Molecular Microbiology (2003) **47**(6), 1485–1494

MicroReview

Mechanisms of iron regulation in mycobacteria: role in physiology and virulence

G. Marcela Rodriguez and Issar Smith*
TB Center, Public Health Research Institute at the International Center for Public Health, 225 Warren Street, Newark, NJ 07103-3535, USA.

Summary

The role of iron in mycobacteria as in other bacteria goes beyond the need for this essential cofactor. Limitation of this metal triggers an extensive response aimed at increasing iron acquisition while coping with iron deficiency. In contrast, iron-rich environments prompt these prokaryotes to induce synthesis of iron storage molecules and to increase mechanisms of protection against iron-mediated oxidative damage. The response to changes in iron availability is strictly regulated in order to maintain sufficient but not excessive and potentially toxic levels of iron in the cell. This response is also linked to other important processes such as protection against oxidative stress and virulence. In bacteria, iron metabolism is regulated by controlling transcription of genes involved in iron uptake, transport and storage. In mycobacteria, this role is fulfilled by the iron-dependent regulator IdeR. IdeR is an essential protein in *Mycobacterium tuberculosis*, the causative agent of human tuberculosis. It functions as a repressor of iron acquisition genes, but is also an activator of iron storage genes and a positive regulator of oxidative stress responses.

Introduction

For most bacteria, iron is an essential, elusive and potentially hazardous element. Iron is essential as a cofactor of enzymes involved in vital cellular functions ranging from respiration to DNA replication. It is elusive because, in the presence of oxygen, at physiological pH, it exists mainly in insoluble ferric complexes. In higher organisms, iron is maintained in solution bound to iron transport and storage proteins such as transferrin, lactoferrin and ferritin; therefore, in a mammalian host, the levels of free iron are far lower than those required for bacterial survival (Weinberg, 1999). Iron is potentially hazardous because of its ability to catalyse the Fenton reaction, which leads to the generation of toxic oxygen radicals from normal products of aerobic metabolism (Imlay *et al*., 1988). As a consequence of these three properties, iron has a decisive role in infectious diseases. On one hand, pathogens have to compete for iron in the host so that they can multiply and establish a successful infection but, on the other hand, they must regulate iron metabolism to prevent excess iron that can initiate a cascade of lethal reactions.

Mycobacteria have solved the problem of iron solubilization and acquisition by secreting high-affinity iron chelators (siderophores) that sequester Fe^{3+}. The ferric–siderophore complexes are transported into the bacteria by an as yet undefined process, and iron is released in the cytoplasm, probably by reduction. Ferrous iron is then available for cellular functions including incorporation into metalloproteins. Recent comprehensive reviews have covered iron acquisition as well as the synthesis and biochemistry of mycobacterial siderophores (De Voss *et al*., 1999; Ratledge, 1999), and this article will focus on regulation of iron metabolism and iron-dependent processes in these bacteria. Regulation of iron metabolism involves sensing intracellular iron concentrations and modulating uptake and storage accordingly. Failure to do so can lead to iron deficiency or to toxic iron overload. Understanding the adaptive response of mycobacteria to changes in iron levels and the mechanisms by which this response is orchestrated will allow the development of new ways of interfering in those events. Interestingly, iron acquisition systems and virulence determinants are often co-regulated in an iron-dependent fashion (Litwin and Calderwood, 1993), a fact that further demonstrates the importance of understanding iron-dependent regulation in pathogenic bacteria.

Mycobacterial response to iron availability

The first experiments to evaluate the response to iron

Accepted 18 November, 2002. *For correspondence. E-mail smitty@phri.org; Tel. (+1) 973 854 3260; Fax (+1) 973 854 3261.

© 2003 Blackwell Publishing Ltd

deprivation in mycobacteria were conducted by Ratledge, Winder and colleagues nearly 40 years ago (Ratledge and Winder, 1962; Winder and O'Hara, 1964). *Mycobacterium smegmatis*, cultured in iron-deficient medium, was shown to undergo several metabolic changes including decreases in DNA–protein ratio, in the levels of porphyrins (haem precursors) and in the activity of iron-containing enzymes such as aconitase and succinate dehydrogenase. Increases in the levels of salicylic acid as well as the activities of several enzymes including a DNA polymerase were also observed. Subsequent studies have examined the production of iron-binding molecules, the changes in protein synthesis and the modulation of gene expression in mycobacteria cultured in low- or high-iron concentrations.

Production of iron-binding molecules in response to iron deficiency

The onset of iron limitation in mycobacteria induces a response aimed at increasing iron acquisition as siderophore production is greatly enhanced (Raghu *et al*., 1993). Mycobacteria produce two classes of siderophores, mycobactins and the exochelins, and details of siderophore structure and biosynthesis have been published elsewhere (Snow, 1965; Gobin *et al*., 1995; Quadri *et al*., 1998; De Voss *et al*., 1999). Pathogenic mycobacteria solely produce mycobactins, whereas saprophytic mycobacteria such as *M. smegmatis* and *Mycobacterium neoarum* produce both mycobactins and exochelins (Ratledge and Ewing, 1996). Mycobactins are salicylate-containing siderophores, and exochelins are peptidic molecules. Mycobactins are found in two forms that differ in the length of an alkyl substitution and hence in polarity and solubility. The less polar form remains cell associated (mycobactin), whereas the more polar one (carboxymycobactin) is secreted into the medium. Mycobacterial siderophores, like most other siderophores, are synthesized by non-ribosomal peptide synthetases. A genetic cluster containing genes required for exochelin synthesis was identified in *M. smegmatis* (Fiss *et al*., 1994; Yu *et al*., 1998; Zhu *et al*., 1998). Three genes from this cluster are essential for exochelin MS biosynthesis: *fxbA*, *fxbB* and *fxbC*. *fxbA* encodes a putative formyltransferase, whereas *fxbB* and *fxbC* encode peptide synthetases. Three open reading frames (ORFs) located upstream of *fxbA* and transcribed in the opposite direction encode proteins that have similarity to the ferric-enterobactin transporters FepD, FepG and FepC of *Escherichia coli.* Based on this homology, it has been proposed that these proteins (FxuA, FxuB and FxuC) might serve as ferric-exochelin transporters. The function of the other two genes in this cluster, *exiT* and *fxuD*, is not clear. ExiT resembles an ABC transporter, whereas FxuD is homologous to a family of proteins involved in iron transport that includes the ferrichrome receptor FhuD from *Bacillus subtilis* and the FepB protein (involved in iron dicitrate transport) of *Synechocystis* sp. Annotation of the complete genome sequence of *M. tuberculosis* identified a cluster of 10 genes, the *mbt* locus that, based on homology with other systems, encodes the appropriate enzymes for the synthesis of the mycobactin and carboxymycobactin core. Three peptide synthetases (MbtB, MbtE and MbtF), two polyketide synthases (MbtD and MbtC), an isochorismate synthase (MbtI) that converts chorismate to salicylate, a salicyloyl-AMP ligase (MbtA) and a hydroxylase (MbtG) are encoded by the *mbt* genes (Quadri *et al*., 1998). The fact that an *M. tuberculosis mbtB* mutant was unable to produce either mycobactin or carboxymycobactin demonstrated that both mycobactin types are generated by the same biosynthetic pathway (De Voss *et al*., 2000). However, gene(s) encoding the enzyme(s) responsible for transferring the alkyl substitutions of different lengths that distinguish mycobactin and carboxymycobactin are not found in this cluster and have not been identified in the *M. tuberculosis* genome.

It is presently unclear why mycobacteria require two siderophores: a cell-associated (mycobactin) and a secreted molecule (carboxymycobactin in pathogenic mycobacteria and carboxymycobactin and exochelin in saprophytic mycobacteria). It has been suggested that the association of mycobactin with the membrane could allow it to participate in iron internalization and/or to serve as a temporary iron-holding molecule to prevent sudden influx of excess iron if the metal suddenly becomes available after a period of iron limitation (Ratledge *et al*., 1982; Ratledge, 1999). Disruption of the production of one type of siderophore independently of the other is necessary to understand the contribution of each to iron uptake. In this regard, examination of iron uptake in the *M. smegmatis* mutants defective in exochelin synthesis (Fiss *et al*., 1994; Yu *et al*., 1998; Zhu *et al*., 1998) would help to address this question.

Salicylic acid is another iron-binding molecule, the production of which is enhanced in mycobacteria maintained in iron-deficient conditions (Ratledge and Winder, 1962), but its function is not fully understood. It has low affinity for iron, which rules out a role as a siderophore (Ratledge *et al*., 1974; Chipperfield and Ratledge, 2000). Although salicylic acid is a precursor for mycobactin biosynthesis (Ratledge and Hall, 1970), this is not its only role, as the requirement for salicylate in a salicylate-requiring auxotrophic mutant of *M. smegmatis* cannot be satisfied by providing mycobactin, carboxymycobactin or exochelin. Furthermore, these mutants require salicylate to assimilate iron from ferri-siderophores fully (Adilakshmi *et al*., 2000).

Protein changes in response to different iron levels

Iron-regulated proteins (IRPs) have been identified by comparing the gel electrophoresis patterns of proteins extracted from mycobacteria grown in low- and high-iron conditions (Hall *et al*., 1987; Lundrigan *et al*., 1997; Wong *et al*., 1999). The IRPs found in the bacterial envelope (iron-regulated envelope proteins or IREPs) have received special attention, as they might be involved in the transport of ferric–siderophore complexes into the cytoplasm. At least five IREPs were detected in membrane extracts from *M. smegmatis* grown in iron-deficient conditions including the extensively studied 29 kDa IREP (Hall *et al*., 1987). This protein can associate directly *in vitro* with ferri-exochelin, and the addition of a polyclonal antiserum generated against it to *M. smegmatis* cells significantly inhibits ferri-exochelin-mediated iron uptake (Dover and Ratledge, 1996). Based on these observations, the 29 kDa protein has been postulated to be a ferri-exochelin receptor in *M. smegmatis*. However, gene inactivation experiments must be done in order to confirm its role in ferric-exochelin uptake.

Induction of the stress-related proteins DnaK and GroEL by H_2O_2 is inhibited in *M. smegmatis* growing under iron limitation (Lundrigan *et al*., 1997). However, these proteins are induced normally by heat and ethanol shock in both iron-deficient and iron-sufficient bacteria. This observation and the fact that iron-deficient bacteria are more sensitive to H_2O_2 suggests a close connection between iron metabolism and oxidative stress response. This relationship has become evident from other experiments that are discussed later in this article.

Studies in *M. tuberculosis* (Calder and Horwitz, 1998; Wong *et al*., 1999), using one- and two-dimensional gel electrophoresis combined with mass spectrometry and sequence information, have shown that the levels of several proteins change in response to iron availability. Among these, a putative cation-transporting ATPase, a mycobacterial homologue of PEPCK (phosphoenolpyruvate carboxykinase) and an NADP-dependent dehydrogenase were induced in bacteria grown in a low-iron medium. On the other hand, FurA (a putative metal regulator), a homologue of EF-Tu and an aconitase were synthesized in higher amounts in bacteria grown in iron-rich medium.

Iron-responsive changes in gene expression

Transcriptional profiling was used to examine the response of *M. tuberculosis* to low- and high-iron conditions. The transcription of 155 ORFs was modulated by the levels of iron in the growth medium. Iron deficiency induced about two-thirds of those genes, while the remainder were upregulated in iron-rich medium (Rodriguez *et al*., 2002). Half the genes induced in low-iron conditions were of unknown function. Among the others, two functional groups were distinguished: one part of the response is clearly focused in overcoming iron deficiency by increasing iron uptake. For instance, the siderophore biosynthesis genes as well as several putative transporters are upregulated by iron deficiency. The other part of the response adjusts the general metabolism of the cell to meet the challenge imposed by iron restriction. It is evident that the bacterium needs to maintain vital processes that depend on iron-containing cofactors, including electron transport, energy metabolism and DNA synthesis. Induction of genes involved in iron sulphur cluster assembly (*nifS* and *nifU*) and a gene encoding a putative haem biosynthesis protein (Rv0693) suggests an attempt by the bacteria to use any available iron for the synthesis of these essential cofactors and the assembly of indispensable iron-containing proteins. Consistent with this is the induction of genes of the biosynthetic pathways for histidine (*hisI*) and cysteine (*cysH*), two critical amino acids that are part of metal-binding centres on haem and non-haem iron-containing proteins. Genes encoding the essential iron-containing proteins, ribonucleoside reductase and a cytochrome P-450 (Rv0766c), are also induced. Increased glycolysis under iron deprivation is suggested by the induction of *pfkA* encoding phosphofructokinase, the most important control point in glycolysis. This could reflect an attempt to increase substrate-level phosphorylation to keep producing ATP in the face of decreased cytochrome biosynthesis and presumably diminished oxidative phosphorylation. Lipid metabolism may also be affected, as several genes encoding enzymes annotated as being involved in this process are induced. As the function of these enzymes, i.e. biosynthetic or degradative, has not been demonstrated, the full significance of these observations is unknown. All these changes could be orchestrated by more than one regulatory network, as genes encoding putative transcriptional regulators and two sigma factors i.e. sigma E and sigma B, are also upregulated under iron deficiency. When cultured in iron-rich medium, *M. tuberculosis* upregulates *bfrA*, *bfrB* and *katG*, indicating that, under those conditions, it is important to increase iron storage capacity and prevent oxidative damage that might be caused by excess iron. *bfrA* and *bfrB* encode two putative iron storage proteins, bacterioferritin and ferritin, respectively, whereas *katG* codes for catalase-peroxidase, which catalyses the dismutation of hydrogen peroxide into water and molecular oxygen. The induction of *katG* is consistent with the need to scavenge hydrogen peroxide to prevent the oxidation of ferrous iron and the generation of oxygen radicals through the Fenton reaction.

Iron-dependent regulation in bacteria

The response to changing iron concentrations is controlled in bacteria at the transcriptional level by specialized iron-dependent regulatory proteins. These can be divided into two distinct groups: the Fur and the DtxR families. Fur (ferric uptake regulator) was identified in *E. coli* and *Salmonella* sp. mutants unable to repress the production of siderophores and their receptor proteins under high-iron conditions (Bagg and Neilands, 1987). Fur homologues are widespread among prokaryotes, including Gram-negative bacteria and Gram-positive species. In general, proteins of the Fur family act as transcriptional repressors, as they bind to Fe^{2+} and to a specific sequence in iron-regulated promoters inhibiting the transcription of downstream genes (de Lorenzo *et al*., 1987). However, Fur can also positively regulate the transcription of some genes in an indirect way (Niederhoffer *et al*., 1990; Gruer and Guest, 1994; Tsolis *et al*., 1995). The mechanism of this indirect action, at least in *E. coli*, is the iron-dependent Fur repression of the synthesis of a small RNA that blocks translation of mRNAs for bacterioferritin and ferritin among other proteins (Masse and Gottesman, 2002). The effect of Fur on the iron-activated ferritin-encoding gene from *Helicobacter pylori* (*pfr*) is another example of this indirect effect. Fur binds to the *pfr* promoter in low iron, and it dissociates from this DNA sequence when iron levels are high. This suggests that the iron-dependent release of Fur from this promoter will derepress its transcription (Delany *et al*., 2001). Fur is a pleiotropic regulator, as it controls not only iron metabolism but diverse processes including oxidative stress response, acid tolerance, chemotaxis, swarming, general metabolism and the synthesis of toxins and other virulence factors (Touati, 2000; Hantke, 2001).

DtxR was identified as the iron-dependent toxin regulator in *Corynebacterium diphtheriae* (Boyd *et al*., 1990; Schmitt and Holmes, 1991). DtxR-like proteins were later identified in other actinomycetes, including streptomycetes, *Brevibacterium lactofermentum* and mycobacteria (Doukhan *et al*., 1995; Gunter-Seeboth and Schupp, 1995; Oguiza *et al*., 1995; Schmitt *et al*., 1995). Although there is no sequence similarity between DtxR and Fur, these two proteins are functional homologues, as DtxR also acts as a metal-dependent transcriptional repressor. It regulates genes encoding iron transport systems, a haem oxygenase, virulence determinants and also genes involved in protecting bacteria from oxidative stress (Schmitt and Holmes, 1991; Schmitt *et al*., 1997; Oram *et al*., 2002; Qian *et al*., 2002). A group of DtxR-like proteins that includes ScaR from *Streptococcus gordonii* (Jakubovics *et al*., 2000), TroR from *Treponema pallidum* (Posey *et al*., 1999), MntR from *B. subtilis* (Que and Helmann, 2000) and *E. coli* (Patzer and Hantke, 2001) controls genes encoding Mn^{2+} transport systems. In *B. subtilis*, MntR is a bifunctional regulator as it represses the Mn^{2+} ABC transporter *mntABC* in high Mn concentrations, while positively regulating it when the levels of Mn are low (Que and Helmann, 2000).

Members of both Fur and DtxR families of metal regulators are found in mycobacteria. The annotation of the *M. tuberculosis* genome sequence revealed two Fur-like proteins, FurA and FurB, and two DtxR homologues, IdeR and SirR. The *Mycobacterium leprae* genome sequence has genes coding for IdeR, FurB, FurA and SirR, but the last two are pseudogenes. In several mycobacteria including *M. tuberculosis*, *M. leprae*, *Mycobacterium marinum* and *M. smegmatis*, *furA* is located immediately upstream of the catalase:peroxidase-encoding gene *katG* (Milano *et al*., 2001; Pym *et al*., 2001; Zahrt *et al*., 2001). In *M. tuberculosis* (Pym *et al*., 2001) and *M. smegmatis* (Zahrt *et al*., 2001), FurA negatively regulates the expression of *katG*, thereby modulating the response to oxidative stress. This effect, however, is iron independent in *M. smegmatis* (Pym *et al*., 2001). Nothing is known about the function of FurB and SirR. Currently, IdeR is the metalloregulator that has been best characterized as to structure and function. As will be discussed below, this protein is the main regulator of iron metabolism in *M. tuberculosis.*

The IdeR protein

IdeR is found in pathogenic and non-pathogenic mycobacteria and is a closely related homologue of DtxR (Doukhan *et al*., 1995). The two ≈230-amino-acid proteins are over 90% identical in the first 180 amino acids. IdeR has two binding sites for divalent metals and three distinct functional domains: the amino-terminal domain containing a helix–turn–helix DNA-binding motif and the dimerization domain that also bears most of the metal-binding residues that are found in the first 180 amino acids, the sequence with the greatest similarity to DtxR. The third domain in the carboxy-terminal region is positioned in the groove between the first two domains and is characterized by having an SH3 (Src homology domain 3)-like fold, suggesting possible interactions with other proteins (Pohl *et al*., 1999; Feese *et al*., 2001). In addition to iron, IdeR binds *in vitro* to other divalent cations including Mn, Zn, Co, Ni and Mg. Crystal structures of IdeR complexed with zinc and cobalt have been obtained and, in both cases, the two metal binding sites are fully occupied. According to the crystal structure, four IdeR monomers form two functional dimers, as observed previously in DtxR (Qiu *et al*., 1995; Schiering *et al*., 1995). Metal binding activates the protein's DNA-binding ability by causing a conformational change in the DNA-binding domains. This change is believed to be mediated by amino acids at the

amino-terminal that also participate in metal binding and therefore link the DNA- and metal-binding domains.

IdeR and regulation of iron metabolism

Based on its homology to DtxR, IdeR was initially predicted to function as an iron-dependent transcriptional repressor. This hypothesis was proved correct when IdeR was shown to bind to the DtxR-binding sequence in the *tox* promoter and to restore iron regulation of the toxin in a *C. diphtheriae dtxR* mutant (Schmitt *et al*., 1995). The role of IdeR in mycobacteria was first explored in the non-virulent mycobacterium *M. smegmatis*. Inactivation of *ideR* resulted in iron-independent production of siderophores (Dussurget *et al*., 1996) and salicylic acid (Adilakshmi *et al*., 2000). A direct role for IdeR as a repressor of siderophore production was supported by the presence of putative IdeR binding sites in the promoter regions of exochelin synthesis and transport genes (Yu *et al*., 1998; Dussurget *et al*., 1999). Binding of IdeR to one of these sequences, the one found directly upstream of the *fxbA* gene, was demonstrated, and iron-dependent repression of *fxbA* was shown to require IdeR, as this gene was no longer iron regulated in an *ideR* mutant strain (Dussurget *et al*., 1999).

IdeR is essential in *M. tuberculosis*, as *ideR* cannot be disrupted unless a second copy of the gene is present or when a secondary suppressor mutation arises (Rodriguez *et al*., 2002). Although the nature of the suppressor mutation remains unknown, complementation of the *ideR* mutation with a wild-type gene demonstrated that IdeR is required for the regulation of iron metabolism in *M. tuberculosis*. In the presence of iron, IdeR binds to a 19 bp inverted repeat consensus sequence or iron box (TTAGG TTAGGCTAACCTAA) at the promoters of genes for siderophore synthesis (*mbtA–J*) and iron storage (*bfrA*), repressing the former and positively regulating the latter (Gold *et al*., 2001). A comparison of the iron-dependent transcriptional profile of the *ideR* mutant with the parental and the complemented strains revealed that about one-third of the iron-regulated genes in *M. tuberculosis* are regulated by IdeR (Rodriguez *et al*., 2002). In addition to genes involved in siderophore production and iron storage (Fig. 1), IdeR controls genes encoding putative transporters, transcriptional regulators, proteins involved in general metabolism, members of the PE/PPE family of conserved

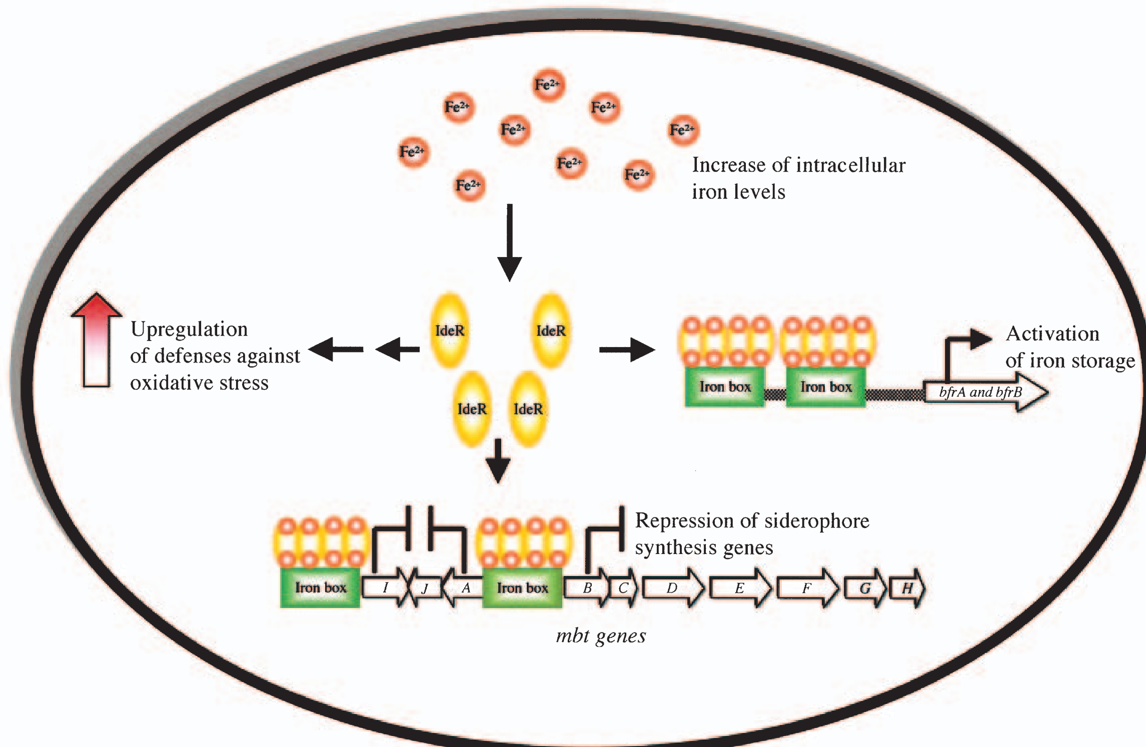

Fig. 1. Iron-dependent regulatory function of IdeR. When intracellular iron levels increase, IdeR combines with Fe^{2+} and binds to specific sequences (iron boxes) in the promoter region of iron-regulated genes modulating their transcription. In these conditions, IdeR–Fe^{2+} downregulates iron uptake by repressing siderophore production and increases iron storage by activating transcription of *bfrA* and *bfrB* encoding bacterioferritin and ferritin. In an indirect manner, IdeR also positively modulates protection against oxidative stress. In low-iron conditions, the IdeR–Fe^{2+} complex is not formed, and IdeR-repressed genes are transcribed while iron storage genes are not expressed.

mycobacterial proteins and the virulence determinant MmpL4 (Camacho *et al*., 1999). Functional characterization of those proteins is expected to identify unknown components of the iron acquisition machinery and to link iron metabolism to diverse aspects of mycobacterial physiology. Like Fur, IdeR is a pleiotropic regulator controlling iron metabolism and also other processes not obviously related to iron metabolism. IdeR can negatively and also positively regulate transcription. However, in the latter case, IdeR activates transcription, unlike Fur, the positive effect of which, as mentioned above, is indirect. Expression of the bacterioferritin-encoding gene *bfrA* requires the binding of Fe^{2+}-activated IdeR to tandem operator sites that are located 100 bp upstream of the *bfrA* transcriptional start point (TSP). Deletion of these IdeR binding sites or inactivation of the *ideR* gene prevents the expression of *bfrA* (Gold *et al*., 2001). The ferritin-encoding gene *bfrB* is also activated by binding of IdeR to a double iron box located 106 bp upstream of the TSP (M. Rodriguez *et al*., unpublished results). According to these results, IdeR activates transcription, presumably by contacting the RNA polymerase and favouring initiation, when it binds to tandem iron boxes that are located, in the examples of *bfrA* and *bfrB*, ≈100 bp upstream from the TSP. Promoters that are repressed by IdeR have a single iron box, generally overlapping the −10 region, and IdeR binding to this sequence would be expected to block access of RNA polymerase (Rodriguez *et al*., 1998; Gold *et al*., 2001).

Regulation of expression of bacterioferritin and ferritin by IdeR is likely to be critical in controlling iron availability in the cytoplasm. Interestingly, bacterioferritin is one of the most abundant proteins detected in *in vivo*-grown *M. leprae* (Pessolani *et al*., 1994) and is a major antigen of *Mycobacterium paratuberculosis* (Brooks *et al*., 1991). *bfrA* and *bfrB* inactivation will be required to assess the importance of these iron storage proteins in the physiology and virulence of *M. tuberculosis.*

IdeR and the oxidative stress response in Mycobacteria

Oxidative stress occurs when abnormally high levels of reactive oxygen species (ROS) are generated, causing DNA, protein and lipid damage. Iron levels and oxidative stress are closely linked in aerobic organisms. On one hand, iron deficiency can lead to oxidative stress by decreasing the activity of iron-containing enzymes such as superoxide dismutase and catalase involved in protection against oxygen radicals. Conversely, increased intracellular free iron available to participate in the Fenton reaction results in the enhanced generation of oxygen radicals and oxidative stress (Imlay *et al*., 1988). Therefore, it is not surprising that many organisms couple regulation of iron metabolism with regulation of protection against oxidative stress. Fur and Fur-like proteins regulate genes induced by oxidative stress including the genes encoding the Mn^{2+} and Fe^{2+} superoxide dismutases (SODs) encoded by *sodA* and *sodB* (Niederhoffer *et al*., 1990), catalase and peroxidase (Hassett *et al*., 1996; Bsat *et al*., 1998), alkyl hydroperoxidase (Bsat *et al*., 1998), 8-hydroxyguanine endonuclease (Lee *et al*., 1998) and the SoxRS and OxyR regulators (Zheng *et al*., 1999).

The control of iron homeostasis and protection against oxidative stress is also coupled in mycobacteria. *M. smegmatis* and *M. tuberculosis ideR* mutants are more sensitive to hydrogen peroxide and superoxide (Dussurget *et al*., 1996; Rodriguez *et al*., 2002). In *M. smegmatis*, this effect may result from the decreased levels of catalase and the major superoxide dismutase, SodA (Dussurget *et al*., 1996), as IdeR is required for the full expression of *katG* and *sodA*. However, this effect is not direct, as there are no IdeR binding sites in the promoters of these genes (Dussurget *et al*., 1998). In the case of *M. tuberculosis*, it is not clear why inactivation of *ideR* results in increased sensitivity to oxidative stress. No difference was found in the expression of genes involved in oxidative stress protection between the *ideR* mutant and the wild-type strains. It is possible that there is an increase in a redox-reactive iron pool in the *ideR* mutant, and this, combined with decreased expression of bacterioferritin and ferritin, may result in increased sensitivity to oxidative stress. In fact, ferritin in *Campylobacter jejuni* (Wai *et al*., 1996) and bacterioferritin A in *Pseudomonas aeruginosa* (Ma *et al*., 1999) are necessary for protection against iron-mediated oxidative stress. The role of IdeR as a positive modulator of the oxidative stress response (Fig. 1) agrees with the upregulation of *ideR* detected in *M. tuberculosis*-infected macrophages (Hobson *et al*., 2002). Although the specific cause of this induction is not known, it might be part of the bacterial response to the macrophages' oxidative defence mechanisms.

Role of iron and iron regulation during infection

Significant efforts have been made in recent years to understand the adaptive response of mycobacteria to the conditions found during infection, and a variety of methods have been used to identify genes that are selectively expressed within macrophages (Barker *et al*., 1998; Triccas *et al*., 1999; Dubnau *et al*., 2002; Hou *et al*., 2002). Current evidence indicates that *M. tuberculosis* is exposed to iron-limiting conditions during infection. For example, the *M. tuberculosis mbtB* gene required for siderophore biosynthesis is induced both *in vitro* under iron deficiency and also in infected macrophages (Gold *et al*., 2001) and in mice (J. Timm *et al*., unpublished observations). An *mbtB* mutant is defective in siderophore production and replicates poorly in infected macrophages (De

Voss *et al.*, 2000). This indicates that *M. tuberculosis* is exposed to a low-iron environment during macrophage infection and requires siderophore biosynthesis for *in vivo* growth. In addition, *M. tuberculosis* expressing a mutant *C. diphtheriae* DtxR protein (E175K), which behaves as a constitutive, iron-independent repressor under some experimental conditions, is attenuated for growth in mice. This effect is thought to result from constitutive repression of iron acquisition systems in this strain, although this was not demonstrated directly (Manabe *et al.*, 1999). Regulation of iron metabolism is also likely to be essential during infection in order to maintain iron homeostasis. Transferrin traffics to the mycobacterial phagosome (Clemens and Horwitz, 1996) in *M. tuberculosis*-infected human macrophages and can serve as iron source for intracellular bacteria (Olakanmi *et al.*, 2000). Intraphagosomal mycobacteria must induce the synthesis of siderophores in order to acquire iron from transferrin but, when sufficient iron has been acquired, regulatory pathways will down-regulate iron uptake in response to the iron-sufficient conditions that they may now face. Consistent with this hypothesis, mycobacteria isolated from infected tissues do not accumulate high levels of mycobactin, implying that the bacilli are not permanently deprived of iron (Kato, 1985; Lambrecht and Collins, 1993) and, as discussed above, certain mycobacterial pathogens grown in animal models have large amounts of bacterioferritin. As IdeR is necessary for an efficient oxidative stress response, it is very likely that this regulator will also be important for resistance of *M. tuberculosis* to the oxidative killing mechanisms encountered during infection of macrophages and later stages in the lung.

Concluding remarks

The ability to respond and adapt to changes in the environment is an essential quality that allows all living organisms to survive and propagate. Mycobacterial pathogens, with their capacity to cause disease and to exist for long periods of time in humans, are remarkable examples of this ability to adapt to changing environments. There is now compelling evidence that iron acquisition is critical for *M. tuberculosis* survival and replication in the infected host. However, given the potential danger involved in having excess iron, each aspect of the metabolism of this metal is tightly regulated. Significant progress has been made in understanding these mechanisms. The major iron regulator IdeR controls iron uptake and storage in *M. tuberculosis* by modulating the expression of genes involved in these processes. It is predicted that characterization of proteins encoded by IdeR-regulated genes will provide a complete understanding of the *M. tuberculosis* iron acquisition machinery. This knowledge should provide new targets for therapeutic intervention against tuberculosis.

Acknowledgements

We thank the members of the Smith laboratory for helpful discussions and critical reading of this review. We also are grateful to Dr Colin Ratledge for sharing unpublished information and ideas. Work from the authors' laboratory discussed in this article was supported by a Parker B. Francis postdoctoral fellowship (awarded to G.M.R.) and NIH research grants AI-44856 and HL-64544 (awarded to I.S.).

References

Adilakshmi, T., Ayling, P.D., and Ratledge, C. (2000) Mutational analysis of a role for salicylic acid in iron metabolism of *Mycobacterium smegmatis*. *J Bacteriol* **182:** 264–271.

Bagg, A., and Neilands, J.B. (1987) Molecular mechanism of regulation of siderophore-mediated iron assimilation. *Microbiol Rev* **51:** 509–518.

Barker, L.P., Brooks, D.M., and Small, P.L. (1998) The identification of *Mycobacterium marinum* genes differentially expressed in macrophage phagosomes using promoter fusions to green fluorescent protein. *Mol Microbiol* **29:** 1167–1177.

Boyd, J., Oza, M.N., and Murphy, J.R. (1990) Molecular cloning and DNA sequence analysis of a diphtheria *tox* iron-dependent regulatory element (*dtxR*) from *Corynebacterium diphtheriae*. *Proc Natl Acad Sci USA* **87:** 5968–5972.

Brooks, B.W., Young, N.M., Watson, D.C., Robertson, R.B., Sugden, E.A., Nielson, K.H., and Becker, S.A.W.E. (1991) *Mycobacterium paratuberculosis* antigen D: characterization and evidence that it is a bacterioferritin. *J Clin Invest* **29:** 1652–1658.

Bsat, N., Herbig, A., Casillas-Martinez, L., Setlow, P., and Helmann, J.D. (1998) *Bacillus subtilis* contains multiple Fur homologues: identification of the iron uptake (Fur) and peroxide regulon (PerR) repressors. *Mol Microbiol* **29:** 189–198.

Calder, K.M., and Horwitz, M.A. (1998) Identification of iron-regulated proteins of *Mycobacterium tuberculosis and* cloning of tandem genes encoding a low iron-induced protein and a metal transporting ATPase with similarities to two-component metal transport systems. *Microb Pathog* **24:** 133–143.

Camacho, L.R., Ensergueix, D., Perez, E., Gicquel, B., and Guilhot, C. (1999) Identification of a virulence gene cluster of *Mycobacterium tuberculosis* by signature-tagged transposon mutagenesis. *Mol Microbiol* **34:** 257–267.

Chipperfield, J.R., and Ratledge, C. (2000) Salicylic acid is not a bacterial siderophore: a theoretical study. *Biometals* **13:** 165–168.

Clemens, D.L., and Horwitz, M.A. (1996) The *Mycobacterium tuberculosis* phagosome interacts with early endosomes and is accessible to exogenously administered transferrin. *J Exp Med* **184:** 1349–1355.

De Voss, J.J., Rutter, K., Schroeder, B.G., and Barry, C.E., III (1999) Iron acquisition and metabolism by mycobacteria. *J Bacteriol* **181:** 4443–4451.

De Voss, J.J., Rutter, K., Schroeder, B.G., Su, H., Zhu, Y., and Barry, C.E., III (2000) The salicylate-derived mycobactin siderophores of *Mycobacterium tuberculosis* are essen-

tial for growth in macrophages. *Proc Natl Acad Sci USA* **97:** 1252–1257.

Delany, I., Spohn, G., Rappuoli, R., and Scarlato, V. (2001) The Fur repressor controls transcription of iron-activated and -repressed genes in *Helicobacter pylori*. *Mol Microbiol* **42:** 1297–1309.

Doukhan, L., Predich, M., Nair, G., Dussurget, O., Mandic-Mulec, I., Cole, S.T., *et al.* (1995) Genomic organization of the mycobacterial sigma gene cluster. *Gene* **165:** 67–70.

Dover, L.G., and Ratledge, C. (1996) Identification of a 29 kDa protein in the envelope of *Mycobacterium smegmatis* as a putative ferri-exochelin receptor. *Microbiology* **142:** 1521–1530.

Dubnau, E., Fontan, P., Manganelli, R., Soares-Appel, S., and Smith, I. (2002) *Mycobacterium tuberculosis* genes induced during infection of human macrophages. *Infect Immun* **70:** 2787–2795.

Dussurget, O., Rodriguez, G.M., and Smith, I. (1996) An *ideR* mutant of *Mycobacterium smegmatis* has a derepressed siderophore production and an altered oxidative-stress response. *Mol Microbiol* **22:** 535–544.

Dussurget, O., Rodriguez, G.M., and Smith, I. (1998) Protective role of the mycobacterial IdeR against reactive oxygen species and isoniazid toxicity. *Tuber Lung Dis* **79:** 99–106.

Dussurget, O., Gomez, M., Timm, J., Yu, S., Jacobs, W., Sabol, S.Z., *et al.* (1999) Transcriptional control of the iron responsive *fxbA* gene by the mycobacterial regulator IdeR. *J Bacteriol* **181:** 3402–3408.

Feese, M.D., Ingason, B.P., Goranson-Siekierke, J., Holmes, R.K., and Hol, W.G. (2001) Crystal structure of the iron-dependent regulator from *Mycobacterium tuberculosis* at 2.0-A resolution reveals the Src homology domain 3-like fold and metal binding function of the third domain. *J Biol Chem* **276:** 5959–5966.

Fiss, E.H., Yu, S., and Jacobs, W.R., Jr (1994) Identification of genes involved in the sequestration of iron in mycobacteria: the ferric exochelin biosynthetic and uptake pathways. *Mol Microbiol* **14:** 557–569.

Gobin, J., Moore, C.H., Jr, Reeve, J., Wong, D.K., Gibson, B.W., and Horwitz, M.A. (1995) Iron acquisition by *Mycobacterium tuberculosis*: isolation and characterization of a family of iron-binding exochelins. *Proc Natl Acad Sci USA* **92:** 5189–5193.

Gold, B., Rodriguez, G.M., Marras, M.P., Pentecost, M., and Smith, I. (2001) The *Mycobacterium tuberculosis* IdeR is a dual functional regulator that controls transcription of genes involved in iron acquisition, iron storage and survival in macrophages. *Mol Microbiol* **42:** 851–865.

Gruer, M.J., and Guest, J.R. (1994) Two genetically-distinct and differentially-regulated aconitases (AcnA and AcnB) in *Escherichia coli*. *Microbiology* **140:** 2531–2541.

Gunter-Seeboth, K., and Schupp, T. (1995) Cloning and sequence analysis of the *Corynebacterium diphtheriae dtxR* homologue from *Streptomyces lividans* and *Streptomyces pilosus* encoding a putative iron repressor protein. *Gene* **166:** 117–119.

Hall, R.M., Sritharan, M., Messenger, A.J.M., and Ratledge, C. (1987) Iron transport in *Mycobacterium smegmatis*: occurrence of iron-regulated envelope proteins as potential receptors for iron uptake. *J Gen Microbiol* **133:** 2107–2114.

Hantke, K. (2001) Iron and metal regulation in bacteria. *Curr Opin Microbiol* **4:** 172–177.

Hassett, D.J., Sokol, P.A., Howell, M.L., Ma, J.-F., Schweizer, H.T., Ochsner, U., and Vasil, M.L. (1996) Ferric uptake regulator (Fur) mutants of *Pseudomonas aeruginosa* demonstrate defective siderophore-mediated iron uptake, altered aerobic growth, and decreased superoxide dismutase and catalase activities. *J Bacteriol* **178:** 3996–4003.

Hobson, R.J., McBride, A.J.A., Kempsell, K.E., and Dale, J.W. (2002) Use of an arrayed promoter-probe library for the identification of macrophage-regulated genes in *Mycobacterium tuberculosis*. *Microbiology* **148:** 1571–1579.

Hou, J.Y., Graham, J.E., and Clark-Curtiss, J.E. (2002) *Mycobacterium avium* genes expressed during growth in human macrophages detected by selective capture of transcribed sequences (SCOTS). *Infect Immun* **70:** 3714–3726.

Imlay, J.A., Chin, S.M., and Linn, S. (1988) Toxic DNA damage by hydrogen peroxide through the Fenton reaction *in vivo and in vitro*. *Science* **240:** 640–642.

Jakubovics, N.S., Smith, A.W., and Jenkinson, H.F. (2000) Expression of the virulence-related Sca (Mn2+) permease in *Streptococcus gordonii* is regulated by a diphtheria toxin metallorepressor-like protein ScaR. *Mol Microbiol* **38:** 140–153.

Kato, L. (1985) Absence of mycobactin in *Mycobacterium leprae*; probably a microbe dependent microorganism implications. *Int J Lepr* **57:** 58–70.

Lambrecht, R.S., and Collins, M.T. (1993) Inability to detect mycobactin in mycobacteria-infected tissues suggests an alternative iron acquisition mechanism by mycobacteria *in vivo*. *Microb Pathog* **14:** 229–238.

Lee, H.S., Lee, Y.S., Kim, H.S., Choi, J.Y., Hassan, H.M., and Chung, M.H. (1998) Mechanism of regulation of 8-hydroxyguanine endonuclease by oxidative stress: roles of FNR, ArcA, and Fur. *Free Rad Biol Med* **24:** 1193–1201.

Litwin, C.M., and Calderwood, S.B. (1993) Role of iron in regulation of virulence genes. *Clin Microbiol Rev* **6:** 137–149.

de Lorenzo, V., Wee, S., Herrero, M., and Neilands, J.B. (1987) Operator sequences of the aerobactin operon of plasmid ColV-K30 binding the ferric uptake regulation (Fur) repressor. *J Bacteriol* **169:** 2624–2630.

Lundrigan, M.D., Arceneaux, J.E., Zhu, W., and Byers, B.R. (1997) Enhanced hydrogen peroxide sensitivity and altered stress response in iron-starved *Mycobacterium smegmatis*. *Biometals* **10:** 215–225.

Ma, J.F., Ochsner, U.A., Klotz, M.G., Nanayakkara, V.K., Howell, M.L., Johnson, Z., *et al.* (1999) Bacterioferritin A modulates catalase A (KatA) activity and resistance to hydrogen peroxide in *Pseudomonas aeruginosa*. *J Bacteriol* **181:** 3730–3742.

Manabe, Y., Saviola, B.J., Sun, L., Murphy, J.R., and Bishai, W.R. (1999) Attenuation of virulence in *Mycobacterium tuberculosis* expressing a constitutively active iron repressor. *Proc Natl Acad Sci USA* **96:** 12844–12848.

Masse, E., and Gottesman, S. (2002) A small RNA regulates the expression of genes involved in iron metabolism in *E. coli*. *Proc Natl Acad Sci USA* **99:** 4620–4625.

Milano, A., Forti, F., Sala, C., Riccardi, G., and Ghisotti, D. (2001) Transcriptional regulation of *furA* and *katG* upon

oxidative stress in *Mycobacterium smegmatis*. *J Bacteriol* **183:** 6801–6806.

Niederhoffer, E.C., Naranjo, C.M., Bradley, K.L., and Fee, J.A. (1990) Control of *Escherichia coli* superoxide dismutase (*sodA* and *sodB*) genes by the ferric uptake regulator (*fur*) locus. *J Bacteriol* **172:** 1930–1938.

Oguiza, J.A., Tao, X., Marcos, A.T., Martin, J.F., and Murphy, J.R. (1995) Molecular cloning, DNA sequence analysis, and characterization of the *Corynebacterium diphtheriae dtxR* homolog from *Brevibacterium lactofermentum*. *J Bacteriol* **177:** 465–467.

Olakanmi, O., Britigan, B.E., and Schlesinger, L.S. (2000) Gallium disrupts iron metabolism of mycobacteria residing within human macrophages. *Infect Immun* **68:** 5619–5627.

Oram, D.M., Avdalovic, A., and Holmes, R.K. (2002) Construction and characterization of transposon insertion mutations in *Corynebacterium diphtheriae* that affect expression of the diphtheria toxin repressor (DtxR). *J Bacteriol* **184:** 5723–5732.

Patzer, S.I., and Hantke, K. (2001) Dual repression by Fe(2+)-Fur and Mn(2+)-MntR of the mntH gene, encoding an NRAMP-like Mn(2+) transporter in *Escherichia coli*. *J Bacteriol* **183:** 4806–4813.

Pessolani, M.V., Smith, D.R., Rivoire, B., McCormick, J., Hefta, S.A., Cole, S.T., and Brennan, P.J. (1994) Purification, characterization, gene sequence, and significance of a bacterioferritin from *Mycobacterium leprae*. *J Exp Med* **180:** 319–327.

Pohl, E., Holmes, R.K., and Hol, W.G.J. (1999) Crystal structure of the iron-dependent regulator (IdeR) from *Mycobacterium tuberculosis* shows both metal binding sites fully occupied. *J Mol Biol* **285:** 1145–1156.

Posey, J.E., Hardham, J.M., Norris, S.J., and Gherardini, F.C. (1999) Characterization of a manganese-dependent regulatory protein, TroR, from *Treponema pallidum*. *Proc Natl Acad Sci USA* **96:** 10887–10892.

Pym, A.S., Domenech, P., Honore, N., Song, J., Deretic, V., and Cole, S.T. (2001) Regulation of catalase-peroxidase (KatG) expression, isoniazid sensitivity and virulence by furA of *Mycobacterium tuberculosi*s. *Mol Microbiol* **40:** 879–889.

Qian, Y., Lee, J.H., and Holmes, R.K. (2002) Identification of a DtxR-regulated operon that is essential for siderophore-dependent iron uptake in *Corynebacterium diphtheriae*. *J Bacteriol* **184:** 4846–4856.

Qiu, X., Verlinde, C.L.M.J., Zhang, L., Schmitt, M.P., Holmes, R.K., and Hol, W.G.J. (1995) Three-dimensional structure of the diphtheria toxin repressor in complex with divalent cation co-repressors. *Structure* **3:** 87–100.

Quadri, L.E.N., Sello, J., Keating, T.A., Weinreb, P.H., and Walsh, C.T. (1998) Identification of a *Mycobacterium tuberculosis* gene cluster encoding the biosynthetic enzymes for assembly of the virulence-conferring siderophore mycobactin. *Chem Biol* **5:** 631–645.

Que, Q., and Helmann, J.D. (2000) Manganese homeostasis in *Bacillus subtilis* is regulated by MntR, a bifunctional regulator related to the diphtheria toxin repressor family of proteins. *Mol Microbiol* **35:** 1454–1468.

Raghu, B., Sarma, G.R., and Venkatesan, P. (1993) Effect of iron on the growth and siderophore production of mycobacteria. *Biochem Mol Biol Int* **31:** 341–348.

Ratledge, C. (1999) Iron metabolism. In *Mycobacteria: Molecular Biology and Virulence*. Ratledge, C., and Dale, J. (eds). London: Blackwell Science, pp. 260–286.

Ratledge, C., and Ewing, M. (1996) The occurrence of carboxymycobactin, the siderophore of pathogenic mycobacteria, as a second extracellular siderophore in *Mycobacterium smegmatis*. *Microbiology* **142:** 2207–2212.

Ratledge, C., and Hall, M.J. (1970) Uptake of salicylic acid into mycobactin S by growing cells of *Mycobacterium smegmatis*. *FEBS Lett* **10:** 309–312.

Ratledge, C., and Winder, F.G. (1962) The accumulation of salicylic acid by mycobacteria during growth on iron-deficient medium. *Biochem J* **84:** 501–506.

Ratledge, C., Macham, L.P., Brown, K.A., and Marshall, B.J. (1974) Iron transport in *Mycobacterium smegmatis*: a restricted role for salicylic acid in the extracellular environment. *Biochim Biophys Acta* **372:** 39–51.

Ratledge, C., Patel, P.V., and Mundy, J. (1982) Iron transport in *Mycobacterium smegmatis*: the location of mycobactin by electron microscopy. *J Gen Microbiol* **128:** 1559–1565.

Rodriguez, G.M., Gold, B., Gomez, M., Dussurget, O., and Smith, I. (1998) Identification and characterization of two divergently transcribed iron regulated genes in *Mycobacterium tuberculosis*. *Tuber Lung Dis* **79:** 287–298.

Rodriguez, G.M., Voskuil, M.I., Gold, B., Schoolnik, G.K., and Smith, I. (2002) *ideR*, an essential gene in *Mycobacterium tuberculosis*: role of IdeR in iron-dependent gene expression, iron metabolism, and oxidative stress response. *Infect Immun* **70:** 3371–3381.

Schiering, N., Tao, X., Zeng, H., Murphy, J.R., Petsko, G.A., and Ringe, D. (1995) Structures of the apo- and the metal ion-activated forms of the diphtheria *tox* repressor from *Corynebacterium diphtheriae*. *Proc Natl Acad Sci USA* **92:** 9843–9850.

Schmitt, M.P., and Holmes, R.K. (1991) Iron-dependent regulation of diphtheria toxin and siderophore expression by the cloned *Corynebacterium diphtheriae* repressor gene *dtxR* in *C. diphtheriae* C7 strains. *Infect Immun* **59:** 1899–1904.

Schmitt, M.P., Predich, M., Doukhan, L., Smith, I., and Holmes, R.K. (1995) Characterization of an iron-dependent regulatory protein (IdeR) of *Mycobacterium tuberculosis* as a functional homolog of the diphtheria toxin repressor (DtxR) from *Corynebacterium diphtheriae*. *Infect Immun* **63:** 4284–4289.

Schmitt, M.P., Talley, B.G., and Holmes, R.K. (1997) Characterization of lipoprotein IRP1 from *Corynebacterium diphtheriae*, which is regulated by the diphtheria toxin repressor (DtxR) and iron. *Infect Immun* **65:** 5364–5367.

Snow, G.A. (1965) The structure of mycobactin P, a growth factor for *Mycobacterium johnei*, *and* the significance of its iron complex. *Biochem J* **94:** 160–165.

Touati, D. (2000) Iron and oxidative stress in bacteria. *Arch Biochem Biophys* **373:** 1–6.

Triccas, J.A., Berthet, F.X., Pelicic, V., and Gicquel, B. (1999) Use of fluorescence induction and sucrose counterselection to identify *Mycobacterium tuberculosis* genes expressed within host cells. *Microbiology* **145:** 2923–2930.

Tsolis, R.M., Baumler, A.J., Stojiljkovic, I., and Heffron, F. (1995) Fur regulon of *Salmonella typhimurium*: identifica-

tion of new iron-regulated genes. *J Bacteriol* **177:** 4628–4637.

Wai, S.N., Nakayama, K., Umene, K., Moriya, T., and Amako, K. (1996) Construction of a ferritin-deficient mutant of *Campylobacter jejuni*: contribution of ferritin to iron storage and protection against oxidative stress. *Mol Microbiol* **20:** 1127–1134.

Weinberg, E.D. (1999) Iron loading and disease surveillance. *Emerg Infect Dis* **5:** 346–352.

Winder, F.G., and O'Hara, C. (1964) Effects of iron deficiency and of zinc deficiency on the activities of some enzymes in *Mycobacterium smegmatis*. *Biochem J* **90:** 122–126.

Wong, D., Lee, B.Y., Horwitz, M.A., and Gibson, B. (1999) Identification of Fur, aconitase and other proteins expressed by *Mycobacterium tuberculosis* under conditions of low and high concentrations of iron by combined two-dimensional gel electrophoresis and mass spectrometry. *Infect Immun* **67:** 327–336.

Yu, S., Fiss, E., and Jacobs, W.R., Jr (1998) Analysis of the exochelin locus in *Mycobacterium smegmatis*: biosynthesis genes have homology with genes of the peptide synthetase family. *J Bacteriol* **180:** 4676–4685.

Zahrt, T.C., Song, J., Siple, J., and Deretic, V. (2001) Mycobacterial FurA is a negative regulator of catalase-peroxidase gene *katG*. *Mol Microbiol* **39:** 1174–1185.

Zheng, M., Doan, B., Schneider, T.D., and Storz, G. (1999) OxyR and SoxRS regulation of *fur*. *J Bacteriol* **181:** 4639–4643.

Zhu, W., Arceneaux, J.E., Beggs, M.L., Byers, B.R., Eisenach, K.D., and Lundrigan, M.D. (1998) Exochelin genes in *Mycobacterium smegmatis*: identification of an ABC transporter and two non-ribosomal peptide synthetase genes. *Mol Microbiol* **29:** 629–639.