the determinative factors for resistance. Selection for both chromosomally and plasmid-encoded resistances are represented. Chromosomal resistance is considered before plasmid options since plasmids specifying resistance to the metal in question may initially be rare or non-existent in the population under selection [13]. While the model is a generalised and simplified view of the generation of metal resistance in bacterial populations it should be sufficiently predictive in its present form to be useful. It can be easily modified to accommodate new information about the factors involved. It can also be improved for particular situations by applying probabilities to the decision-making options. Particularly useful would be information about environmental effects on selection [4,22,23], since this would allow the application of the resistance selection model to specific environments.

In summary, the selection model should allow predictions to be made about the evolution of metal resistance in bacterial populations, particularly the genetic basis and mechanisms of resistance. If relevant information is known it will also explain the presence and basis of resistance in present populations. Metal interaction with cells and the evolution of metal resistance involves many factors and is a highly complex phenomena. The interaction of, and selection for resistance to, other toxic substances, such as antibiotics and toxic analogues, involve similar principles although fewer factors may be involved. Therefore, selection for resistance to other toxicants could be modelled using a subset of the factors influencing metal resistance. Potentially then, models for selection of resistance to any substance could be derived from the metal resistance selection model.

Review of known resistance mechanisms by the selection model

The best studied examples of metal resistance systems include *mer* operon encoded mercury resistance in Gram-negative bacteria, cadmium resistance in *Staphylococcus aureus*,

arsenite/arsenate resistance in *E. coli*. In these examples sufficient information is known about the resistance factors to allow predictions from the selection model to be made to compare with the reported mechanisms (Table 3).

Arsenite resistance in *E. coli* is a good example of a mechanism which could have been predicted by the selection model; the decision path is marked in Fig. 1(B). In this system an inner-membrane pump (ArsA, ArsB) removes arsenite from the cytosol. An additional component, ArsC, reduces arsenate (AsV) to arsenite (AsIII) giving resistance to both toxic species. Arsenite is toxic to the cytosol where it acts on sulphydryl groups to inactivate cellular components. The cell is required to exclude arsenite and should opt for an export system. In this case the system is regulated by arsenite such that a metabolic load is not placed on the cell in the absence of the toxic species. In line with the results the model predicts export-mediated resistance, marginally over a detoxification system, as the preferred mechanism. In contrast, enterobacterial tellurite resistance, with the same decision path as arsenite in the model, may utilise the detoxification option [31].

Another example is the mechanism of mercury resistance encoded by the inducible *mer* operon which involves mercury binding, transport and detoxification (Fig. 3) [9]. Hg(II) entering the cell envelope may be bound in the periplasmic space by a Hg(II)-binding protein. The bound mercury is then presented to a mercury transport system located in the cytoplasmic membrane which allows facilitated diffusion of mercury into the cell. Intracellular Hg(II) is then reduced by cytoplasmic mercuric reductase to non-toxic Hg(0), which subsequently volatilises from the cell. This mechanism apparently protects the cytoplasmic membrane from mercury damage. It is thus effectively equivalent to an externally operating detoxification system, which is the mechanism predicted for inducible mercury resistance from the metal resistance selection model (Table 3). The elaborate *mer* operon encoded system appears to be necessary to channel Hg(II) to the mercuric reductase which requires a cytoplasmic nicotinamide co-factor,

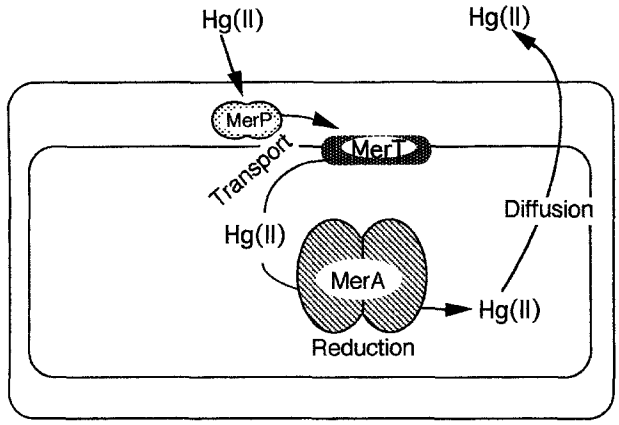

Fig. 3. The mechanism of mercury resistance encoded by the *mer* operon of Tn*501* (S. Silver, pers. comm.).

thereby necessitating its cytoplasmic location, while protecting the cytoplasmic membrane from Hg(II) toxicity. The targets which require protection are the cell membrane and essential sulphydryl groups of membrane-located proteins (Table 2), so that resistance is unlikely to occur by a single chromosomal mutation. Resistance could be achieved by acquiring a plasmid-mediated external detoxification or barrier system. A mercury-specific barrier would almost certainly need to contain a high concentration of sulphur ligands, however, these may be prone to oxidative impairment and availability of sulphur can be limited in many bacterial environments. In contrast, a detoxification system requires a vanishingly low concentration of sulphur ligands, due to catalytic turnover, which is more compatible with sulphur limitations. Also, compared to barriers, detoxification mechanisms allow for rapid induction of resistance, which would be favoured where mercury levels quickly rise to levels which are bactericidal to non-resistant bacteria, such as has occurred in hospitals. A plasmid-encoded

TABLE 3

Evaluation of four known resistance mechanisms

Resistance	Major predictive factors[a]	Predicted mechanism	Observed mechanism type
Plasmid-coded AsO_2^-, AsO_4^{3-}, *E. coli*	intracellular toxicity, nutrient-rich environment	export or detoxification	export [53]
Chromosome-coded AsO_4^{3-}, *E. coli*	single major uptake system, intracellular toxicity	decreased uptake	decreased uptake [62]
Plasmid-coded Hg(II), Gram-negative bacteria	membrane level toxicity, high toxicity	extracellular detoxification	extra-membrane binding, uptake, internal detoxification [9]: equivalent to prediction
Plasmid-coded Cd(II), *S. aureus*	intracellular toxicity	export or detoxification	export [41]

[a] Toxicity factors from Table 2.

external detoxification system, or equivalent, is therefore the mechanism of choice for dedicated resistance to mercury.

Similarly, the reported mechanisms for cadmium resistance in *S. aureus* and chromosome-coded arsenate resistance in *E. coli* fall within the classes of mechanisms predicted by the selection model (Table 3). In some cases, such as arsenite resistance, the model can make fairly precise predictions about the likely resistance mechanism. In other cases a few alternatives may be predicted.

While the examples of resistance to the metals not required physiologically are well understood, knowledge of resistance to trace metals is less complete. Among the latter group copper provides the best studied examples, with the *pco* and *cop* coded resistance mechanisms [10,12,48]. The selection model indicates that such mechanisms may be highly complex due to: 1) the need for them to comply with the homeostasis requirement; and 2) the dichotomy of the common valencies of copper, with Cu(I) being a class B ion, while the less toxic Cu(II) is of the borderline class. The former factor predicts interaction with components of cellular copper metabolism, while the latter suggests that the mechanisms of resistance for the two ions may be generally similar, but differences may occur since they both have different sites of toxicity (only Cu(I) affecting the cell outside the cytoplasm) and vary in relative prevalence according to environment. These factors may help explain the complicated phenotypes observed, and therefore why *pco* and *cop* carry so many genes (8 and 6, respectively) [12].

In conclusion, the mechanisms found to be responsible for a number of bacterial metal resistances are consistent with predictions from the selection model. This suggests that the main factors involved in selection for metal resistance in bacteria have been identified in producing the model. That is, in its present form the model offers an essentially valid explanation for metal resistance mechanisms in bacteria.

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