Out of the iron age: new insights into the critical role of manganese homeostasis in bacteria

Nicholas S. Jakubovics and Howard F. Jenkinson

Overview

Manganese (Mn) is required for the growth and survival of most, if not all, living organisms. Until recently, relatively little was known about how bacteria take up trace nutrients such as Mn, nickel (Ni), copper (Cu) and zinc (Zn), or about how they regulate intracellular levels of these in response to availability and demand. Over the last 5 years a large number of systems involved in these processes have been identified and characterized. This has led to new insights into transition metal ion homeostasis in Gram-positive and Gram-negative bacteria. Metal ions, including Mn, iron (Fe), cobalt (Co), Ni, Cu and Zn, are both essential and potentially toxic. Therefore, homeostatic regulation of their intracellular concentrations is critical.

In prokaryotic cells, which lack internal compartmentalization, metal ion homeostasis is maintained primarily by tight regulation of metal cation flux across the cytoplasmic membrane. Studies on transition metal ion homeostasis in bacteria have focused mainly on Fe metabolism (reviewed by Braun & Killmann, 1999). The acquisition of Fe poses unique problems because of the poor solubility of Fe(III) species that predominate around neutral pH in oxygenated environments. Furthermore, Fe is tightly sequestered by eukaryotic proteins, so the ability of bacteria to wrest Fe from the host is fundamental to the pathogenesis of many infectious diseases. Despite these potential difficulties, many bacteria accumulate relatively high levels of Fe. For example, laboratory-cultured Escherichia coli cells contain greater than fivefold more Fe than Mn (Posey & Gherardini, 2000). Fe is required as an essential cofactor for many proteins, including components of the respiratory chain (cytochromes, cytochrome oxidase), tri-carboxylic acid cycle (aconitase, succinate dehydrogenase) and oxidative defence systems [catalase, peroxidase, superoxide dismutase (SOD)]. Microbial Fe acquisition has been extensively studied and shown to occur by at least three very different strategies. These include the production of small molecules (siderophores) to chelate extracellular Fe$^{3+}$, the direct capture of Fe$^{3+}$ from host Fe-containing proteins such as transferrin, lactoferrin or haem proteins, and the uptake of Fe$^{2+}$ by the Feo transport system (Braun & Killmann, 1999). Within cells, unbound Fe$^{2+}$ (and Cu$^{+}$) ions are especially noxious because they catalyse Fenton-type reactions that lead to the production of damaging hydroxyl radicals (Pierre & Fontecave, 1999). By contrast, Mn(II) is highly soluble and does not catalyse hydroxyl radical formation (Chetón & Archibald, 1988).

Interestingly, new experimental and genomic sequence data suggest that some micro-organisms may have no requirement for Fe, having evolved metabolic and survival strategies that can be accommodated by Mn. For example, the Lyme disease pathogen Borrelia burgdorferi appears to have an obligate requirement for Mn (Posey & Gherardini, 2000). In the absence of Fe, the cell is limited in reactions that can be carried out; for example, aerobic respiration is impossible without cytochromes. However, the ability to forgo Fe may confer a selective advantage in natural environments in which there is intense competition for available Fe. An absolute requirement for Mn, whether in place of or in addition to Fe, would necessitate acquisition of strategies to effectively compete for Mn. Indeed, high-affinity uptake of Mn$^{2+}$ was reported in E. coli over three decades ago (Silver & Lusk, 1987), but the candidate gene for a high-affinity transporter was identified only last year (Kehres et al., 2000). Also, bacterial transcriptional regulators that respond to Mn$^{2+}$ have only recently been discovered, with homologous protein families shown to be present across the eubacteria (Posey et al., 1999; Jakubovics et al., 2000; Que & Helmann, 2000). This review will summarize some of the intracellular functions of Mn and discuss recent
advances that have been made in understanding the mechanisms utilized by bacteria for Mn uptake and homeostasis.

**Cellular functions of Mn**

Mn metalloenzymes have many diverse functions within bacterial cells (Christianson, 1997; Yocum & Pecoraro, 1999), some of which are summarized in Table 1. The ionic radius of Mn$^{2+}$ (0.76 Å) in aqueous solutions lies between that of Mg$^{2+}$ (0.65 Å) and Ca$^{2+}$ (0.99 Å), and is close to that of Fe$^{2+}$ (0.76 Å) and several other transition metal ions. It is therefore not surprising that Mn$^{2+}$ and other cations may be interchangeable in the metal-binding sites of many proteins. Most commonly, Mn and Mg are interchangeable on account of the similarities between chelate structures of these ions. However, Mn is essential for certain metabolic pathways. For example, oxygenic photosynthesis in cyanobacteria requires a tetra-Mn cluster present in the reaction centre complex of photosystem II (Table 1; Yocum & Pecoraro, 1999), and a number of enzymes, including MnSOD, mangani-catalase and arginase, specifically require Mn$^{2+}$ for activity (Christianson, 1997). In addition, glycolysis cannot proceed fully without 3-phosphoglycerate mutase (PGM) which, in several Gram-positive endospore-forming bacteria, is active only when associated with Mn$^{2+}$ (Chander et al., 1998). The pH-dependent dissociation of Mn$^{2+}$ from PGM represents a novel signalling mechanism for rapid enzyme inactivation (see below). It has been suggested that similar mechanisms may be responsible for the regulation of other strictly Mn$^{2+}$-dependent enzymes, such as arginase (Kuhn et al., 1995). In addition, it appears that Mn has an important role in bacterial signal transduction. The recently identified *E. coli* proteins PrpA and PrpB belong to a family of Mn$^{2+}$-containing serine/threonine protein phosphatases that are present widely in eukaryotes where they modulate complex signalling pathways (Barford, 1996). PrpA and PrpB are linked to the activity of a two-component sensor, CpxAB, and regulation of the periplasmic stress response protease HtrA/DegP (Missiakas & Raina, 1997). The involvement of Mn in signalling during *Bacillus* sporulation is discussed below.

The biological importance of Mn is not restricted to enzyme-mediated catalysis. For example, Mn$^{2+}$ can detoxify a variety of reactive oxygen species (ROS), protecting cells that lack enzymic defences (discussed below). Additionally, non-enzymic Mn$^{2+}$ is crucial for the proper function of a variety of bacterial products, including secreted antibiotics (see Archibald, 1986), and contributes to the stabilization of bacterial cell walls (Doyle, 1989).

**Bacillus development**

Early work in the 1950s demonstrated that Mn is unique amongst the transition metals in promoting both endospore formation and spore germination in *Bacillus* (Charney et al., 1951; Gould, 1969). Recent genetic studies have shown that Mn homeostasis is essential for efficient sporulation in *Bacillus subtilis* (Que & Helmmann, 2000), and that Mn appears to be important at several stages in the developmental cycle (Fig. 1). Sporulation in *B. subtilis* involves a complex sequence of transcriptional changes, controlled by several different σ factors, which are themselves subject to spatial and temporal regulation (Kroos et al., 1999). The initial decision to sporulate is triggered by starvation, and is transcriptionally evoked by σ^A_. At this time, cells are exquisitely sensitive to ROS, so that exposure of bacteria

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**Table 1. Selected functions of Mn within bacterial cells**

<table>
<thead>
<tr>
<th>Process or pathway</th>
<th>Enzyme or protein</th>
<th>Selected reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosynthesis</td>
<td>Mn-stabilizing protein (PSII-O)</td>
<td>Morgan et al. (1998)</td>
</tr>
<tr>
<td>Gluconeogenesis</td>
<td>PEP synthase</td>
<td>Chao et al. (1993)</td>
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<tr>
<td>Glycolysis</td>
<td>3-Phosphoglycerate mutase</td>
<td>Chander et al. (1998)</td>
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<tr>
<td>Sugar metabolism</td>
<td>l-Fucose isomerase</td>
<td>Seemann &amp; Schulz (1997)</td>
</tr>
<tr>
<td>Amino acid metabolism</td>
<td>Arginase</td>
<td>Sekowska et al. (2000)</td>
</tr>
<tr>
<td>Aromatic acid metabolism</td>
<td>Muconate cycloisomerase</td>
<td>Neidhart et al. (1990)</td>
</tr>
<tr>
<td>Peptide cleavage</td>
<td>Aminopeptidase P</td>
<td>Yocum &amp; Pecoraro (1999)</td>
</tr>
<tr>
<td>Nucleic acid degradation</td>
<td>Ribonuclease III</td>
<td>Ohtani et al. (2000)</td>
</tr>
<tr>
<td>Signal transduction</td>
<td>Serine/threonine protein phosphatases 1 and 2</td>
<td>Missiakas &amp; Raina (1997)</td>
</tr>
<tr>
<td>Oxidative stress defence</td>
<td>Mangani-catalase</td>
<td>Whittaker et al. (1999)</td>
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<tr>
<td></td>
<td>MnSOD</td>
<td>Fridovich (1995)</td>
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[1710]
Fig. 1. Roles of Mn in the developmental cycle of Bacillus. Mn homeostasis is essential for efficient initiation of sporulation in the vegetative cell (1). The switch from medial to polar septum formation (2) is influenced by the Mn-requiring SpoIIIE protein. Following septation, Mn accumulates in the developing forespore. SpoIIIE localizes to both poles of the cell, but exclusively activates $\sigma^F$ in the forespore. A drop in pH of around 1 unit accompanying forespore development (3) causes dissociation of Mn$^{2+}$ from phosphoglycerate mutase (PGM), inactivating the enzyme (PGM i) and leading to accumulation of the storage molecule 3-phosphoglyceric acid (3-PGA) (4). Production of the inner (shaded) and outer (thick line) layers of the spore coat (5) requires superoxide dismutase (MnSOD). Germination of the mature spore (6) involves a rapid increase in pH, leading to the activation of PGM and the mobilization of 3-PGA reserves. Protease production at this stage is stimulated by Mn, and high levels of Mn$^{2+}$-dependent pyrophosphatase (MnPPiase) are associated with an increase in metabolic activity. Chromosomal DNA is represented here by wavy lines.

To paraquat, which generates intracellular superoxide ($O_2^-$), at a concentration that does not affect vegetative growth, results in sporulation arrest (Inaoka et al., 1999). MnSOD has been shown to play a crucial protective role since inactivation of the sodA gene markedly reduces the paraquat concentration required to inhibit sporulation, while provision of sodA on a multicopy plasmid results in increased sporulation efficiency in the presence of paraquat (Inaoka et al., 1999). In the switch from symmetric to asymmetric cell division, which signals commitment to sporulation, Mn$^{2+}$ is directly involved as a cofactor of the SpoIIIE serine phosphatase (Schroeter et al., 1999). This is a bifunctional protein that influences polar septum formation through interactions with FtsZ (King et al., 1999), and that separately activates $\sigma^F$ in the forespore by dephosphorylation of SpoIIAA (Kroos et al., 1999). It is not yet clear how SpoIIIE activates $\sigma^F$ in the forespore, but not in the mother cell, since SpoIIIE and $\sigma^F$ are present in both cell types (King et al., 1999). It has been suggested that an inhibitor may bind to SpoIIIE in the forespore, thus delaying activation until the septum is complete (Arigoni et al., 1999). However, since Mn$^{2+}$ accumulates specifically within the developing forespore, it is possible that $\sigma^F$ activation by SpoIIIE may be Mn$^{2+}$-regulated.

Mn$^{2+}$ also influences spore composition, structure and germination. Bacillus endospores contain a storage reservoir of 3-phosphoglyceric acid (3-PGA) that is metabolized following germination. Accumulation of 3-PGA within the developing forespore occurs following acidification of the forespore compartment, with concomitant dissociation of the essential Mn$^{2+}$ cofactor from PGM (Kuhn et al., 1995; Chander et al., 1998) (Fig. 1). Upon initiation of spore germination, PGM is rapidly reactivated following an increase in intracellular pH, and 3-PGA is degraded (Kuhn et al., 1995; Chander et al., 1998). During the process of spore coat assembly, there is evidence that MnSOD may be involved in generating hydrogen peroxide (H$_2$O$_2$), which is required for the $\alpha,\alpha$-dityrosine cross-linking of the coat structural protein CotG (Henriques et al., 1998). During germination, Mn$^{2+}$ is involved in the activation of PGM, proteases and inorganic pyrophosphatase (Gould, 1969; Kuhn & Ward, 1998). Following recent advances in understanding of Mn homeostasis in B. subtilis (Que & Helmann, 2000), more detailed information on the functions of Mn in sporulation is anticipated in the near future. It is notable that Mn$^{2+}$ has recently been identified as an essential cation for cell cycle progression in Saccharomyces cerevisiae (Loukin & Kung, 1995).

Oxidative stress
One of the major challenges facing bacteria growing in oxygenated environments is to efficiently resist or repair...
damage caused by ROS such as O$_2^\cdot$, H$_2$O$_2$ and hydroxyl radicals (‘OH). Oxidative stress may be induced endogenously, for example by the oxidation of flavoproteins during respiration, or exogenously, by phagocytic production of ROS (Miller & Britigan, 1997). Therefore, enzymes for protection against reactive oxygen, including SODs, catalases and peroxidases, are ubiquitous in bacteria. Fe is intrinsically linked to oxidative damage, primarily through the ability of Fe$^{2+}$ to reduce H$_2$O$_2$ (Touati, 2000). One strategy for minimizing oxidative damage therefore involves limiting intracellular Fe content. In fact, there is good evidence that Lactobacillus plantarum and B. burgdorferi have dispensed with Fe requirements (Archibald, 1986; Posey & Gherardini, 2000), and also that several species of streptococci can grow in the absence of Fe (Spatafora & Moore, 1998; Niven et al., 1999; Jakubovics et al., 2000). Where bacteria grow in the absence of Fe they appear to have an absolute requirement for Mn$^{2+}$.

Bacteria have acquired elaborate defence mechanisms that co-ordinate Fe-sensing and Mn-sensing with oxidative stress responses (Bsat et al., 1998; Storz & Imlay, 1999). While Fe$^{2+}$ is toxic and can promote ‘OH radical formation, Mn$^{2+}$-containing compounds react with O$_2^\cdot$, H$_2$O$_2$ and ‘OH, without generating deleterious free radical species (Chetcon & Archibald, 1988; Stadtman et al., 1990). Mn is essential for the detoxification of ROS in most bacteria, principally as a cofactor for MnSOD (SodA). However, several groups of lactic acid bacteria incorporate high levels of intracellular Mn$^{2+}$ as a protectant in place of enzymic SOD (Archibald, 1986). Simple Mn(II) salts can also substitute for SOD activity in SOD-deficient laboratory mutants, providing increased resistance to toxic effects of O$_2^\cdot$ anions (Inaoka et al., 1999). The precise mechanism by which non-enzymic Mn$^{2+}$ scavenges O$_2^\cdot$ in bacteria is not understood, but requires considerably higher intracellular Mn$^{2+}$ levels than those needed for efficient MnSOD-mediated protection. In natural environments, enzymic SOD is probably dispensable only in bacteria such as L. plantarum that grow on Mn$^{2+}$-rich plant material.

Resistance to the toxic effects of superoxide anions more usually involves the concerted activities of two or more SOD enzymes. E. coli possesses three SOD activities: periplasmic Cu/ZnSOD (SodC) and two cytosolic proteins, FeSOD (SodB) and MnSOD (SodA) (Fridovich, 1993). The precise function of Cu/ZnSOD is unclear at present, but it has been suggested that this protein may protect against exogenous O$_2^\cdot$. Disruption of the sodA and sodB genes in E. coli leads to enhanced sensitivity of cells to O$_2^\cdot$ and generates nutritional auxotrophies resulting from inactivation of dihydroxyacid dehydratase by O$_2^\cdot$ (Kuo et al., 1987). On the other hand, it appears that B. subtilis (Inaoka et al., 1999) and most streptococci and enterococci (e.g. Niven et al., 1999), may produce only MnSOD. Inactivation of the sodA gene in Streptococcus pyogenes sensitizes these cells to O$_2^\cdot$ (Gibson & Caparon, 1996), and in Streptococcus pneumoniae reduces virulence in intranasal infection of mice (Yesilkaya et al., 2000). The cytoplasmic MnSOD in Strept. pyogenes is also secreted to the cell surface (Gerlach et al., 1998), where it may interact with exogenous O$_2^\cdot$. One of the most likely explanations for reduced virulence of bacteria that have defects in Mn$^{2+}$ uptake is that they are impaired in ability to cope with oxidative stress. Another important function of enzymic Mn is in the detoxification of H$_2$O$_2$ by mangani-catalase. Mangani-catalases have been described in L. plantarum and Thermus thermophilus (Whittaker et al., 1999), and recently in a range of Gram-negative bacteria (Robbe-Saule et al., 2001).

### Mn$^{2+}$ transport systems

Mn homeostasis in bacteria depends upon regulation of Mn$^{2+}$ transport. Early work in E. coli demonstrated that Mn$^{2+}$ uptake was mediated by a high-affinity import system with a $K_m$ of approximately 0·2 nM and a $V_{\text{max}}$ of 1–4 nmol min$^{-1}$ per 10$^{12}$ cells (reviewed by Silver & Lusk, 1987). This system could not be inhibited by Ca$^{2+}$ or Mg$^{2+}$, but was sensitive to Fe$^{2+}$ or Co$^{2+}$ inhibition. Uptake systems for Mn$^{2+}$ were also identified in a range of Gram-positive bacteria and, in contrast to E. coli, Mn$^{2+}$ transport in B. subtilis, L. plantarum and Staphylococcus aureus was competitively inhibited by Cd$^{2+}$ (Silver & Lusk, 1987). The maximal rate of Mn$^{2+}$ uptake in L. plantarum was found to be unusually high [$V_{\text{max}}$ of 24 µmol min$^{-1}$ (g protein$^{-1}$)], consistent with the high concentration of internal Mn$^{2+}$ (30–35 mM) in this organism (Archibald, 1986). The mechanisms of Mn$^{2+}$ uptake were unknown until Bartsevich & Pakrasi (1995) discovered the mntCAB operon encoding an ATP-dependent transporter in Synechocystis sp. PCC 6803, a cyanobacterium with a high Mn$^{2+}$ requirement for photosynthesis. Subsequently, the pace of research on bacterial Mn$^{2+}$ uptake has intensified, with characterization of a number of transporters for Mn$^{2+}$. These transport systems are sufficiently structurally conserved to allow the location of the genes encoding them within all the currently sequenced bacterial genomes. At least three types of Mn$^{2+}$ import systems have now been identified. The unusual transporter from L. plantarum is a P-type ATPase (MntA), the only one to date that has been demonstrated to have high specificity for Mn$^{2+}$ (Hao et al., 1999). The other two types of Mn$^{2+}$ uptake system conform to either the ATP-binding cassette (ABC) transporter superfamily or to the natural resistance-associated macrophage protein (Ntramp) family.

### ABC transporters

The mntCAB operon of Synechocystis encodes a solute-binding extracytoplasmic protein (MntC), a cytoplasmic ATP-binding component (MntA) and a transmembrane protein (MntB). Protein MntC shares 32% amino acid sequence identity with ScaA in Streptococcus gordoni, previously identified as a lipoprotein associated with an ABC transporter of unknown function (Kolenbrander et al., 1994). ScaA-like proteins have been found across the streptococci and enterococci (Burnette-Curley et al.,...
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1995; Berry & Paton, 1996; Singh et al., 1998; Kitten et al., 2000). They constitute a family of proteins originally designated Lral (Jenkinson, 1994) and now recognized as a new cluster of solute-binding proteins for metal ion transport (Dintilhac et al., 1997). In Strep. gordonii (Kolenbrander et al., 1998) and Strep. pneumoniae (Dintilhac et al., 1997), the sacCBA and psaBCA operons encode the components of ABC-type uptake systems for Mn\(^{2+}\). The \(K_m\) of the Strep. gordonii Sca transporter was estimated at 0.1–0.3 \(\mu\)M (Kolenbrander et al., 1998), somewhat lower than that of the Mnt permease in Synechocystis sp. PCC 6803 (Bartsevich & Pakrasi, 1995). In addition to the PsA (Mnt\(^{2+}\)) transporter, Strep. pneumoniae expresses an ABC-type Zn\(^{2+}\) permease, encoded by the adcCBA genes, and this is thought to transport Mn\(^{2+}\) also (Dintilhac et al., 1997). Mutations in the Strep. gordonii sca genes, or in the Strep. pneumoniae psa genes, affect Mn\(^{2+}\) uptake and reduce the abilities of cells to undergo DNA-mediated transformation (Dintilhac et al., 1997; Kolenbrander et al., 1998).

The form of Mn\(^{2+}\) that is transported by the ABC-type permeases has not been determined. Future studies will be facilitated by assays of substrate binding by purified solute-binding proteins or reconstituted transport systems. The cation specificity of the Strep. pyogenes Lral lipoprotein MtsA was investigated by ligand blotting (Janulczyk et al., 1999) and shown to be relatively broad, with specificity for Zn\(^{2+}\), Fe\(^{3+}\) or Cu\(^{2+}\), but surprisingly not for Mn\(^{2+}\). The X-ray crystal structure of pneumococcal PsA has been determined to 2.0 \(\text{Å}\) resolution and Zn\(^{2+}\), rather than Mn\(^{2+}\), was bound to the recombinant protein (Lawrence et al., 1998). Thus, evidence for direct binding of Mn\(^{2+}\) ions to the solute-binding components of ABC transporters is not convincing. The possibility that Mn\(^{2+}\) is bound and transported as a chelated complex cannot be ruled out. Uptake of Mn\(^{2+}\) by the P-type ATPase of L. plantarum is stimulated by citrate and other tricarboxylic acids, although citrate does not seem to be imported (Archibald, 1986; Hao et al., 1999).

**Nramps**

Mn\(^{2+}\) uptake in bacteria can be inhibited by uncouplers (Silver & Lusk, 1987), and the discovery in bacteria of proteins belonging to the Nramp transporter family (Kehres et al., 2000; Que & Helmann, 2000), accounts for this observation. The Nramp proteins form a class of pH-dependent divalent transition metal cation transporters that are required for intestinal Fe uptake and for host macrophage cell resistance to chronic bacterial infections (Gruenheid & Gros, 2000). The substrate specificity and cation uptake kinetics of the E. coli Nramp homologue MntH (Kehres et al., 2000) suggest that this transporter corresponds to the Mn\(^{2+}\) uptake system originally described over 30 years ago. Several other bacterial Nramps have been characterized to date, and inspection of genomic sequence data shows that they are widespread throughout many diverse groups of eubacteria. A primary role for the Nramp-like proteins in E. coli and Salmonella typhimurium may be to help protect cells against ROS, a function that is particularly important during infection of the host (Kehres et al., 2000; see below).

**Cation export**

Transition metal cations, including Co\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\) and Zn\(^{2+}\), are actively extruded from bacterial cells (Silver, 1996) and regulation of export systems contributes significantly to the homeostatic control of these metal ions. In many cases, genes encoding metalloregulatory proteins are adjacent to those encoding the export apparatus. In Synechocystis sp. PCC 6803, an operon containing nine ORFs encodes the components required for the independent sensing and export of Ni\(^{2+}\), Co\(^{2+}\) and Zn\(^{2+}\) (García-Domínguez et al., 2000). It is possible that regulated export systems for Mn\(^{2+}\) exist, but no system for export of Mn\(^{2+}\) by bacteria has yet been demonstrated.

**Transcriptional regulation**

The central regulator of Fe transport pathways in E. coli is Fur (Escolar et al., 1999). Fur is a metalloprotein that, in the presence of Fe\(^{3+}\) or Mn\(^{2+}\), binds to the operator regions of at least 20 genes involved in diverse cell functions, including siderophore production, transmembrane Fe transport, motility, virulence factor expression and the oxidative stress and acid shock responses. In Corynebacterium diphtheriae, Fe-dependent gene expression was shown to be controlled by DtxR protein (Holmes, 2000), a metallorepressor with <15% amino acid identity to Fur. DtxR regulates the expression of a number of genes involved in siderophore production, haem degradation and diphtheria toxin biosynthesis. Until recently, transcriptional responses of bacteria to transition metals other than Fe were relatively poorly understood. This picture has changed dramatically over the past 5 years, with the identification of novel metalloregulators responsive to Mn\(^{2+}\), Co\(^{2+}\), Cu\(^{2+}\) and Zn\(^{2+}\). Thus, at least three distinct classes of metalloproteins control Zn\(^{2+}\) transport. In B. subtilis and E. coli, a high-affinity Zn\(^{2+}\) uptake transporter is regulated in response to Zn\(^{2+}\) by the Zur metalloregulator protein, which shares around 27% identity with Fur (Gaballa & Helmann, 1998). Cyanobacterial Zn\(^{2+}\) resistance is modulated by SmtB and ZiaR, both members of the ArsR metalloregulator family (Thelwell et al., 1998). ZnTR, a Zn\(^{2+}\)-dependent regulator of the MerR protein family, negatively controls expression of the Zn\(^{2+}\) efflux pump ZntA in Staph. aureus (Singh et al., 1999). MerR family proteins CorR of Synechocystis sp. PCC 6803 (García-Domínguez et al., 2000) and CueR of E. coli (Stoyanov et al., 2001) control the export of Co\(^{2+}\) and Cu\(^{2+}\), respectively. These responses to Co\(^{2+}\), Cu\(^{2+}\) and Zn\(^{2+}\) seem to be confined to cation homeostasis and there is no evidence yet that they directly affect the expression of genes involved in other cell processes.

Mn\(^{2+}\)-sensing occurs widely in bacteria and influences
both Mn\(^{2+}\) homeostasis and genes involved in the oxidative stress response. Several regulatory proteins related to *C. diptheriae* DtxR have recently been shown to respond uniquely to Mn\(^{2+}\) (Posey et al., 1999; Jakubovics et al., 2000; Que & Helmann, 2000). These proteins provide a common mechanism for the control of Mn\(^{2+}\) permease production. When intracellular levels of Mn\(^{2+}\) rise, the metalloregressor (designated Sca in *Strep. gordonii*) binds with high affinity to the transporter operon promoter, thus inhibiting transcription. Under limiting Mn\(^{2+}\), the apoprotein dissociates from the promoter control region such that repression is rapidly relieved, with upregulation of transcription and corresponding increase in permease levels (Jakubovics et al., 2000). In *B. subtilis*, the metalloregressor MntR is a bifunctional protein. It activates transcription of *mntABCD* encoding the ABC transporter in low Mn\(^{2+}\), but represses expression of the Nramp transporter encoded by *mntH* in Mn\(^{2+}\)-replete conditions (Que & Helmann, 2000). Disruption of the negative regulatory control mechanisms on Mn\(^{2+}\) uptake may sensitize cells to Mn\(^{2+}\) toxicity. For example, overexpression of *mntH* encoding the Nramp transporter on a high-copy-number plasmid in *E. coli* or *Sal. typhimurium* decreases the growth rates of cultures in media containing >10 \(\mu\)M Mn\(^{2+}\) (Kehres et al., 2000).

Likewise, constitutive derepression of *mntH* expression in *B. subtilis*, as a result of inactivating the metalloregulator gene *mntR*, greatly sensitizes the cells to Mn\(^{2+}\) (Que & Helmann, 2000). Interestingly, a *mntA* knockout mutant in the ABC transporter was also more sensitive to Mn\(^{2+}\), presumably because the MntH channel was upregulated in this strain (Que & Helmann, 2000). Thus, the co-ordinated expression and activities of the transporters are necessary for the cells to be able to respond to fluctuations in external Mn\(^{2+}\).

It is not yet known whether the Mn\(^{2+}\)-specific DtxR-like proteins have global effects on transcription. Oxidative stress response genes are controlled in response to Mn\(^{2+}\) by the PerR metalloregressor, which shares about 28% identity with Fur protein. *B. subtilis* PerR responds to both Fe\(^{2+}\) and Mn\(^{2+}\) in controlling transcription at \(\geq\) four loci, collectively known as the peroxide regulon (Bsat et al., 1998). PerR-repressed genes encode a variety of oxidative stress response proteins, including the vegetative cell catalase, alkyl hydroperoxide reductase, haem biosynthetic enzymes and a protective DNA-binding protein, MrgA.

Recent studies with *L. plantarum* have provided further evidence for an important role of Mn\(^{2+}\)-sensing and signalling in bacteria. Expression of only the P-type ATPase MntA, but also of two glycolytic enzymes, enolase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), is repressed in Mn\(^{2+}\)-rich medium (Hao et al., 1999). Surface-bound enolase and GAPDH have been shown to bind human tissue proteins in a variety of organisms, and in group A streptococci they are implicated in the ability of the bacteria to modulate host cell functions (Pancholi & Fischetti, 1998). It is tempting to speculate, therefore, that Mn\(^{2+}\) concentrations might influence expression of surface proteins that are relevant for colonization or virulence.

**Mn\(^{2+}\) homeostasis and virulence**

Clear links have been established between expression of the Lrl family of putative metal-ion-binding proteins and the course of bacterial infections. Antibodies to EfaA, the metal-ion-binding protein of the Efa permease in *Enterococcus faecalis*, are elevated in patients with enterococcal endocarditis (Jiang et al., 1997). Mutants of *Streptococcus parasanguinis* and *Strep. pneumoniae* in which the Lrl polypeptide genes are inactivated are avirulent in animal models of endocarditis (Burnette-Curley et al., 1995) and sepsis (Berry & Paton, 1996). Essential roles for the ABC-type Mn\(^{2+}\) transporters have recently been shown in virulence of *E. faecalis* (Singh et al., 1998), *Streptococcus mutans* (Kitten et al., 2000) and *Yersinia pestis* (Bearden & Perry, 1999). The solute-binding lipoprotein component of the streptococcal ABC transporter for Mn\(^{2+}\) is upregulated under Mn\(^{2+}\)-limiting conditions (Jakubovics et al., 2000), such as those in serum where Mn\(^{2+}\) levels are characteristically around 20 nM (Krachler et al., 1999). Thus, these proteins may be essential for Mn\(^{2+}\) homeostasis and for survival of bacteria in the animal host.

Roles for the bacterial Nramps in virulence are less well established. These proteins might be necessary for the acquisition of divalent cations by bacteria within phagosomes during chronic infections. However, disruption of *mntH* in *Sal. typhimurium*, while delaying mortality in a mouse model, does not affect survival of bacteria within macrophages (Kehres et al., 2000). There is, on the other hand, a direct correlation between Mn\(^{2+}\) homeostasis and sensitivity of bacteria to oxidative stress. Inactivation of the Nramp transporter genes in *B. subtilis*, *E. coli* or *Sal. typhimurium* enhances the sensitivities of these bacteria to H\(_2\)O\(_2\) compared with the corresponding wild-type strains (Kehres et al., 2000; Que & Helmann, 2000). In *Strep. gordonii*, the activity of MnSOD is 50% reduced in *scaA* or *scaC* mutants that lack the functional ABC-type Mn\(^{2+}\) transporter (N. S. Jakubovics, A. W. Smith & H. F. Jenkinson, unpublished). Taken collectively, these recent data indicate that bacterial systems for acquiring Mn\(^{2+}\) are crucial for protection against reactive oxygen. Since ROS play such an important role in host defence against bacterial infection (Miller & Britigan, 1997), it is logical to hypothesize that an essential function of Mn\(^{2+}\) sequestration by infecting bacteria is to activate defence systems against both externally and internally derived ROS.

A model for the role of Mn\(^{2+}\) homeostasis in the pathogenesis of streptococcal infections is shown in Fig. 2. The main features of this proposal centre on the mechanisms by which streptococci are able to resist the toxic effects of ROS. Exogenous O\(_2\) is probably detoxified by cell-surface MnSOD (Gerlach et al., 1998). Internal ROS are produced following diffusion of molecular oxygen into bacterial cells from the blood-
stream. \( \text{H}_2\text{O}_2 \) is generated intracellularly predominantly by the activities of pyruvate oxidase (Spx; Pericone et al., 2000) and other oxidases, which also generate \( \text{O}_2^\cdot \) (Fig. 2). The major enzyme capable of detoxifying \( \text{O}_2^\cdot \) in streptococci is MnSOD. Since streptococci lack catalase, most \( \text{H}_2\text{O}_2 \) generated by oxidases and MnSOD is released by the cells, causing haemolysis or tissue damage (Barnard & Stinson, 1996). Internal protection against \( \text{H}_2\text{O}_2 \) is afforded by peroxidases such as thiol peroxidase (Tpx), or is extruded from the cell (dashed line with arrowhead).

**Future perspectives**

\( \text{Mn}^{2+} \) is an essential cofactor for many enzymes, some of which, for example MnSOD, are essential for bacterial growth and survival under oxidative stress. In *Bacillus* spp. it has long been recognized that \( \text{Mn}^{2+} \) is required for sporulation, but research into the topic has lain dormant until recently. With new insights into the mechanisms of \( \text{Mn}^{2+} \) transport, regulation of \( \text{Mn}^{2+} \)-dependent gene expression and the importance of \( \text{Mn}^{2+} \) in oxidative stress defence, future advances in these areas are predicted. Several questions regarding the basic functions of \( \text{Mn}^{2+} \) homeostasis in bacteria remain unanswered. For example, how does \( \text{Mn}^{2+} \) protect against reactive oxygen in the absence of SOD? What is the physiological significance of this non-enzymic protection mechanism in bacteria? Are there other types of \( \text{Mn}^{2+} \) transporters to be discovered? Do efflux pumps fine-tune intracellular \( \text{Mn}^{2+} \) levels and thus contribute to \( \text{Mn}^{2+} \) homeostasis?

In the immediate future, it will be interesting to identify other \( \text{Mn}^{2+} \) transporters that are related to the P-type ATPase of *L. plantarum*, which has an unusually high capacity for \( \text{Mn}^{2+} \) import (Hao et al., 1999). Also, the discovery of Nramp transporters in bacteria has underscored the similarities between prokaryotic and eukaryotic systems for essential cation uptake. Determining the relative significance of multiple \( \text{Mn}^{2+} \) import systems in homeostasis in individual species presents a major challenge for future research. Significantly, the observation that \( \text{Mn}^{2+} \) acquisition by bacteria is linked to virulence in the host suggests that \( \text{Mn}^{2+} \) transport is a potential new therapeutic target for control of bacterial infection.

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