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METAL ION TRANSPORT AND REGULATION IN *MYCOBACTERIUM TUBERCULOSIS*

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TABLE OF CONTENTS

1. ABSTRACT

The regulation of metal ion concentrations is central to the physiology of the interaction between pathogenic bacteria and their hosts. Apart from the NRAMP orthologue, MntH, metal ion transporters in *Mycobacterium tuberculosis* have not been studied. Mn, the physiological substrate of MntH in other bacteria, may play an important role as a structural and redox-active cofactor in a wide range of metabolic processes. Fe, Cu and Zn play structural and catalytic roles in metalloenzymes involved in oxidative stress responses. Fe and Mg are required for growth in macrophages. Genomic analyses reveal 28 sequences encoding a broad repertoire of putative metal ion transporters (or transporter subunits), representing 24% of all transporters in this organism. These comprise 8 families of secondary active transporters and 3 families of primary active transporters, including 12 'P' type ATPases. Potential metal ion specificities include K^+, Na^+, Cu^{2+}, Cd^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, Co^{2+}, Ni^{2+}, $Fe^{2+/3+}$, Hg^{2+}, AsO_2^- and AsO_4^{2-}. 17 of these transporters are also encoded as complete open reading frames in *Mycobacterium leprae*, suggesting a role in intracellular survival. Iron transcriptionally regulates a diverse set of genes via the iron-dependent DNA-binding proteins, Fur and IdeR. Changes in Fe and Mg concentrations signal entry into the intracellular compartment and potentially trigger up-regulation of virulence determinants. The plethora of putative transport systems encoded by the *M. tuberculosis* genome contrasts strikingly with the paucity of experimental data on these systems. The detailed analysis of the temporal pattern of *M. tuberculosis* transporter gene expression during infection will provide important insights into the basic biology of intracellular parasitism and may help to shape novel therapeutic strategies.

2. INTRODUCTION

Trans-membrane ion transport is a neglected subject in studies on mycobacterium species in general and *M. tuberculosis* in particular. To date, the only transmembrane metal ion transporter for which functional data exist, is the Natural Resistance Associated Macrophage Protein (NRAMP) family orthologue, MntH, previously called Mramp (1). Most studies on metal ion uptake in *M. tuberculosis* have focussed on siderophore-mediated iron acquisition (recently reviewed (2)), but the mechanisms by which iron is translocated across the cytoplasmic membrane are unknown. Much less is understood about any aspect of the acquisition of other metal ions.

The capacity to survive within a specialised phagosomal compartment is central to the pathogenicity of *M. tuberculosis*. Evidence for the significance of metal ion transport in this micro-environment (and possibly for that of Manganese (Mn) in particular) comes from studies on the NRAMP family. Mammalian NRAMP1 and NRAMP2, which utilise proton gradients to energise divalent cation transport (3-5), are important determinants of intra-phagosomal divalent cation composition. They are recruited to the phagosomal membrane following the phagocytosis of diverse intracellular pathogens including Mycobacteria, Salmonella and Leishmania (6-8). NRAMP1 mutations in mice are robustly associated with differential susceptibility to these pathogens during the early phase of infection (9, 10), while NRAMP1 polymorphisms have also been implicated in susceptibility to tuberculosis and leprosy (or leprosy type) in some human populations (11-16). While NRAMP2 is a major $iron_{II}$ (Fe^{2+}) transporter, the physiologically important divalent cation(s) transported by NRAMP1 and the direction of transport remain controversial. Recent studies have demonstrated that NRAMP1 can transport Mn, Fe, Cobalt (Co) and Zinc (Zn) (4, 5, 17, 18), but the absence of any direct measurements of the relative affinities of this transporter for these cations has so far precluded definitive conclusions as to which might be the most physiologically relevant in this context. In one study (18), NRAMP1 exhibited an apparently greater selectivity for Mn compared to NRAMP2, but was indistinguishable from NRAMP2 with respect to Fe transport, suggesting that NRAMP1 might have a predilection for Mn.

Bacterial NRAMP orthologues are represented in all major bacterial groups apart from the Archaea (19). It has been suggested that competition with the host for limiting concentrations of essential trace metals within the phagosomal compartment may be a common physiological imperative amongst diverse intraphagosomal pathogens (20). The possibility that Mn *per se* might play an important role in the interaction between intraphagosomal pathogens and host cells is reinforced by the discovery that the two bacterial NRAMP orthologues for which detailed kinetic characterisations are available (*E. coli* and *S. enterica* serovar Typhimurium), are highly selective Mn transporters. This conclusion is based on the observation that both exhibit K_Ts for Mn in the sub micromolar range (21, 22), commensurate with physiologically plausible ambient Mn concentrations. Their affinities for Fe^{2+}, on the other hand, are greater than 30 microM, higher than any free Fe^{2+} concentrations likely to be encountered *in vivo*. This may not, however, be the case for all prokaryotic Nramp orthologues under all conditions. The *M. tuberculosis* NRAMP orthologue (Mramp) transports Fe, Zn and Mn (1, and Agranoff, Kehres *et al*, unpublished observations) but reliable predictions regarding its preferred substrate await detailed kinetic studies.

The evolution of mechanisms for Fe scavenging may be a key characteristic of successful bacterial pathogens. Sequestration of Fe and Zn by mammals is a common, non-specific response to bacterial infection (23). Conversely, availability of Fe in an accessible form has been associated in humans with increased susceptibility to a wide variety of infectious agents. Many important human pathogens possess high affinity siderophores. In at least some cases, inactivation of these mechanisms by genetic manipulation leads to attenuation of pathogenicity. Infection and inflammation-induced alterations in host-availability of other trace metals such as Zn, Copper (Cu), Selenium (Se), Magnesium (Mg) are also well described (23-25) and bacteria have evolved sensing mechanisms to register these alterations.

In this article, we review our current understanding of the physiological importance of divalent metal cations for *M. tuberculosis* and the significance of these processes in its interaction with the host. We draw attention, in particular, to the striking knowledge gap reflected by the contrast between the plethora of putative metal ion transport systems encoded in the genome and the dearth of experimental data on all but a couple of these.

3. THE PHYSIOLOGICAL ROLES OF METAL IONS IN *M. TUBERCULOSIS*

Divalent cations of the transition metal series participate in complex redox reactions by virtue of their variable oxidation states - a property exploited by a variety of enzyme systems with key physiological functions. These include the respiratory chain cytochromes which incorporate Fe and Cu, and enzymes involved in responses to oxidative stress. In *M. tuberculosis*, the capacity to withstand the defensive oxidative burst generated by host mononuclear cells may be an essential adaptation to intracellular survival.

M. tuberculosis possesses two superoxide dismutases, SodA and SodC containing respectively, Fe and a Cu/Zn pair (26). The former is one of the most abundant proteins found in virulent *M. tuberculosis* culture supernatants (27). By analogy with functionally characterised orthologues, SodA is probably involved in combating intra-cytoplasmic oxidative stresses arising from endogenous oxidative phosphorylation. SodC has been localised to a periplasmic compartment consistent with its proposed role in the detoxification of host-generated toxic free radicals (28), and contributes to resistance to oxidative stress in liquid culture and in activated macrophages (29). Other Fe-containing *M. tuberculosis* metalloenzymes involved in the oxidative stress response include the catalase-peroxidase, KatG, AhpC, an alkyl hydroperoxide reductase and the thioredoxin proteins TrxA and TrxB (30).

The physiological functions of Mn^{2+} in *M. tuberculosis* (and in bacteria in general) are still poorly understood, although a role for bacterial Mn-cofactored enzymes in a diverse range of metabolic functions including free radical detoxification, central carbon metabolism, signal transduction and growth regulation, is becoming apparent (31). These include Mn possesses a unique redox chemistry in biological systems, compared to other transition metals. In contrast to Fe^{2+}, the relative stability conferred by the symmetry of its half-filled d-shell ($3d^5$) makes it much less prone, in aqueous solution, to oxidation from the Mn^{2+} to the Mn^{3+} state. Consequently, its ability to generate toxic free radicals - for example, by the reduction of H_2O_2 in the Fenton reaction - is much lower than that of Fe^{2+}, and cells can therefore tolerate far higher concentrations of free Mn^{2+}. However, its redox potential is profoundly influenced by its ligand environment. This phenomenon is exploited in radical detoxifying enzymes such as Mn cofactored SodA, where conformational alterations modify the effective redox potential allowing dismutation of superoxide radicals under appropriate circumstances. SodA orthologues in many bacteria utilise Mn, but SodA in *M. tuberculosis* appears to be Fe-cofactored (26). In some cases, free Mn^{2+} present at very high concentrations (milimolar range) may exert a direct protective effect via non-enzymatic superoxide scavenging and peroxide dismutating activity. Lactobacilli, for example, utilise high cytoplasmic Mn^{2+} concentrations to withstand oxidative stresses without the requirement for SODs or Fe co-factored catalase/peroxidases (32), while *Borrelia burgdorferi* (33) and *Streptococcus suis* (34) also maintain very high levels of cytoplasmic Mn^{2+}, possibly serving the same physiological functions. The very few known bacterial metalloproteins containing Mn (apart from some SodA proteins) are the serine/threonine phosphatases, PrpA, PrpB present in some enterobacteria but without obvious orthologues in *M. tuberculosis*, enolase, a key glycolytic enzyme which is represented in *M. tuberculosis* (Rv1023) and GpmM, a Mn-dependent phosphoglyceromutase not present in *M. tuberculosis*. On the other hand, because of its liganding geometry, Mn^{2+} may substitute for Mg^{2+} as a non-structural cofactor for several enzymes including the *M. tuberculosis* adenylyl cyclase, Cya, (35) and serine/threonine kinase PknA (involved in morphological regulation during cell division) (36). It can also substitute

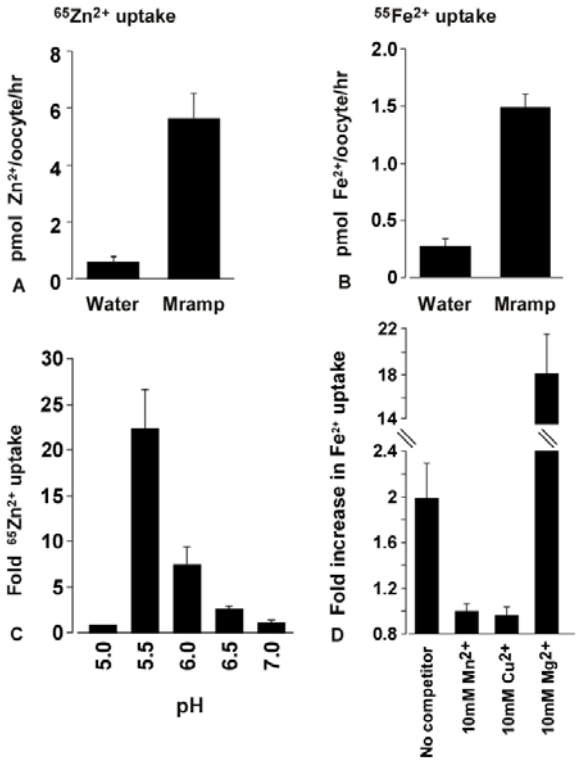

Figure 1. Metal ion transport in *Xenopus laevis* oocytes expressing *M. tuberculosis* MntH (Mramp). Oocytes were injected with 5ng capped cRNA encoding MntH, and $^{65}Zn^{2+}$ and $^{55}Fe^{2+}$ uptake assays were performed after 48-96hrs incubation. **A.** $^{65}Zn^{2+}$ uptake in water and MntH cRNA-injected oocytes. **B.** $^{55}Fe^{2+}$ uptake in water and MntH cRNA-injected oocytes. **C.** Effect of pH on $^{65}Zn^{2+}$ uptake. **D**. Effects of other divalent cations on $^{55}Fe^{2+}$ uptake (modified from (1)).

for Ca^{2+} in stimulating autophosphorylation of TrcS, the histidine kinase component of the TrcS/TrcR two-component regulatory system (37), a potentially important signa1 transduction mechanism. It is likely that several other enzyme systems in *M. tuberculosis* will prove to be Mn^{2+} dependent (31).

The siderophores of *M. tuberculosis* constitute a family of soluble and cell envelope associated, low molecular weight (744-800 Da) molecules known as mycobactins (38). Their water-solubilities vary by virtue of the length and polarisation of their acyl side chains (2). Mycobactins are synthesised by non-ribosomal peptide synthases encoded by the *mbt* gene cluster (39). They exhibit very high affinities for Fe^{3+} and soluble mycobactins (or 'exomycobactins') can acquire Fe^{3+} from 40% saturated transferrin, lactoferrin and ferritin (40). They appear to be important for survival within macrophages as inactivation of *mbtB*, encoding one of the critical enzymes in the biosynthetic pathway, significantly impairs growth in THP1 cells and in iron-limited liquid media (41) (although how far an immortalised murine cell line reflects the situation in human macrophages/monocytes, is debatable).

It is assumed that iron is transferred from soluble, to cell wall-bound, mycobactins but a major gap in our understanding of Fe acquisition in *M. tuberculosis* is the mechanism by which it is subsequently translocated across the cytoplasmic membrane. In some bacteria, Fe is passed on to a periplasmic iron-binding protein e.g. FhuD in *E. coli*, and thence to a multi-subunit ATP-binding cassette (ABC)-type integral membrane transport ATPase. In others, the entire siderophore-iron complex is translocated, also via an ABC traffic ATPase (42). In *M. tuberculosis*, while putative ABC-type ATPases are present in abundance (at least 25), possible Fe-transporting ABC complexes are represented only by the binding protein subunits FecB and FecB2 in the apparent absence of corresponding ATP-binding domain and transmembrane subunits (43).

Mg^{2+} has many important physiological functions in bacteria. Growth of *M. tuberculosis* is greatly restricted by low Mg^{2+} concentrations (<50 microM) under conditions of acidic pH (< 6.5) (29) but Mg^{2+} transport has not yet been formally studied. Major candidates for important *M. tuberculosis* Mg^{2+} transporters are CorA and MgtE (and possibly one or more of the 'P' type ATPases), discussed further below.

4. MNTH – A DIVALENT TRANSITION METAL TRANSPORTER IN THE NRAMP FAMILY

The *mntH* ORF in *M. tuberculosis* (Rv0924c) encodes a predicted 428 amino acid protein, exhibiting sequence identities of between 31% and 74% with orthologues in other bacteria. We have detected *mntH* mRNA both in *M. tuberculosis* grown in liquid media as well as from intracellular *M. tuberculosis* isolated from THP1 cells, a macrophage-like cell line, and have obtained preliminary functional data on this protein by expressing it in *Xenopus* oocytes (1) and sf21 insect cells (Agranoff, Kehres *et al*, unpublished observations). Like its host-encoded orthologues, MntH is a proton dependent divalent cation transporter which stimulates the specific uptake of $^{65}Zn^{2+}$ and $^{55}Fe^{2+}$ in oocytes, maximally between pH values of 5.5-6.5 (Figure 1A, B and C). This range accords well with estimates of the pH within the mycobacterial phagosome (44), suggesting that MntH might be important in the intracellular environment. Mn^{2+} and Cu^{2+} in excess (10mM) inhibit this specific uptake suggesting that they compete for transport or binding by MntH (Figure 1D). Mg^{2+} appears to stimulate Fe^{2+} uptake, but this may be a result of non-specific effects on oocytes.We have also demonstrated direct transport of Mn^{2+} by insect cells expressing MntH (not shown).

As discussed earlier, there is convincing evidence that Mn^{2+} is the physiological substrate of MntH in *E. coli*, and *S. typhimurium*, and, by extension, this may well be so, too, for *M. tuberulosis*. In the former organisms, as well as *B. subtilis*, MntH orthologues are known to be up-regulated by low Mn^{2+} concentrations, acting via the Mn^{2+}-dependent repressor, MntR (45, 46). This regulator is distantly related to the DtxR family of iron-dependent regulators, DNA-binding proteins which, when loaded with their metal cofactor, bind to specific palindromic recognition sequences in gene promoter regions (see below). However, *M. tuberculosis* does not encode an MntR homologue, nor

is there an obvious MntR box upstream of *mntH*. The *E. coli* and *S. typhimurium* orthologues are additionally regulated by Fe (via the iron-binding repressor, Fur) and, in the case of *S. typhimurium*, also by peroxide, via the regulator, OxyR, with corresponding Fur and OxyR boxes in the promoter region (21, 47). However, scrutiny of the *M. tuberculosis mntH* upstream region reveals no consensus binding sites corresponding to either of these regulators.

We are currently studying the regulation of MntH expression and its contribution to intraphagosomal survival and pathogenicity. *mntH* knockout mutants were recently reported as showing no discernible phenotype in liquid culture, macrophages or mice (48, 49). This may be a consequence of the deployment of compensatory transporters (the large number of 'P' type ATPases, for example, may provide an ample repertoire of substrate specificities). Alternatively, the physiological stimulus for MntH function may not have been identified.

5. THE POTENTIAL *M. TUBERCULOSIS* METAL ION TRANSPORTER REPERTOIRE

M. tuberculosis encodes 28 putative metal ion transporters (or transporter subunits) based on sequence comparisons with functionally characterised transporters in other bacteria (50). Table 1 presents a classification of these sequences and table 2 compares *M. tuberculosis* cation transporters as a proportion of all encoded transporters, with this proportion in a selection of completely sequenced prokaryotic genomes. Cation transporters in *M. tuberculosis* represent 24% of all encoded transporter sequences. This broad repertoire may confer the versatility required for adaptation to both intra- and extracellular niches during infection and may also reflect the proposed divergence of *M. tuberculosis* from a free living environmental ancestor in the relatively recent past (51).

Table 1 reveals a predominance of primary active transporters (belonging to 3 families) in which ATP hydrolysis provides the energetic driving force for cation transport. Eight families of secondarily active metal ion transporters are encoded by *M. tuberculosis*, each represented by only a single member. The disproportion between primary and secondary active transporters is due to the presence of 12 'P' type ATPases, a greater number than in any other sequenced prokaryotic genome. Although *M. tuberculosis* encodes at least 25 complete members of the multi-subunit ABC family (43), those involved in metal ion transport in other bacteria are represented only incompletely (and possibly non-functionally) in *M. tuberculosis*, by genes encoding the isolated substrate binding protein components, FecB and FecB2. In *E. coli*, FecB binds $iron_{III}$ dicitrate before delivering it to the rest of the ABC complex. While Paulsen *et al* ((50)) list Rv3041 as a possible associated nucleotide binding subunit, we were unable to find a convincing basis in homology to group this ORF with nucleotide binding subunits of known Fe transporting ABC protein complexes. The third family of primary active transporters are the arsenical efflux proteins (Ars family), comprising ArsA, ArsB and ArsB2. ArsB and ArsB2 are integral membrane subunits whose homologues in other bacteria function either in conjunction with the ATPase subunit (ArsA) as heterodimeric primary active efflux pumps or independently as secondarily active transporters energetically coupled to the membrane potential (52).

A comparison of these transporters with those of *Mycobacterium leprae* affords an insight into the extent of functional redundancy (with respect to parasitism) in *M. tuberculosis*. The *M. leprae* genome contains counterparts of many *M. tuberculosis* genes. However, almost half of all *M. leprae* ORFs are pseudogenes, arguably the result of a dramatic 'proteomic streamlining' in which proteins unnecessary for an obligate parasitic lifestyle have been 'jettisoned' through a process of gene deletion and decay (53). 17 of the 28 *M. tuberculosis* metal ion transporters are either not present at all in *M. leprae* or are represented by pseudogenes (table 1). The remainder may be precisely those which have a critical function in some aspect of intracellular survival. This 'residual set' of metal transporter genes consists of *mntH*, *corA*, *chaA*, *CPA-2*, 4 of the 12 'P' type ATPases, *arsB* (the secondarily active arsenical exporter), *fecB* and the possibly associated ATPase subunit, Rv3041c. These considerations might justify making these proteins the focus of initial studies on metal ion transport during infection by *M. tuberculosis*.

5.1. 'P' type ATPases

The 'P' type ATPases are a superfamily of ubiquitous primary active transporters (54, 55). Table 3 illustrates a currently accepted subclassification (54). Most are metal cation transporters apart from a subfamily of eukaryotic amino-phospholipid translocases. Prokaryotic 'P' type ATPases transport a range of divalent metal cations encompassing Cu^{2+}, Ag^{2+}, Cd^{2+}, Zn^{2+} and Mn^{2+}. *M. tuberculosis* encodes more 'P' type ATPases than any other class of metal transporter (table 4).

Three of the 5 classes of 'P' type ATPases are represented in *M. tuberculosis*; the complete set of 12 encompasses 7 probable type IB or heavy metal pumps (*ctps A, B, C, D, G, J* and *V*), 3 possible type IIA ATPases (*ctps F, H* and *I*), 1 type IA or kdpB-type transporter and 1 unclassifiable sequence (*ctpE*) (table 1). Figure 2 illustrates their striking variation in overall length and in the lengths of individual domains. Of the 7 type IB or 'heavy metal' ATPases, only ctpA and ctpB possess a recognisable N-terminal heavy metal binding motif containing paired cysteine residues (56). However, short ORFs encoded immediately upstream of *ctps C, G* and *V* contain possible histidine-rich heavy-metal binding regions at their C-termini, suggesting that these may function as metal-binding subunits (57).

The *M. tuberculosis* heavy metal 'P' type ATPases can be sub-classified into 3 groups, based on alignments of the first 100 amino acids in their N-termini (not shown). These comprise: i. ctpA and ctpB which share 47.2% identity and cluster with known Cu^{2+} transporters, ii. ctps C,G, and V sharing 40-50% identity and iii. ctpD and J

Table 1. Classification of *M. tuberculosis* metal ion transporters

Transporter class	Transporter family	Abbrev-iation	TC No.	Gene name	Rv No.	Substrate	Energetics	Comments	*M. leprae* orthologue	% amino-acid identity
Secondarily active:										
	Proton dependent Mn^{2+} transporter	MntH/ Nramp	2.A55.3.1	*mntH*	Rv0924c	Mn^{2+}, Fe^{2+}, Zn^{2+}/H^{+} symporter	PMF		ML2098	67
	Metal Ion transporter	MIT	2.45	*corA*	Rv1239c	? $Mg^{2+}/Co^{2+}/Fe^{2+}$ uptake	?		ML1080	79
	Cation Diffusion Facillitator	CDF	2.4	-	Rv2025c	? $Cd^{2+}/Zn^{2+}/Co^{2+}$	?		Pseudogene ML1977	-
	Ni^{2+}, Co^{2+} transporter	NiCoT	2.A.52	*nicT*	Rv2856	Ni^{2+} / Co^{2+}	PMF		Pseudogene ML1571	-
	Arsenical resistance-3	ACR3		*arsC*	Rv2643	? AsO^{3-}	? PMF		-	-
	Ca^{2+}: cation antiporter	CaCA	2.19	*chaA*	Rv1607	? Ca^{2+}	? PMF		ML1267	56
	Monovalent cation: proton antiporter-1	CPA-1	2.36	*yjcE*	Rv2287	? Na^{+}/H^{+} antiporter	? PMF		Pseudogene ML1792	-
	Monovalent cation: proton antiporter-2	CPA-2	2.37	*kefB*	Rv3236c	?K^{+} or Na^{+}/H^{+} antiporter	? PMF		ML0782	71
Primary active:										
	P' type ATPase	P-ATPase	3.3	*ctpA*	Rv0092	? Heavy metal (?$Cu^{+/2+}$)	ATP hydrolysis	Type IB	ML1987	74.4
				ctpB	Rv0103c	? Heavy metal (?$Cu^{+/2+}$)	" "	Type IB	ML2000	76.5
				ctpC	Rv3270	? Heavy metal (?Cd^{2+}/Fe^{2+})	" "	Type IB	ML0747	74
				ctpD	Rv1469	? Heavy metal (?Cd^{2+})	" "	Type IB	Pseudogene ML1819	-
				ctpE	Rv0908	?	" "	? Type II or novel subclass	Pseudogene ML2115	-
				ctpF	Rv1997	? Ca^{2+}/Mg^{2+}	" "	Type IIA		-
				ctpG	Rv1992c	? Heavy metal (?Cd^{2+})	" "	Type IB		-
				ctpH	Rv0425c	?	" "	? Type IIA (V. extended N-terminal region)	Pseudogene ML1933	-
				ctpI	Rv0107c	?	" "	" "	ML2671	81
				ctpJ	Rv3743c	? Heavy metal (?Cd^{2+})	" "	Type IB	-	-
				ctpV	Rv0969	? Heavy metal (?$Cu^{+/2+}$)	" "	Type IB	-	-
				kdpB	Rv1030	? K^{+} uptake	" "	Type IA	-	-
	ATP-binding cassette	ABC	3.1	*fecB*	Rv0265	?Fe^{3+}		Substrate binding protein subunit	ML1729	75.6
				fecB2	Rv3044	?Fe^{3+}		Substrate binding protein subunit	Pseudogene ML2548	-
				-	Rv3041c	?Fe^{3+}	ATP hydrolysis	ATPase subunit	ML1726	75
	Arsenical efflux	Ars	3.A.4	*arsA*	Rv2084	? AsO^{3-} efflux	ATP hydrolysis		-	-
				arsB	Rv2685	? AsO^{3-} efflux	? PMF		ML1036	69
				arsB2	Rv3578	? AsO^{3-} efflux	? PMF		Pseudogene ML0331	-
Unclassified:	Mg^{2+} transporter	MgT	99.19	*mgtE*	Rv0362	?Mg^{2+}, Co^{2+} uptake	?		-	-
	MerTP mercuric ion permease	MerTP	99.2	*merT*	Rv2877c	? Hg^{2+} uptake	?		Pseudogene ML1585	-

With the exception of MntH, the substrate specificities, where stated, are speculative and based on those of functionally characterised orthologues in other organisms. *M. leprae* orthologues were identified by TBLASTN sequence comparisons with the *M. leprae* genome database (http://genolist.pasteur.fr/Leproma/) and % amino acid identities are stated for the largest regions of overlap for intact open reading frames. CorA (Rv1239) is the major high capacity constitutive Mg^{2+} importer in *E. coli* and *S. typhimurium* (70). Homologues of Rv2025c in the soil bacteria *Alcaligenes spp.* function as divalent cation/proton antiporters in the efflux of Cd^{2+}, Zn^{2+} and Co^{2+} (71). Functionally characterised members of the NiCoT family catalyse proton motive force (PMF)-driven uptake of either Ni^{2+} or Co^{2+} in Gram +ve and Gram -ve bacteria (72). ArsC is homologous to a yeast exporter of arsenite (73). ChaA is a member of a large family, represented in all 3 domains of life, which mediate Ca^{2+} efflux in exchange for either H^{+} or Na^{+}. YjcE is a member of another large group of metal cation/proton exchangers which play a role in cytoplasmic pH regulation and mammalian water fluxes (74). The CPA-2 family, represented by KefB, includes the *E. coli* KefB and KefC proteins which mediate glutathione-gated K^{+} efflux, affording protection against toxic electrophilic metabolites (75). In some bacteria, eg. *E. hirae*, homologous transporters may act as Na^{+}/H^{+} antiporters (76). Single representatives of each of 2 transporter families, unclassified on the basis of energetics, are present: the bacterial Mg^{2+}/Co^{2+} importer MgtE, functionally characterised in *Bacillus firmus* (77) and the bacterial Hg^{2+} importer, MerT (71). Based on (50). PMF – Proton Motive Force.

Table 2. Metal ion transporters as a proportion of total numbers of transporters in a representative selection of genomes

Organism	Metal ion transporters as % of all transporters in genome
Escherichia coli	11
Chlamydia trachomatis	13
Bacillus subtilis	14
Pseudomonas aeruginosa	16
Thermotega maritima	16
Rickettsia prowazekii	18
Mycoplasma pneumoniae	18
Mycoplasma genitalium	20
Streptococcus pyogenes	20
Mycobacterium leprae	21
Haemophilus influenza	22
Campylobacter jejuni	23
Vibrio cholerae	23
Mycobacterium tuberculosis	24
Pyrococcus horikoshii	24
Helicobacter pylori	27
Archaeoglobus fulgidus	27
Borrelia burgdorferii	28
Treponema pallidum	29
Neisseria meningitidis AZ2491	31
Synechocystis PCC6803	33
Methanococcus jannaschii	38
Aquifex aeolicus	44
Methanobacterium thermoautotrophicum	47

Table 3. Sub-classification of 'P' type ATPases

Subgroup	Substrate category
Type I	
• IA	Bacterial KdpB ATPases (K^+ uptake)
• IB	Heavy metal ATPases
Type II	
• IIA	SERCA pumps
• IIB	PMCA pumps
• IIC	Mammalian Na^+/K^+, H^+/K^+
• IID	Fungal pumps of uncertain function
Type III	
• IIIA	HA pumps of fungi and plants
• IIIB	Bacterial Mg^{2+} pumps
Type IV	Aminophospholipid transferases
Type V	Eukaryotic pumps of undefined function

This classification is based on that of Axelsen (54). SERCA - Sarco(Endo)plasmic Reticulum Calcium ATPase, PMCA - Plasma Membrane Calcium ATPase, HA - Proton ATPase. Cations transported by the heavy metal pumps (type IB) encompass Cd^{2+}, Zn^{2+}, $Cu^{+/2+}$, Ag^+ and Mn^{2+}.

(40.7%). The latter 2 groups cluster with known Cd^{2+} / Zn^{2+} transporters. *Between* groups there is only 3.7-16.7% identity within the first 100 amino acids and it is tempting to speculate that such variation may reflect adaptations to different substrate specificities. One study has shown up-regulation of ctpC, together with its upstream ORF, *irp10*, in Fe-depleted growth conditions (0.5 microM) (57), raising intriguing questions about the relationship between these transporters and Fe. No Fe transporting bacterial 'P' type ATPases have so far been described.

The 3 putative type IIA ATPases (ctps F, H and I) resemble one another from the TGE or 'phosphatase site' motif onwards. However, the N-terminal regions of ctpH and ctpI are at least twice as long as corresponding regions in any of the others. This unusual feature has not been described previously in any other 'P' type ATPase. *ctpI* has a close orthologue in *M. leprae* (81% amino acid identity in a 1287 amino acid overlap), while the counterpart of *ctpH* is represented by a pseudogene. Database searches reveal a *ctpH* orthologue in *Streptomyces coelicolor*. In contrast to the considerable N-terminal variability between ctps F, H and I, their C-terminal halves all contain conserved residues associated with Ca^{2+} binding. The conserved motifs, XNXGE and QXXWXNXXTD, located in transmembrane segments M5 and M6 respectively, have been linked with Ca^{2+} binding in eukaryotic type IIA (SERCA) pumps (58, 59). They are also partly conserved in the Mg^{2+} transporting ATPases, MgtA and MgtB, of *S. typhimurium.* Whether the physiological substrate of these *M. tuberculosis* proteins is Mg^{2+} or Ca^{2+}, awaits experimental elucidation.

ctpE exhibits some features of both the type I and type II ATPases. However, it possesses neither heavy metal signature motifs nor the SERCA associated M5/M6 motifs discussed above. Its closest affiliations appear to be with the type II sequences as reflected by the PEGL motif in the M4 ion channel which is virtually characteristic of type II ATPases (54, 55, 59) and a hydropathy profile consistent with the proposed membrane topology of this subfamily.

6. REGULATION AND SIGNALLING BY Fe AND Mg

M. tuberculosis encodes representatives of 3 families of trans-acting DNA-binding proteins which mediate the effects of Fe on gene regulation; these are FurA/B, IdeR and SirR. Fur (Ferric Uptake Regulator) is the major mediator of gene regulation by Fe in low G+C content bacteria and, when iron-bound, functions as both a repressor and activator of gene expression (60). In *M. tuberculosis*, FurA is the major repressor of the downstream encoded, catalase-peroxidase, KatG (61). Its role in this context may be compensatory for the absence in *M. tuberculosis* of OxyR, an important regulator of the oxidative stress response, including KatG expression, in other bacteria (62). It may also regulate other virulence determinants (61). Fur is itself up-regulated by high iron concentrations (70 microM)(63).

IdeR (iron dependent regulator) is a member of the DtxR (diphtheria toxin regulator) family of iron repressors (64) and the major iron-dependent regulator in

Table 4. Comparison of numbers of 'P' type ATPase sequences (total and subclasses) encoded by *M. tuberculosis* with those in representative selection of completed prokaryotic genomes

Organism	No. of 'P' type ATPases			Total
	Type IA	Type IB	Type II (a,b)	
M. tuberculosis	1	7	4[1]	12
Synechococcus spp.	1	4	4	9
M. thermoautotrophicum	1	3	2	6
Streptomyces coelicolor	0	4	1	5
Yersinia pestis	1	3	1	5
Staphylococcus aureus MRSA 252	2	3	0	5
M. leprae	0	3	1	4
B. subtilis	0	3	1	4
E. coli	1	4	1	4
Salmonella typhi	1	1	2	4
H. pylori	0	3	0	3
Bordetella pertussis	1	2	0	3
A. aeolicus	0	2	1	3
A. fulgidus	0	2	0	2
Neisseria meningitidis A	0	2	0	2
Campylobacter jejuni	0	2	0	2
M. genitalium	0	0	1	1
M. pneumoniae	0	0	1	1
H. influenza	0	1	0	1
C. trachomatis	0	1	0	1
T. pallidum	0	1	0	1
T. maritima	0	1	0	1
M. jannaschii	0	0	1	1
R. prowazekii	0	0	0	0
B. burgdorferii	0	0	0	0
P. horikoshii	0	0	0	0

[1] this includes ctpE (Rv0908) which, while most closely resembling the type II sequences, nevertheless differs sufficiently to merit a subclass of its own (54). The numbers quoted refer to the number of apparently intact open reading frames and do not include pseudogenes.

Gram positive organisms with high G+C content. There are putative IdeR binding sites in the upstream regions of more than 40 genes in *M. tuberculosis*, including those involved in biosynthesis of siderophores, aromatic amino acids, cell wall structural components and iron storage proteins (30, 65). There is evidence from quantitative reverse transcription PCR (RT-PCR) experiments for the role of IdeR as a repressor of *HisE* (involved in histidine biosynthesis), *mbtA*, *mbtB*, *mbtI* (involved in the synthesis of siderophores), *bfd* (implicated in iron storage) and *Rv3402c* (an unknown protein with some similarities to enzymes involved in multiple synthetic pathways) (65). It also acts as both an activator and repressor of *bfrA*, which encodes a bacterioferritin subunit. *MbtB*, *MbtI* and *Rv3402c* were up-regulated on entry into THP1 cells, suggesting that the intracellular environment is iron-limited. Many of the other genes preceded by IdeR boxes do not have an obvious relationship to Fe metabolism. SirR is a representative of yet another family of iron-dependent regulators, first described in *Staphylococcus* epidermidis (66). To date, its function in *M. tuberculosis* is unknown.

2D protein electrophoresis has demonstrated the Fe-dependent expression of at least 27 distinct proteins from *M. tuberculosis* grown in high and low Fe conditions (70 microM and 1 microM, respectively) (63). High Fe-induced proteins included Fur, and proteins apparently homologous to an aconitase (another potential transcriptional regulator), EF-Tu (a helper protein involved in protein synthesis), LSR2 (a dominant T cell antigen), Hsp16.3 (an α-crystallin homologue), an NADPH-dependent dehydrogenase and a peptidyl-prolyl *cis-trans* isomerase (PPI). Proteins up-regulated under low iron conditions included PEPCK (homologous to many GTP-dependent phosphoenol pyruvate carboxykinases in other species). Fe therefore regulates more than the set of genes involved in its acquisition and storage, pointing to its role as an important signal for the co-ordinated deployment of diverse mechanisms associated with rapid adaptation to changes in environment, including entry into host compartments.

Mg may also have an important signalling function with respect to entry into an intra-phagosomal environment. *M. tuberculosis* encodes a protein, MgtC, which is essential for growth in liquid media under conditions of low Mg^{2+} (<50 microM) and acidic pH (<6.5). Mutants in which MgtC was inactivated, exhibited diminished survival in macrophages and decreased virulence in mice, compared to wild-type controls (67)

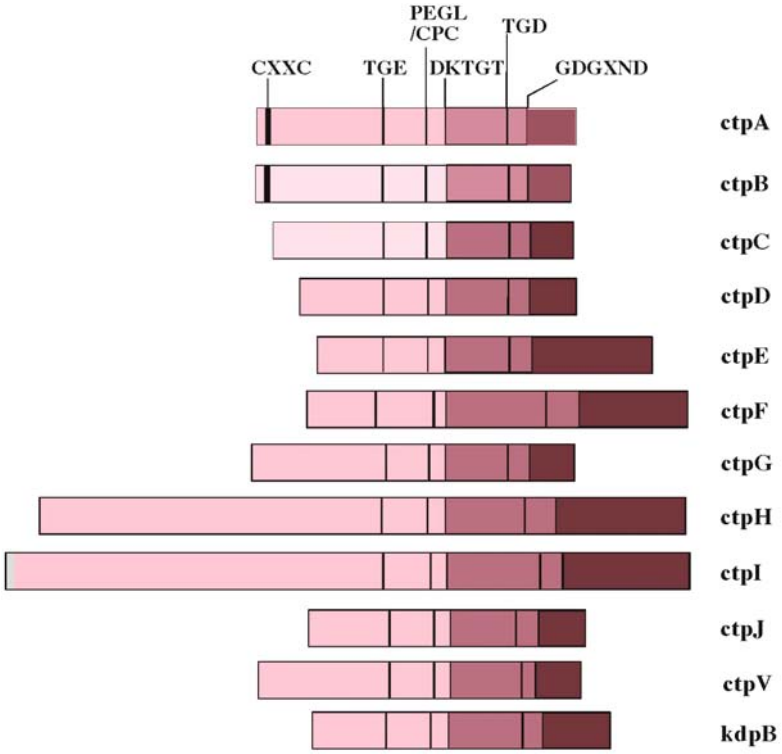

Figure 2. Schematic alignment of the 12 *M. tuberculosis* 'P' type ATPase sequences. The phosphorylation sites have been aligned to facilitate comparison of the sizes of the various domains, which are drawn approximately to scale. The large cytoplasmic loop is in mid-pink, and the domains N- and C-terminal to this are in light and dark pink respectively. The locations of conserved motifs are indicated by vertical lines. CXXC - N-terminal heavy metal binding motif, TGE - phosphatase site, PEGL/CPC - ion channel motif, DKTGT - phosphorylation site, TGD-GDGXND - ATP-binding domain.

suggesting that these are the conditions encountered in the phagosome. MgtC is closely related to an orthologue in *S. typhimurium*, which is similarly essential for growth at low Mg^{2+} concentrations, intra-phagosomal survival and virulence, and is encoded as part of the pathogenicity island, SPI 3 (68). It is regulated in *S. typhimurium*, by the 2-component system PhoP/PhoQ, in response to changes in Mg^{2+} concentration. Although a predicted integral membrane protein, MgtC does not appear to be directly involved in Mg^{2+} transport itself (69) and in any case, it seems probable that Mg^{2+} requirements can be adequately furnished by the activity of CorA, a transporter also present in *M. tuberculosis.* MgtC may be play a part in a more general co-ordinated response to phagocytosis signalled by a change in Mg^{2+} levels. No other Mg^{2+} regulated genes in *M. tuberculosis* are known at present. There are also no data on the influence of other metals on *M. tuberculosis* gene expression. We are currently undertaking studies to address these important questions.

7. PERSPECTIVE

The lack of functional studies on the vast majority of potential *M. tuberculosis* metal ion transporters raises more questions than answers but highlights a fertile area for future study. What is clear, is that metal ions are of unquestionable significance both as essential metabolic cofactors and as physiological signals. Studies on bacterial NRAMP orthologues have raised the prominence of Mn transport as a potentially key process in the interplay between intracellular pathogens and their hosts, although the Mn selectivity of *M. tuberculosis* MntH has yet to be determined. Genomic analyses indicate that *M. tuberculosis* encodes a large and diverse repertoire of potential metal transporters. This apparent redundancy in metal transport capability might provide the versatility required for survival in varying host habitats at different stages in the infective process. The extra-cellular and intra-cellular environments of *M. tuberculosis* pose different physiological challenges. We know relatively little about the cationic composition of the mycobacterial phagosome other than its mildly acidic pH and, indirectly, that it is probably low in Mg^{2+} and Fe^{2+}. We know even less about how its metal ion composition changes over time. However, given the highly dynamic character of the phagosomal compartment, it is a plausible supposition that *M. tuberculosis* may need to deploy different transporters even over the short time frame of phagosomal maturation. The detailed analysis of the temporal pattern of *M. tuberculosis* transporter gene expression during infection and the interaction between these processes and host encoded metal ion transport will provide important insights into the basic biology of intracellular parasitism and may help to shape novel therapeutic strategies. If the 'streamlined' genome of *M. leprae* does indeed encode the minimal gene set required for intracellular survival, then

the overlap in transporters encoded by the two organisms may provide a fruitful starting point. A thorough analysis of even this delimited set of 17 transporters is a major challenge. However, new technologies such as DNA microarrays, proteomic analyses and efficient techniques for homologous recombination in *M. tuberculosis* provide the necessary tools to address these questions.

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9. REFERENCES

1. Agranoff D, I. M. Monahan, J. A. Mangan, P. D. Butcher & S. Krishna: Mycobacterium tuberculosis expresses a novel pH-dependent divalent cation transporter belonging to the Nramp family. *J Exp Med* 190, 717-724 (1999)
2. De Voss J. J, K. Rutter, B. G. Schroeder & C. E. Barry III: Iron acquisition and metabolism by mycobacteria. *J Bacteriol* 181, 4443-4451 (1999)
3. Gunshin H, B. Mackenzie, U. V. Berger, Y. Gunshin, M. F. Romero, W. F. Boron, S. Nussberger, J. L. Gollan, & M. A. Hediger: Cloning and characterisation of a mammalian proton-coupled metal-ion transporter. *Nature* 388, 482-488 (1997)
4. Jabado N, A. Jankowski, S. Dougaparsad, V. Picard, S. Grinstein & P. Gros: Natural resistance to intracellular infections: natural resistance-associated macrophage protein 1 (Nramp1) functions as a pH-dependent manganese transporter at the phagosomal membrane. *J Exp Med* 192, 1237-48 (2000)
5. Goswami T, A. Bhattacharjee, P. Babal, S. Searle, E. Moore, M. Li & J. M. Blackwell: Natural-resistance-associated macrophage protein 1 is an H^+/bivalent cation antiporter. *Biochem J* 354, 511-9 (2001)
6. Gruenheid S, F. Canonne-Hergaux, S. Gauthier, D. J. Hackam, S. Grinstein & P. Gros: The Iron Transport Protein NRAMP2 Is an Integral Membrane Glycoprotein That Colocalizes with Transferrin in Recycling Endosomes. *J Exp Med* 189, 831-841 (1999)
7. Gruenheid S, E. Pinner, M. Desjardins & P. Gros: Natural resistance to Infection with Intracellular Pathogens: The *Nramp1* Protein is Recruited to the Membrane of the Phagosome. *J Exp Med* 185, 717-730 (1997)
8. Searle S, N. A. Bright, T. I. A. Roach, P. G. P. Atkinson, C. H. Barton, R. H. Meloen & J. M. Blackwell: Localisation of Nramp1 in macrophages: modulation with activation and infection. *J Cell Sci* 111, 2855-2866 (1998)
9. Skamene, E. Schurr, E.& P. Gros: Infection genomics: *Nramp1* as a major determinant of natural resistance to intracellular infections. *Annu Rev Med* 49, 275-287 (1998)
10. Govoni G & P. Gros: Macrophage NRAMP1 and its role in resistance to microbial infections. *Inflamm Res* 47, 277-84 (1998)
11. Bellamy R, C. Ruwende, T. Corrah, K. P. W. J. McAdam, H. C. Whittle & A. V. S. Hill: Variations in the *NRAMP1* gene and susceptibility to tuberculosis in West African patients. *N Engl J Med* 338, 640-644 (1998)
12. Greenwood C. M. T, T. M. Fujiwara, L. J. Boothroyd, M. A. Miller, D. Frappier, E. A. Fanning, E. Schurr & K. Morgan: Linkage of tuberculosis to chromosome 2q35, including NRAMP1, in a large aboriginal Canadian family. *Am J Hum Genet* 67, 405-416 (2000)
13. Abel L, F. O. Sánchez, J. Oberti, N. V. Thuc, L. V. Hoa, V. D. Lap, E. Skamene, P. H. Lagrange & E. Schurr: Susceptibility to leprosy is linked to human *NRAMP1* gene. *J Infect Dis* 177, 133-145 (1998)
14. Ryu S, Y. K. Park, G. H. Bai, S. J. Kim, S. N. Park & S. Kang: 'UTR polymorphisms in the NRAMP1 gene are associated with susceptibility to tuberculosis in Koreans. *Int J Tuberc Lung Dis* 4, 577-580 (2000)
15. Cervino A. C, S. Lakiss, O. Sow & A. V. Hill: Allelic association between the NRAMP1 gene and susceptibility to tuberculosis in Guinea-Conakry. *Ann Hum Genet* 64, 507-12 (2000)
16. Meisner S. J, S. Mucklow, G. Warner, S. O. Sow, C. Lienhardt & A. V. Hill: Association of NRAMP1 polymorphism with leprosy type but not susceptibility to leprosy per se in west Africans. *Am J Trop Med Hyg* 65, 733-5 (2001)
17. Kuhn D. E, B. D. Baker, W. P. Lafuse & B. S. Zwilling: Differential iron transport into phagosomes isolated from the RAW264.7 macrophage cell lines transfected with Nramp1Gly169 or Nramp1Asp169. *J Leukocyte Biol* 66, 113-9 (1999)
18. Forbes J. R & P. Gros: Iron, manganese, and cobalt transport by Nramp1 (Slc11a1) and Nramp2 (Slc11a2) expressed at the plasma membrane. *Blood* 102, 1884-92 (2003)
19. Cellier M, I. Bergevin, E. Boyer & E. Richer: Polyphyletic origins of bacterial Nramp transporters. *Trends Genet* 17, 365-369 (2001)
20. Agranoff D & S. Krishna: Metal ion homeostasis and intracellular parasitism. *Mol microbiol* 28, 403-412 (1998)
21. Kehres D. G, M. L. Zaharik, B. B. Finlay & M. E. Maguire: The NRAMP proteins of *Salmonella typhimurium* and *Escherichia coli* are selective manganese transporters involved in the response to reactive oxygen. *Mol microbiol* 36, 1085-1100 (2000)
22. Makui H, E. Roig, S. T. Cole, J. D. Helmann, P. Gros & M. F. M. Cellier: Identification of the Escherichia coli K-12 Nramp orthologue (MntH) as a selective divalent metal ion transporter. *Mol microbiol* 35, 1065-1078 (2000)
23. Beisel W. R: Magnitude of the host nutritional responses to infection. *Am J Clin Nutr* 30, 1236-1247 (1977)
24. Shenkin A: Trace elements and inflammatory response: implications for nutritional support. *Nutrition (suppl)* 11, 100-105 (1995)
25. Srinivas U, J. H. Braconier, B. Jeppsson, M. Abdulla, B. Akesson & P. A.Ockerman: Trace element alterations in infectious diseases. *Scand J Clin Lab Invest* 48, 495-500 (1988)
26. Zhang Y, R. Lathigra, T. Garbe, D. Catty & D.Young: Genetic analysis of superoxide dismutase, the 23 kilodalton antigen of Mycobacterium tuberculosis. *Mol microbiol* 5, 381-91 (1991)
27. Tullius M. V, G. Harth & M. A. Horwitz: High extracellular levels of Mycobacterium tuberculosis glutamine synthetase and superoxide dismutase in actively growing cultures are due to high expression and extracellular stability rather than to a protein-specific export mechanism. *Infect Immun* 69, 6348-63 (2001)

28. Wu C. H, J. J. Tsai-Wu, Y. T. Huang, C. Y. Lin, G. G. Lioua & F. J. Lee: Identification and subcellular localization of a novel Cu,Zn superoxide dismutase of Mycobacterium tuberculosis. *FEBS Lett* 439, 192-6 (1998)
29. Piddington D. L, F. C. Fang, T. Laessig, A. M. Cooper, I. M. Orme & N. A. Buchmeier: Cu,Zn superoxide dismutase of Mycobacterium tuberculosis contributes to survival in activated macrophages that are generating an oxidative burst. *Infect Immun* 69, 4980-7 (2001)
30. Dussurget O & I. Smith: Interdependence of mycobacterial iron regulation, oxidative-stress response and isoniazid resistance. *Trends microbiol* 6, 354-358 (1998)
31. Kehres D. G & M. E. Maguire: Emerging themes in manganese transport, biochemistry and pathogenesis in bacteria. *FEMS microbiol Rev* 27, 263-90 (2003)
32. Archibald, F: Manganese: its acquisition by and function in the lactic acid bacteria. *Crit Rev microbiol* 13, 63-109 (1986)
33. Posey J. E & F. C. Gherardini: Lack of a role for iron in the Lyme disease pathogen. *Science* 288, 1651-3 (2000)
34. Niven D. F, A. Ekins & A. A. al-Samaurai: Effects of iron and manganese availability on growth and production of superoxide dismutase by Streptococcus suis. *Can J microbiol* 45, 1027-32 (1999)
35. Reddy S. K, M. Kamireddi, K. Dhanireddy, L. Young, A. Davis & P. T. Reddy: Eukaryotic-like adenylyl cyclases in Mycobacterium tuberculosis H37Rv: cloning and characterization. *J Biol Chem* 276, 35141-9 (2001)
36. Chaba R, M. Raje & P. K. Chakraborti: Evidence that a eukaryotic-type serine/threonine protein kinase from Mycobacterium tuberculosis regulates morphological changes associated with cell division. *Eur J Biochem* 269, 1078-85 (2002)
37. Haydel S. E, N. E. Dunlap & W. H. Benjamin, Jr: In vitro evidence of two-component system phosphorylation between the Mycobacterium tuberculosis TrcR/TrcS proteins. *microb Pathog* 26, 195-206 (1999)
38. Gobin J, C. H. Moore, J. R. Reeve Jr, D. K. Wong, B. W. Gibson & M. A. Horwitz: Iron acquisition by Mycobacterium tuberculosis: isolation and characterization of a family of iron-binding exochelins. *Proc Natl Acad Sci U S A* 92, 5189-93 (1995)
39. Quadri L. E, J. Sello, T. A. Keating, P. H. Weinreb & C. T. Walsh: Identification of a Mycobacterium tuberculosis gene cluster encoding the biosynthetic enzymes for assembly of the virulence-conferring siderophore mycobactin. *Chem Biol* 5, 631-45 (1998)
40. Gobin J. & M. A. Horwitz: Exochelins of Mycobacterium tuberculosis remove iron from human iron- binding proteins and donate iron to mycobactins in the M. tuberculosis cell wall. *J Exp Med* 183, 1527-32 (1996)
41. De Voss J. J, K. Rutter, B. G. Schroeder, H. Su, Y. Zhu, & C. E. Barry III: The salicylate-derived mycobactin siderophores of *Mycobacetrium tuberculosis* are essential for growth in macrophages. *Proc Natl Acad Sci USA* 97, 1252-1257 (2000)
42. Braun V & H. Killmann: Bacterial solutions to the iron-supply problem. *Trends Biochem Sci* 24, 104-9 (1999)
43. Braibant M, P. Gilot & J. Content: The ATP binding cassette (ABC) transport systems of Mycobacterium tuberculosis. *FEMS microbiol Rev* 24, 449-67 (2000)
44. Sturgill-Koszycki S, P. H. Schlesinger, P. Chakraborty, P. L. Haddix, H. L. Collins, A. K. Fok, R. D. Allen, S. L. Gluck, J. Heuser & D. G. Russell: Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. *Science* 263, 678-681 (1994)
45. Que Q & J. D. Helmann: 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 (2000)
46. Patzer S. I & K. Hantke: 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, 4806-13 (2001)
47. Kehres D. G, A. Janakiraman, J. M. Slauch & M. E. Maguire: Regulation of Salmonella enterica serovar Typhimurium mntH transcription by H(2)O(2), Fe(2+), and Mn(2+). *J Bacteriol* 184, 3151-8 (2002)
48. Domenech P, A. S. Pym, M. Cellier, C. E. Barry & S.T. Cole: Inactivation of the Mycobacterium tuberculosis Nramp orthologue (mntH) does not affect virulence in a mouse model of tuberculosis. *FEMS microbiol Lett* 207, 81-6 (2002)
49. Boechat N, B. Lagier-Roger, S. Petit, Y. Bordat, J. Rauzier, A. J. Hance, B. Gicquel & J. M. Reyrat: Disruption of the gene homologous to mammalian Nramp1 in Mycobacterium tuberculosis does not affect virulence in mice. *Infect Immun* 70, 4124-31. (2002)
50. Paulsen I. T, L. Nguyen, M. K. Sliwinski, R. Rabus & M. H.Saier Jr: microbial genome analyses: comparative transport capabilities in eighteen prokaryotes. *J Mol Biol* 301, 75-100 (2000)
51. Cole S. T, R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Gas, C. E. Barry 3rd, F. Tekaia, K. Badcock, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. Davies, K. Devlin, T. Feltwell, S. Gentles, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, B. G. Barrell, & et al.: Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. *Nature* 393, 537-44 (1998)
52. Kuroda M, S. Dey, O. I. Sanders & B. P. Rosen: Alternate energy coupling of ArsB, the membrane subunit of the Ars anion-translocating ATPase. *J Biol Chem* 272, 326-331 (1997)
53. Cole S. T, K. Eiglmeier, J. Parkhill, K. D. James, N. R. Thomson, P. R. Wheeler, N. Honore, T. Garnier, C. Churcher, D. Harris, K. Mungall, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. M. Davies, K. Devlin, S. Duthoy, T. Feltwell, A. Fraser, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, C. Lacroix, J. Maclean, S. Moule, L. Murphy, K. Oliver, M. A. Quail, M. A. Rajandream, K. M. Rutherford, S. Rutter, K. S. S. Seeger, M. Simmonds, J. Skelton, R. Squares, S. Squares, K. Stevens, K. Taylor, S. Whitehead, J. R. Woodward & B. G. Barrell: Massive gene decay in the leprosy bacillus. *Nature* 409, 1007-11 (2001)
54. Axelsen K. B & M. G. Palmgren: Evolution of substrate specificities in the P-type ATPase superfamily. *J Mol Evol* 46, 84-101 (1998)
55. Fagan M. J & M. H. Saier Jr: P-type ATPases of eukaryotes and bacteria:sequence analyses and construction of phylogenetic trees. *J Mol Evol* 38, 57-99 (1994)
56. Lutsenko S & J. H. Kaplan: Organization of P-type ATPases: significance of structural diversity. *Biochemistry* 34, 15607-15613 (1995)
57. Calder K. M & M. A. Horwitz: Identification of iron-regulated proteins of *Mycobacterium tubercuolosis* 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 (1998)
58. Clarke D, T. Loo, G. Inesi & D. MacLennan: Location of high affinity Ca-binding sites within the predicted transmembrane domain of the sarcoplasmic reticulum Ca ATPase. *Nature* 339, 476-478 (1989)
59. Moller J, B. Juul & M. le Maire: Structural organisation, ion transport and energy transduction of P-type ATPases. *Biochim Biophys Acta* 1286, 1-51 (1996)
60. Escolar L, J. Perez-Martin & V de Lorenzo: Opening the iron-box: transcriptional metalloregulation by the Fur protein. *J Bacteriol* 181, 6223-6229 (1999)
61. Pym A. S, P. Domenech, N. Honore, J. Song, V. Deretic & S. T. Cole: Regulation of catalase-peroxidase (KatG) expression, isoniazid sensitivity and virulence by furA of Mycobacterium tuberculosis. *Mol microbiol* 40, 879-89 (2001)
62. Zahrt T. C, J. Song, J. Siple & V. Deretic: Mycobacterial FurA is a negative regulator of catalase-peroxidase gene katG. *Mol microbiol* 39, 1174-85 (2001)
63. Wong D. K, B. Y. Lee, M. A. Horwitz & B. W. Gibson: 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, 327-36 (1999)
64. Schmitt M. P, M. Predich, L. Doukhan, I. Smith & R. K. Holmes: Characterisation 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 (1995)
65. Gold B, G. M. Rodriguez, S. A. Marras, M. Pentecost & I. Smith: 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, 851-65 (2001)
66. Hill P. J, A. Cockayne, P. Landers, J. A. Morrissey, C. M. Sims & P. Williams: SirR, a novel iron-dependent repressor in *Staphylococcus epidermidis*. *Infect Immun* 66, 4123-4129 (1998)
67. Buchmeier N, A. Blanc-Potard, S. Ehrt, D. Piddington, L. Riley & E. A.Groisman: A parallel intraphagosomal survival strategy shared by mycobacterium tuberculosis and Salmonella enterica. *Mol microbiol* 35, 1375-82 (2000)
68. Blanc-Potard A.B & E. A. Groisman: The Salmonella *selC* locus contains a pathogenicity island mediating inramacrophage survival. *EMBO J* 16, 5376-5385 (1997)
69. Moncrief M. B & M. E. Maguire: Magnesium and the role of MgtC in growth of Salmonella typhimurium. *Infect Immun* 66, 3802-9 (1998)
70. Smith R.L & M.E. Maguire: microbial magnesium transport: unusual transporters searching for identity. *Mol microbiol* 28, 217-226 (1998)
71. Silver S & L.T. Phung: Bacterial Heavy Metal Resistance: New Surprises. *Annu Rev microbiol* 50, 753-789 (1996)
72. Degen O, M. Kobayashi, S. Shimizu & T. Eitinger: Selective transport of divalent cations by transition metal permeases: the *Alcaligenes eutrophus* HoxN and the *Rhodococcus rhodococcus* NhlF. *Arch microbiol* 171, 139-145 (1999)
73. Wysocki R, P. Bobrowicz & S. Ulaszewski: The Saccharomyces cerevisiae ACR3 gene encodes a putative membrane protein involved in arsenite transport. *J Biol Chem* 172, 30061-30066 (1997)
74. Orlowski J & S. Grinstein: Na^+/H^+ exchangers of mammalian cells. *J Biol Chem* 272, 22373-22376 (1997)
75. Ferguson G. P, A.W. Munro, R. M. Douglas, D. McLaggan & I. R. Booth: Activation of potassium channels during metabolite detoxification in *Escherichia coli*. *Mol microbiol* 9, 1297-1303 (1993)
76. Reizer J, A. Reizer & M. H. Saier Jr: The putative Na+/H+ antiporter (NapA) of *Enterococcus hirae* is homologous to the putative K+/H+ antiporter (KefC) of *Escherichia coli*. *FEMS microbiol Lett* 94, 161-164, (1992)
77. Smith R. L, L. J. Thompson & M. E. Maguire: Cloning and characterisation of MgtE, a putative new class of Mg^{2+} transporter from *Bacillus firmus*. *J Bacteriol* 177, 1233-1238 (1995)

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