ever, two significant deviations were observed. First, halobacterial DpsA exhibits from position 164 to 167 a linear break splitting helix D into two smaller parts (see Figure 1B). Because at these positions there is a gap in the sequence alignment we suggest a modelling artifact and expect that an uninterrupted helix can be derived from the secondary-structure calculation. The second difference is based on the fact that the N-terminal part of halobacterial DpsA has no equivalent in the structure of *Listeria* ferritin and resulted in a random-coil structure. From secondary-structure calculations it can be shown that a helical structure in this segment is very unlikely because of many bend-forming amino acids.

**Regulation of the dpsA/ferritin gene**

*H. salinarum* was grown under different growth conditions (iron-starvation, normal medium and additional iron). The Northern-blot analyses of total RNA isolations performed after various periods of growth seem to indicate regulation at the transcriptional as well as the post-transcriptional level.

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**References**


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**Mercury transport and resistance**


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**Abstract**

Resistance to mercuric ions in bacteria is conferred by mercuric reductase, which reduces Hg(II) to Hg(0) in the cytoplasmic compartment. Specific mercuric ion transport systems exist to take up Hg(II) salts and deliver them to the active site of the reductase. This short review discusses the role of transport proteins in resistance and the mechanism of transfer of Hg(II) between the mercury-resistance proteins.

**Key words:** heavy metal ion transport, membrane protein, mercuric ion resistance.

**Introduction**

Mercury resistance is the most widespread of all anti-microbial-resistance determinants, occurring in a wide variety of Gram-negative and Gram-positive bacterial genera, in mesophiles, halophiles and some extremophiles [1]. Mercury resistance can be to inorganic mercury and a wide range of organomercurials, so-called 'broad-spectrum' resistance, or to inorganic mercury and a very limited number of organomercurials, 'narrow-spectrum' resistance. The systems that are most studied and best understood are those from Gram-negative bacteria, in which the mercury-resistance (*mer*) determinants are often encoded on mobile genetic elements and can spread through the
population [2]. In both Gram-negative and Gram-positive bacteria mercury resistance is due to reduction of toxic mercuric ions, Hg(II), to relatively non-toxic and potentially volatile elemental mercury, Hg(0). For this reduction to occur, mercuric ions must be brought into the cytoplasmic compartment. This short account will focus on narrow-spectrum mercury-resistance systems in Gram-negative bacteria.

The genetic structure of mer determinants

One of the simplest mer determinants is that of transposon Tn501, originally isolated from Pseudomonas aeruginosa [3,4]. This contains the structural genes for mercuric reductase (merA) and the transport proteins merT and merP flanked by merR and merD, which are involved in regulation of expression of the structural genes in response to mercuric salts. These are in the order merRTTAPAD, with the merR gene being expressed divergently from the merTPAD genes and regulating the mer promoter between them. Recently, another gene, merE, has been identified distal to merD and has been suggested as another possible transport function, although experimental proof is lacking. The MerR regulator is one of the best-studied of a family of regulators responding to environmental stimuli and activating gene expression. Although primarily an activator in the presence of Hg(II), MerR causes some repression of mer gene expression in the absence of mercury salts and it autoregulates its own synthesis.

Other Gram-negative mer determinants contain additional genes. For example, the Tn21 mer operon on plasmid R100 is very similar to that of Tn501, but contains an additional gene, merC, and has the operon structure merRTPCADE [5]. The mer determinant of plasmid pMER327/419 contains the gene merF in the equivalent location to merC in Tn21 [6]. Broad-spectrum mer determinants, such as that of pDU1358, also contain the merB gene, encoding organomercury lyase [2].

Mechanism of mercuric ion resistance

Detoxification of mercuric ions is catalysed by the NADPH-dependent cytoplasmic flavoenzyme mercuric reductase, which in free solution catalyses reaction (1):

\[
RS-Hg^{II}SR' + NADPH + H^+ = Hg^0 + RSH + R'SH + NADP^+ \quad (1)
\]

where RSH and R'SH represent donor thiol ligands. This dimercaptran substrate may be protein-bound Hg(II) or Hg(II)-glutathione adducts; it is unlikely that free Hg^{II} will exist in the 5–10 mM concentrations of reduced glutathione in the bacterial cytoplasm. A number of studies of the detailed mechanism of mercuric reductase have been undertaken by enzymologists and the details of the reaction mechanism are well understood [7]. However, the mechanism of delivery of the mercuric ion to the reductase protein is not understood (see below).

The activity of mercuric reductase in vivo can be detected by volatilization of radioactive mercury, ^{203}Hg, from the culture medium due to the high vapour pressure of Hg(0). The rate-limiting step for mercury volatilization has long been known to be the uptake of Hg(II) into the cell [8], so volatilization can be used as an indirect measure of transport/uptake of Hg(II). Standard uptake assays cannot be used with mercuric salts, due to the high affinity of mercuric salts for a variety of cell-surface and intracellular ligands. Volatilization and resistance studies have led to our current understanding of the mechanism of mercuric ion uptake and detoxification shown in Figure 1.

MerP, a small periplasmic protein, is thought to scavenge Hg(II) in the periplasmic compartment and subsequently pass the Hg(II) atom to the inner-membrane transporter, MerT. From MerT the Hg(II) is passed to mercuric reductase, in which the substrate-binding site is at the C-terminus (Cys-558, Cys-559 in Tn501 mercuric reductase). Here the Hg(II) is reduced to Hg(0) by electron transfer from FADH\_2 in the active site.

Mechanism of Hg(II) transfer between proteins

We proposed in 1991 [9] a mechanism for Hg(II) transfer from MerP to MerT. We proposed that Hg(II) formed a temporary 3-co-ordinate bridging structure between the two cysteines in MerP and either Cys-24 or Cys-25 in MerT, which was subsequently resolved, via a 3-co-ordinate bridge between one of the MerP cysteines and both Cys-24 and Cys-25 of MerT, to a product in which Hg(II) was bound just to the two MerT cysteines (Figure 2). The properties of MerP and MerT...
cysteine mutants [10] provided some evidence for this, especially in that a single mutation of MerP Cys-17Ser abolished mercuric ion uptake, whereas a MerP deletion did not, presumably because the mutant MerP became locked in a non-productive complex.

Mercuric reductases from a variety of Gram-negative sources contain a conserved domain of about 100 amino acids at the N-terminus of the protein, which has sequence similarity to MerP [11]. It was proposed that transfer of Hg(II) from MerT to mercuric reductase occurs initially to this

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**Figure 1**

Diagram of mercuric ion resistance in Gram-negative bacteria, showing the three transport systems, MerPT, MerC and MerF

Solid circles are cysteines known or presumed to act as Hg(II) ligands; the dotted line shows the presumed path of the mercuric ion through the MerPT transport system and the reductase; the redox active cysteines of mercuric reductase are not marked.

**Figure 2**

Proposed mechanism of Hg(II) transfer between paired thiol residues in proteins
N-terminal domain [4] and that subsequent intermolecular transfer of Hg(II) to the C-terminal substrate-binding site occurred. Mutants in which the N-terminal Cys-10 and Cys-13 of mercuric reductase were converted to alanine or to serine showed no effect on mercuric reductase activity or mercury resistance in vitro or in vivo at high or low copy number of the mer determinant ([12] and K. J. Glendinning and N. L. Brown, unpublished work). The reason for the conservation of this N-terminal structural domain is not known; the conditions under which this domain confers a selective advantage may not have been identified.

The mercury-transport proteins

While the function of the MerT and MerP proteins has been well studied, MerC and MerF have received less attention. It was shown that MerF and MerC were mercury-transport proteins [13,14] and we predicted that MerT, MerC and MerF had, respectively, three, four and two transmembrane regions [14]. The cysteines in the first transmembrane region of MerC were shown to be required for uptake of Hg(II) [15], as is the case for MerT [10].

Our recent data have shown that the topologies of MerT, MerC and MerF are indeed as predicted (J. R. Wilson, Y.-C. Shih and N. L. Brown, unpublished work) and that MerF also requires cysteines in the first transmembrane region (Cys-21, Cys-22) for mercuric ion resistance and volatilization. We have further shown necessary roles for charged residues in the second transmembrane helix in all three proteins. We anticipate that these proteins function as multimers, although this has not yet been demonstrated. It appears that these three proteins have very similar functions and mechanisms, in spite of the differences in their topologies.

The reasons that mer determinants possibly have two or three transporters, apparently functioning in a similar manner, are unclear. Attempts have been made to link the level of mercuric ion pollution in the environment with the selection for, as measured by the presence of, Tn501- or Tn21-like determinants (e.g. [16]), but the data are not conclusive.

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