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Structure, properties and regulation of magnesium transport proteins

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Introduction

Magnesium is unique among the biological cations. Its size, charge density, structure in aqueous solution and its aqueous chemistry are generally different from all other cations, monovalent or divalent. Just as magnesium itself is different, the proteins that transport it are different, even unique. Mg^{2+} transport has been extensively studied in mammalian and other systems. For reviews the reader is referred to the article by Romani and Maguire in this issue and a recent comprehensive review by Romani & Scarpa (2000). Despite this extensive study, only a single gene for a Mg^{2+} transport protein has been cloned to date from any eukaryote. In contrast, three distinct families of prokaryotic Mg^{2+} transport proteins have been identified and cloned: MgtE, CorA, and MgtA/B. This review will concentrate on these three prokaryotic Mg^{2+} transporter types from the viewpoints of their structure, transport properties and regulation.

Mg^{2+} chemistry

Mg^{2+} is highly unusual among the biologically relevant cations in the geometry of the Mg^{2+} cation and strength its ionic interaction with water and other ligands (see review by Maguire & Cowan in this issue). Because magnesium lacks *d* electrons to participate in ligand coordination, it has significantly less bond angle flexibility than most other cations. In aqueous solution and in biological systems, Mg^{2+} is almost always hexacoordinate, binding water or other ligands in a regular octahedral geometry with all bond angles close to 90° (Cowan 1991; Diebler *et al.* 1969; Huang & Cowan 1994; Martin 1990). Mg^{2+} strongly prefers oxygen as a ligand. It can frequently interact with nitrogen, as in chlorophyll, but interactions with sulfur are unknown in biological systems. Magnesium binds its waters of hydration orders of magnitude more tightly than do Na^{+}, K^{+} or Ca^{2+}. Indeed, as reviewed elsewhere in this issue by Cowan, many of Mg^{2+}'s activities as an enzyme cofactor are mediated through precise spatial coordination of a bound water molecule rather than through interaction with the cation itself (Black & Cowan 1997; Cowan 1993, 1998; Jou & Cowan 1991; Suga *et al.* 1998).

Mg^{2+} has the largest hydrated radius of any common cation; it's ionic radius, i.e., minus waters of hydration, is among the smallest seen with divalent cations. Because of these size considerations, the volume change between hydrated and ionic Mg^{2+} is almost 400-fold. Thus, any protein transporting Mg^{2+} must be capable of initially interacting with a rather large cation. Then, assuming that Mg^{2+} like other cations is transported in its ionic form, Mg^{2+} must pass through a pore that is quite small. Consequently, transport (or interaction with an enzyme) must therefore involve a large initial binding site and/or more elaborate means of dehydration than are commonly found in other cation transporters. It is these atypical geometric and energetic features of magnesium chemistry that explain why magnesium transporters, as far as they have been characterized, tend to be novel types of proteins.

Prokaryotic magnesium transport

Three classes of magnesium transporter have been cloned from Bacteria and Archaea, MgtE, CorA, and MgtA/B (for reviews see Smith & Maguire 1995b, 1998). Detailed information is available only for the three transporters – CorA, MgtA, and MgtB – cloned from *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium). The transporters were cloned by complementation of the Mg^{2+} growth requirement of a *S.* Typhimurium strain engineered to

lack all Mg^{2+} transport. This strain, MM281, requires 100 mM supplemental Mg^{2+} in the growth medium because it has insertions in its three Mg^{2+} transporters, CorA, MgtA and MgtB (Hmiel *et al.* 1986, 1989; Snavely *et al.* 1989, 1991a). Introduction of the gene for any one of the three *S*. Typhimurium Mg^{2+} transporters or of the gene for a Mg^{2+} transporter from another organism relieves both the growth and transport phenotype.

MgtE magnesium transporters

Genomics

In screening microbial genomic libraries for additional CorA-like transporters, the MgtE class of Mg^{2+} transporter was unexpectedly cloned from *Bacillus firmus* OF4 (Smith *et al.* 1995) and *Providencia stuarti* (Townsend *et al.* 1995). At least 36 MgtE-like proteins can be identified currently in DNA sequence databases using a reasonably stringent BLAST search. Except for a short stretch of about 100 amino acids in the middle of the protein that resemble Archaeal inosine monophosphate dehydrogenases, MgtE transporters do not resemble any known class of proteins. The proportion of organisms carrying MgtE sequences in Bacteria *versus* Archaea is essentially equivalent, reflecting the total number of genomes sequenced to date. The phylogenetic relationships of their amino acid sequences follow quite closely the taxonomic relationships of the organisms containing them.

Initial database searches for eukaryotic MgtE homologs were negative. In retrospect, this was probably because sequence from most eukaryotic genomes was essentially random, reflecting cloning of individual genes rather than *a priori* genomic sequencing. This has changed however with the recent completion of sequencing of several eukaryotic genomes. Unlike the CorA and possibly the MgtA/B class of Mg^{2+} transporters, potential homologs of the prokaryotic MgtE Mg^{2+} transporter are widespread in eukaryotes including humans. The eukaryotic homologs appear more closely related to the Archaeal MgtE homologs where there is over 20% identity and over 40% similarity between Archaeal and human amino acid sequences. No eukaryotic MgtE homolog has been cloned and tested for Mg^{2+} transport to date. Nonetheless, there is no other known function of the MgtE class of proteins; moreover, while the percent identity from prokaryote to human is relatively low, somewhat closer homologs exist in other eukaryotes such as *Caenorhabditis elegans*. Finally, we have previously shown that when an Archaeal CorA homolog is expressed in *S.* Typhimurium (Smith *et al.* 1998a), it has virtually identical transport properties with the *S.* Typhimurium CorA even though they share only 16% overall identity (see below for discussion of the CorA transport system.). Thus, it seems likely that at least one class of mammalian Mg^{2+} transporters has now been identified.

Physiology

When expressed from multicopy plasmids in *S.* Typhimurium, the MgtE proteins of *B. firmus* OF4 and *P. stuarti* (Smith *et al.* 1995; Townsend *et al.* 1995) exhibit similar K_m's and V_{max}'s of about 70 μM and 0.50 nmol min^{-1} 10^8 $cells^{-1}$ for $^{57}Co^{2+}$ uptake, respectively. Mg^{2+} inhibits with an apparent K_i of 50 μM. Sr^{2+}, Mn^{2+}, Ca^{2+}, and Zn^{2+} inhibit with K_i's ranging from 80 μM down to 20 μM, respectively, though it has not been determined if any of these cations is transported. Ni^{2+} does not inhibit. Nothing is known about the regulation of MgtE expression. Recently, an MgtE homolog was cloned from *Aeromonas hydrophila* and shown to be involved in biofilm formation and adherence in this opportunistic pathogen (Merino *et al.* 2001). Thus, like CorA (*vide infra*), MgtE may be involved in bacteria pathogenesis.

Structure

MgtE transporters appear to have 4 or more likely 5 transmembrane (TM) domains with a large hydrophilic domain at the N-terminus residing in the cytosol (Smith *et al.* 1995; Townsend *et al.* 1995). Putative helical transmembrane segments contain a few modestly conserved charged residues and several well conserved residues bearing hydroxyl side chains. Because introduction of a single gene is sufficient to induce Mg^{2+} uptake, it can be inferred that MgtE does not require another protein for transport, but it is unknown whether MgtE transporters function as homo-oligomers or monomers. MgtE sequences lack recognizable NTP binding motifs and therefore likely depend on the transmembrane electrochemical gradient to provide energy for Mg^{2+} transport.

CorA magnesium transporters

The genetic locus for the CorA transporter of *Escherichia coli* was identified by Silver and colleagues in 1969 (Silver 1969) and some transport properties described. CorA from *S.* Typhimurium was the first Mg^{2+} transport system to be cloned in 1985 (Hmiel *et al.* 1986). Like the subsequently discovered MgtE, it represents a totally novel class of protein with no homology to any other type of transporter or membrane protein.

Genomics

CorA is ubiquitous within both the Bacteria and the Archaea. Among the many microbial genomes now available, CorA is absent in only a few species, generally those with the smallest genomes (Kehres *et al.* 1998) where MgtE appears to take its place. CorA's virtually universal distribution indicates that it is the primary Mg^{2+} transporter of both the Bacteria and the Archaea. As these two Kingdoms of life comprise the largest biomass on Earth, CorA is thus the Earth's most abundant Mg^{2+} transporter.

Some species have multiple CorA-like sequences, usually 2 and sometimes 3 (Kehres *et al.* 1998; Smith & Maguire 1995a). Analysis indicates that there are two major branches of the CorA family. One subclass is termed the 'MPEL' group, where MPEL represents a highly conserved sequence between TM domain 2 (TM2) and TM3. This subclass is closely related to the originally cloned *S.* Typhimurium CorA. The second subclass is less coherent, and we have termed them 'CorA-II' sequences. They likely diverged from CorA relatively early in evolution. Preliminary data from our laboratory and from the laboratory of R.L. Smith suggest that CorA-II proteins are efflux systems although the identity of the ion(s) transported physiologically is not known. They do not transport Mg^{2+}.

There is a surprising lack of correlation between CorA molecular phylogeny and the generally accepted underlying organismal phylogeny derived from 16S rRNA sequence (Kehres *et al.* 1998). This could imply that there have been multiple lateral transfers of *corA* between organisms, even possibly between Archaea and Bacterial as has recently been suggested for catalase-peroxidase genes (Faguy & Doolittle 2000). The lack of phylogenetic concordance suggests that CorA is a very ancient protein in prokaryotes, predating the divergence of Bacteria and Archaea.

Faint homology to CorA has been claimed for some yeast proteins (Bui *et al.* 1999; MacDiarmid & Gardner 1998) including two aluminum resistance loci ALR1 and ALR2 and a mitochondrial RNA splicing factor, MRS2p. The similarity is weak and is confined to a small portion of the transmembrane domains. No direct evidence for Mg^{2+} transport has been presented although the presence or absence of each of these proteins does cause a change in Mg^{2+} content of the cell or organelle. The phenotype of mutant strains in each of these loci is partially complemented by introduction of the *S.* Typhimurium *corA* gene. Regardless of the function of these putative homologs, the ever-growing amount of eukaryotic DNA sequence available clearly indicates that close homologs of the CorA Mg^{2+} transporter are unlikely to exist in eukaryotes.

Physiology

S. Typhimurium CorA is a constitutive protein whose promoter does not respond to changes in extracellular magnesium concentration or to any other stimuli so far tested (Smith *et al.* 1998b; Tao *et al.* 1998). Nothing is known about transcriptional regulation of CorA from other organisms or about regulation of the CorA-II branch of the family. It is likely that most if not all *corA* genes are constitutively expressed since the CorA Mg^{2+} transporter can be considered a basic 'housekeeping' gene, essential for cell function.

Transport parameters have been established for CorA systems from the Bacteria *S.* Typhimurium, *E. coli*, and *Haemophilus influenzae*, and the *Archaeon Methanococcus jannaschii*. The bacterial CorA's all mediate the influx of Mg^{2+}, Co^{2+}, and Ni^{2+} (Hmiel *et al.* 1986; Snavely *et al.* 1989). CorA exhibits an affinity for Mg^{2+} of 15–20 μM. The affinities for Co^{2+} and Ni^{2+} are 20–40 and 200–400 μM, respectively, clearly within the toxic range for organisms such as *S.* Typhimurium. Thus uptake of Co^{2+} and Ni^{2+} is unlikely to be important physiologically although since the requirement of a cell for these two cations is very small, their 'leakage' through CorA might provide some or all of the cell's requirements under some environmental conditions. The maximal rate of Mg^{2+} uptake by CorA is > 1 nmol min^{-1} 10^8 $cells^{-1}$. Given the size of *S.* Typhimurium and its cellular content of Mg^{2+}, this rate would double cell Mg^{2+} in less than 60 sec if influx were unabated. Uptake of $^{28}Mg^{2+}$ at 37 °C is linear for only 15–20 sec after which it plateaus; in contrast, uptake of Co^{2+} and Ni^{2+} are not markedly slower than that of

Mg^{2+}, but the rate remains linear for at least 15 min. These observations imply the conclusion that the cell can control the function of the CorA protein itself or that CorA is a channel that desensitizes during Mg^{2+} uptake. The sequence of CorA contains no recognizable ATP binding site and since transport via CorA is sensitive to membrane potential, the likely mechanism of Mg^{2+} uptake is either through CorA functioning as a Mg^{2+} channel driven by the inward electrochemical Mg^{2+} potential or as a Mg^{2+}/H^{+} antiporter driven by the overall electrochemical gradient.

The *S.* Typhimurium CorA can also mediate Mg^{2+} efflux but cannot efflux Ni^{2+} or Co^{2+}. The significance of this activity is not known as it only occurs under conditions unlikely to occur outside of the laboratory except in sea water, i.e., a very high extracellular Mg^{2+} concentration (Gibson *et al.* 1991; Snavely *et al.* 1989). In the absence of a functional CorA protein, no Mg^{2+} efflux can be detected under a variety of conditions, thus demonstrating both that CorA is the only apparent Mg^{2+} efflux protein of *S.* Typhimurium and that Mg^{2+} efflux is not essential to cell viability. Efflux via CorA requires extracellular Mg^{2+} concentrations in the millimolar range, two orders of magnitude above the K_m for influx. Thus the efflux does not represent a Mg^{2+}-Mg^{2+} exchange process since the influx rate is already saturated before any significant amount of efflux can be detected.

M. jannaschii is an Archaeal microbe isolated from deep sea vents (Jones *et al.* 1983). It contains an apparent corA gene, the same length as that of *S.* Typhimurium, but only 12% identical to it in the soluble, periplasmic domain and 19% identical in the membrane domain. Strikingly, when this distantly related protein is expressed in *S.* Typhimurium, it exhibits transport properties virtually identical to those of *S.* Typhimurium CorA (Smith *et al.* 1998a). Normal conditions for this Archaeal protein would be 85 °C, 250 atmospheres of pressure, a greatly different membrane environment and an extracellular environment of sea water which contains 55 mM Mg^{2+} (Jones *et al.* 1983). Yet the *M. jannaschii* CorA exhibits an identical affinity for Mg^{2+} and other divalent cations compared to those for the *S.* Typhimurium CorA. Its rate of influx is somewhat less than that of CorA, although this is certainly in part because of its insertion into a quite different lipid environment. As would be expected for a protein from a thermophile, it is considerably more stable to temperature, retaining maximal activity to at least 65 °C. Since such a distant homolog can function under such different, it seems likely that all members of the CorA branch of this protein family are capable of transporting Mg^{2+} into cells.

This laboratory has recently identified selective and potent inhibitors of the CorA family of Mg^{2+} transporters (Kucharski *et al.* 2000). The Mg^{2+} cation binds 6 waters, forming an inner, relatively tightly bound hydration shell of almost 5 Å in diameter, far larger than for other divalent cations. Cations of similar size but with much greater stability exist. Certain transition metals who prefer a hexacoordinate liganded state including Co, Ru, and Ni can *covalently* bond to a variety of ligands which replace the waters of hydration. The most common of these ligands are the ene-amines and ammines. Thus, Co(III)hexaammine consists of a trivalent Co atom covalently bonded to six ammines (NH_3) with a geometry and size identical to a hydrated Mg^{2+} cation (Basolo & Pearson 1967; Meek & Ibers 1970). Such substituted cations can mimic the action of hydrated Mg^{2+} in many enzyme active sites (Black & Cowan 1997; Cowan 1993; Huang & Cowan 1994). We tested Co(III)-, Ru(II)-, and Ru(III)-hexaammines and found that they were potent inhibitors of CorA but not of other Mg^{2+} transporters and other Mg^{2+}-binding proteins, whether from *S.* Typhimurium or *M. jannaschii*. The hexaammines that inhibit have affinities for CorA about 3–10-fold greater than Mg^{2+} itself. Other cation hexaammines and some related complexed cations did not inhibit CorA. Structural data shows that those compounds that inhibit are all roughly 5 Å in diameter or slightly less, but that larger complexed cations could not inhibit at all. We interpret the data to indicate that the initial binding site for Mg^{2+} in the periplasmic domain of CorA is at least 5 Å in diameter and binds a fully hydrated cation. Thus, unlike many binding sites for Mg^{2+} and other cations, CorA interacts initially with the fully hydrated cation and then during transport presumably strips the hydration shell. Finally, the ability of the cation hexaammines to inhibit *M. jannaschii* CorA with affinities identical to those for the *S.* Typhimurium CorA implies, despite only 12% identity in the soluble, periplasmic domain, that the structure of the Archaeal and Bacterial CorA's are very similar.

Structure

CorA's have a variably conserved N-terminal hydrophilic domain of about 240 amino acids followed by a fairly well conserved hydrophobic domain. The large majority of CorA's have only a short soluble

sequence of 6 amino acids at the C-terminus. The membrane domain of *S.* Typhimurium CorA has been shown experimentally to consist of 3 TM domains, separated by very short loops (Smith *et al.* 1993b). This places the C-terminus in the cytosol and the N-terminus in the periplasm. This topology is highly unusual if not unique among membrane transporters. CorA is thus a two-domain protein: a large N-terminal periplasmic domain and a smaller C-terminal membrane domain. Three transmembrane segments suggests that CorA must function as an oligomer. Genetic data indicate that the CorA protein alone is sufficient for transport, and preliminary data suggest that CorA is pentameric (M.A. Szegedy, L.M. Kucharski and M.E. Maguire, unpublished).

At over 25 kDa (about 240 amino acids in length) the soluble domain of CorA is the largest known N-terminal sequence to be translocated across the plasma membrane without a signal peptide. It contains an unusually high percentage of charged amino acids and is predicted to have a pI of about 4. A truncated protein consisting of the entire soluble domain has been purified using a 6× His tag. The purified protein appears to retain structure, and, as predicted by various computer algorithms, is virtually all α-helix as measured by circular dichroism. Crystallization efforts are in progress.

Only one CorA homolog of over 50 now known contains even a single charge in TM2 and TM3, though the presence of multiple hydroxyl-bearing residues renders both of these TM domains amphipathic. In TM1 of the *S.* Typhimurium CorA there is a single Glu residue; it is not conserved in other CorA's. This single charged Glu residue in TM1 can be mutated to alanine without detectable effect on CorA transport (Smith *et al.* 1998c). Thus, the *S.* Typhimurium CorA and presumably other CorA's are able to transport Mg^{2+} across the membrane bilayer without electrostatic interactions. This lack of negatively charged residues within the membrane domain sets CorA quite apart from virtually all other cation transporters, most of which require multiple Glu and Asp residues within the membrane domain for transport. In contrast, CorA mediates the influx of the most charge dense of the biological cations without use of a single negatively charged residue within the membrane.

What residues within the membrane are involved? Site-directed mutagenesis (Smith *et al.* 1998c; Szegedy & Maguire 1999) suggests that three conserved residues on a single face of the α-helix of both TM2 and TM3 are important to transport and seem to participate directly in substrate binding. These mutagenesis studies also indicate that all the residues in a highly conserved 'YGMNF' sequence near the C-terminal end of TM2 are essential for transport. This region appears to play a role in maintaining a proper critical loop conformation between TM2 and TM3 rather than participating in direct substrate binding.

As noted above, many, perhaps most, Bacteria and Archaea carry paralogs of CorA. As noted above, CorA Mg^{2+} transporters have no charged residues in TM2 or TM3. The number of charged residues in TM1 varies from 0 to 4 in *M. jannaschii*. In contrast, the CorA-II class, which phylogenetically branch from the CorA transporters all carry multiple (up to 9) charged residues in the sequence corresponding to TM1 in the *S.* Typhimurium CorA. Clearly such a stretch of charged amino acids would not be stable within the hydrophobic membrane environment and suggests that the CorA-II class of transporters possess only 2 TM domains, corresponding to TM2 and TM3 in the *S.* Typhimurium CorA. This prediction has been tested by topology mapping and found to be correct, the CorA-II family has only 2 TM segments (R.L. Smith, personal communication). The presence of only 2 TM domains in the CorA-II family has the interesting corollary that the N-terminal soluble domain, rather than being in the periplasm, would be in the cytosol. It would be of interest to determine whether it has the same 3-dimensional structure as the periplasmic domain of the CorA family.

Physiology

Because CorA is expressed constitutively and is the cell's primary transporter for an essential cation, *corA* could be referred to as a housekeeping gene. The usual implication of such a designation is that the gene plays no other role than to supply it's product. Yet this does not seem to be the case for *corA*. Mutation in the *corA* gene do not elicit any significant growth phenotype for *S.* Typhimurium when grown in the laboratory on either rich or minimal medium. As discussed below, the organism has 2 other Mg^{2+} transporters, MgtA and MgtB with which to obtain Mg^{2+} which can supply sufficient Mg^{2+} for even rapid growth in rich media. Nonetheless, *corA* appears to influence a number of functions within the cell. Specifically, *S.* Typhimurium *corA* mutants are markedly defective for invasion of and survival and proliferation within macrophages (J. Lin, L.M. Kucharski and M.E. Maguire, submitted for

publication). Moreover, the virulence of a *corA* mutant strain is significantly attenuated in the mouse infection model. The basis for this decreased virulence is not yet known, but we have determined that a number, though not all, of genes regulated by the Mg^{2+} receptor PhoQ and its response regulator (transcription factor) PhoP are derepressed. Although the PhoPQ signal transduction system is still functional and changes in extracellular Mg^{2+} concentration can alter gene transcription, the transcription of some PhoPQ-regulated genes increases markedly, independent of the extracellular Mg^{2+} concentration. This suggests that there is some alteration in intracellular Mg^{2+} homeostasis that has widespread effects on cell function. The lack of a growth phenotype when tested on defined media simply underscores the idea that the laboratory is not the normal environment for *S.* Typhimurium. The basis for this alteration in gene transcription and its downstream effects are under investigation.

MgtA/MgtB Mg^{2+} transporters

Genomics

The MgtA/B class of Mg^{2+} transporters are not a well-defined set of proteins from a phylogenetic standpoint. *S.* Typhimurium MgtA and MgtB are siblings. Their transport properties are not strikingly different. *mgtA* is the endogenous *Salmonella* P-type ATPase Mg^{2+} transporter while *mgtB* is a foreign gene. It is part of the *mgtCB* operon (see below) carried on *Salmonella* Pathogenicity Island 3, an insertion of unknown origin acquired by one branch of the *Salmonella enterica* family via horizontal transfer and which carries a number of genes important for virulence (Blanc-Potard & Groisman 1997; Snavely *et al.* 1991b; Tao *et al.* 1995). The phylogenetic distributions of *mgtA* and *mgtB* differ markedly as would be expected for an endogenous versus an acquired genetic element. Southern blot experiments and available genomic sequences suggest that MgtA may be widespread in the Gram-negative Enterobacteriaciae. Although Mg^{2+} transport has not been demonstrated, apparently close homologs exist in Gram-positive bacteria and in some but by no means all extremophiles. Despite its apparent occurrence in only distantly related organisms, MgtA is far from ubiquitous like CorA. Indeed, the large majority of sequenced Bacterial and Archaeal genomes do not contain a P-type ATPase closely related to either MgtA or MgtB. In contrast to MgtA, MgtB was only found in three of nine Enterobacterial species tested (Blanc-Potard & Groisman 1997). The significance of this varied and limited distribution of the Mg^{2+} transport P-type ATPases is unknown.

Structure

Based on sequence alignments, the MgtA/B class of Mg^{2+} transporter belong to the P-type ATPase superfamily (Carafoli & Brini 2000; Scarborough 1999). They are most similar to the yeast H^+-ATPases and the mammalian Ca^{2+}- ATPases of the sarco(endo)plasmic reticulum. Interestingly, there are few if any examples of bacterial P-type ATPases that transport Ca^{2+}, suggesting the possibility that P-type Mg^{2+} transport ATPases gave rise in evolution to eukaryotic Ca^{2+}-ATPases.

MgtB was the first P-type ATPase, bacterial or mammalian, whose complete membrane topology was unequivocally determined (Smith *et al.* 1993a). As had been predicted for the large majority of eukaryotic P-type ATPases, MgtB was shown to have ten TM domains with both its N- and C-termini in the cytoplasm. This experimental data has been confirmed by subsequent studies on other P-type ATPases and especially by the recent crystal structure of the sarco(endo)plasmic Ca^{2+}-ATPase (Toyoshima *et al.* 2000; Zhang *et al.* 1998). The presence of 10 TM domains in a prokaryotic P-type ATPase is unusual. The large majority of other prokaryotic P-type ATPases are about 300 amino acids shorter than MgtA/MgtB and lack the 4 C-terminal TM domains. With the exception of the Kdp K^+-ATPase of *E. coli*, MgtA and MgtB are also different from all other P-type ATPases, prokaryotic or eukaryotic, in that their apparent primary function is to mediate the *influx* of a cation (Maguire *et al.* 1992). Other P-type ATPases mediate the *efflux* of their primary substrate, only occasionally mediating the influx of a secondary substrate, e.g., the Na^+, K^+-ATPase. The mechanistic basis for this difference is not apparent.

The mammalian Na^+, K^+-and Ca^{2+}- P-type ATPases have been shown to possess six highly conserved negatively charged residues within the membrane domains that are apparently responsible for binding cation during membrane passage (Clarke *et al.* 1989; MacLennan *et al.* 1997). These residues are also conserved in MgtA and MgtB; however, mutagenesis of *S.* Typhimurium MgtB suggests there are puzzling differences in addition to intriguing similarities. Indeed, only two of the six conserved residues appear to have

any role whatsoever in transport (D.G. Kehres, L.M. Kucharski and M.E. Maguire, unpublished) and one of those two residues is uncharged.

Physiology

The MgtA/B class of Mg^{2+} transporters are fundamentally different from other known Mg^{2+} transporters in that their expression is regulated by a specific signal transduction system while the CorA and MgtE classes are all apparently constitutively expressed. *S.* Typhimurium MgtA and MgtB are repressed as the concentration of Mg^{2+} in the growth medium increases; both are induced to an enormous extent (at least 1000-fold) upon Mg^{2+} deprivation (Tao *et al.* 1995, 1998). This induction is mediated by the PhoPQ two-component regulatory system (Groisman *et al.* 1989; Miller *et al.* 1989, 1991). PhoPQ regulated genes are induced (technically, derepressed) upon invasion of macrophage or epithelial cells, and MgtA and MgtB among those genes induced upon *S.* Typhimurium invasion of a mammalian host cell. Many of these PhoPQ-regulated genes are important for virulence. Nonetheless, despite this regulation while within the macrophage, neither MgtA nor MgtB appear to have a major role in *S.* Typhimurium pathogenesis (Blanc-Potard & Groisman 1997). For a more detailed discussion of the regulation of the *mgtA* and *mgtB* genetic loci see reference (Smith & Maguire 1998).

MgtA and MgtB both transport Mg^{2+} with K_m's of 5–20 μM essentially equivalent to that of CorA. Their true V_{max}'s are hard to measure because of their regulation (Snavely *et al.* 1989; Tao *et al.* 1995, 1998). The apparent V_{max} of each transporter is perhaps 10- to 100-fold lower than the 'channel'-like throughput of CorA. The two proteins differ very slightly in the spectrum of cations that inhibit each and in pH and temperature dependence. For example, MgtB is extremely temperature sensitive, being fully active at 37 °C but completely inactive in terms of transport at 20 °C whereas MgtA exhibits a normal response to changes in temperature, being slightly active even at 4 °C. No physiological basis for these differences is yet apparent.

Several puzzles remain regarding the actual physiological role of MgtA/B transporters. With a K_m similar to that of the primary CorA Mg^{2+} uptake system, they apparently do not fulfill the classic role of a second transporter for a substrate, that of a scavenger system expressed only to transport an important nutrient in less than optimal environments. They mediate cation influx, but their homologs primarily efflux ion. Perhaps the Mg^{2+} transport P-type ATPases efflux some undetermined substrate, and Mg^{2+} is simply a counterion. Otherwise there is no obvious physiological reason to use ATP to mediate the *influx* of Mg^{2+} since there is a steep electrochemical gradient driving Mg^{2+} into the cell. Perhaps Mg^{2+} uptake is not the primary role of the MgtA/B class of proteins in Bacteria? A hint that *S.* Typhimurium MgtB may perform some additional function comes from the phenotype of a E337A mutant. When transformed into the Mg^{2+} transport deficient *S.* typhimurium strain MM281, this mutant will not support growth on LB agar plates in the absence of 100 mM magnesium supplementation, yet it transports Mg^{2+} with kinetics indistinguishable from the wild type protein (D.G. Kehres, L. Kucharski and M.E. Maguire, unpublished). Other mutants of MgtB, even some with very low transport capacity, fully complement the growth requirement for supplemental Mg^{2+}. Could the E337A mutation be compromising the export half of some bi-directional transport cycle?

Phylogenetically, Mg^{2+} transport P-type ATPases are relatively sparse. The majority of Bacteria and Archaea with extensive or complete genomic sequence possess only *corA*. A substantial minority of about one-third also have an apparent *mgtE* homolog in addition to *corA*. Those few microbes that do not carry a *corA* gene carry *mgtE*. In contrast, only a small number of Bacteria and no Archaea possess a close homolog of MgtA or MgtB. No microbe possesses only a P-type ATPase as its sole Mg^{2+} transporter (assuming of course there is not an as yet identified fourth class of Mg^{2+} transporter). This suggests that most cells have no compelling physiological need for such a transport system. Conversely, since microbes generally do not carry genes they do not need, it likewise suggests that those cells that possess a Mg^{2+} transport P-type ATPase have a specific need, a physiological niche that such an enzyme helps it fill.

The MgtC protein

In *S.* Typhimurium, the *mgtB* gene is the second gene of a 3 gene operon. While other prokaryotic P-type ATPases reside in an operon structure, the *mgtCB-Orf X* operon is the only known to date in which the other genes are apparently unrelated, functionally or by sequence, to the transporter. The operon comprises

the right end of *Salmonella* Pathogenicity Island 3 (SPI-3) but interestingly has normal GC content and normal codon usage for *Salmonellae* (Blanc-Potard *et al.* 1999; Blanc-Potard & Groisman 1997; Snavely *et al.* 1991b). Thus, it is apparently a separate addition to SPI-3 acquired presumably from a source different than that (unknown) from which the remainder of the genes in SPI-3 were derived. Orf X, the third gene of this operon is of unknown function and has no homology to other known genes.

The first gene of this operon, *mgtC*, is of some relevance. Since it is part of the same operon as *mgtB*, it is also subject to regulation by extracellular Mg^{2+} via the PhoPQ two-component system (Snavely *et al.* 1991a; Tao *et al.* 1995). A few other Bacterial species have apparent homologs of MgtC, but their phylogenetic distribution does not allow for any conclusions about its origin, and the function of these homologs is not known (Blanc-Potard & Groisman 1997). The expressed protein is about 23 kDa in size and is very hydrophobic with 5 or 6 TM segments. Extensive genetic and transport work has shown that MgtC does not form a β-subunit for the MgtB P-type ATPase, similar to the β-subunits of many eukaryotic P-type ATPases (Tao *et al.* 1995, 1998).

Using intraperitoneal injection of *S.* Typhimurium into mice, Blanc-Potard and Groisman (1997) showed that *mgtC* is an essential virulence gene for *S.* Typhimurium and suggested that it may be a fourth Mg^{2+} transporter in *S.* Typhimurium. They also demonstrated that the *mgtC* homolog of *Mycobacterium tuberculosis* has a similar phenotype (Buchmeier *et al.* 2000). However, we subsequently showed that expression of *mgtC* alone in the Mg^{2+} transport deficient MM281 strain of *S.* Typhimurium does not give detectable Mg^{2+} transport and does not relieve the requirement for Mg^{2+} supplementation in the growth medium (Moncrief & Maguire 1998), although the latter is slightly reduced by such expression. This suggests that MgtC is not a Mg^{2+} transporter. Curiously, although Mg^{2+} deprivation markedly and rapidly induces transcription of both *mgtC* and *mgtB*, only MgtB protein can be detected for several hours after transcription of the operon. The reason for this marked delay in MgtC translation and indeed its function remain a mystery as does its function in virulence of *S.* Typhimurium.

Conclusions and perspective

The properties of prokaryotic magnesium transporters reflect the unique chemistry of their substrate. (*i*) CorA has no homology to other known transporters and mediates influx of Mg^{2+} without use of charged residues in the membrane domain, unlike other cation transporters. (*ii*) Although the MgtA/B class of Mg^{2+} transporters are P-type ATPases, they mediate influx of cation rather than efflux, are phylogenetically much closer to eukaryotic than prokaryotic P-type ATPases, and do not appear to transport cation using the same intramembrane residues as transporters of their class. (*iii*) Finally, the MgtE class of Mg^{2+} transporter, like CorA, has no homology to other known transport proteins. These results support our hypothesis that Mg^{2+} transporters will most likely be unique transport proteins or at least highly unusual members of known classes of transport proteins (Grubbs & Maguire 1987; Maguire 1990).

What about the relationship of these prokaryotic Mg^{2+} transporters to eukaryotic systems? There are no clear homologs of either CorA or MgtE in any eukaryote, although recent genomic sequence data suggests that some distant MgtE homologs may exist. Of the multitudinous P-type ATPases described in eukaryotes, none have been shown to transport Mg^{2+}. Given the extent of microbial genomic sequence now available from a wide phylogenetic distribution and the recognition of a CorA or MgtE homolog in every organism for which extensive genomic sequence is available, it is highly unlikely that another widely distributed class of Mg^{2+} transporter exists among the prokaryotes. Thus, the Eukarya appear to have evolved quite different Mg^{2+} transport systems than the Bacteria and Archaea. Why this has occurred is completely unknown.

The properties of the prokaryotic Mg^{2+} transporters are obviously not yet completely defined. Energetics and molecular details of MgtE are completely unstudied. With MgtA and MgtB, despite delineation of basic transport properties and definition of some important intramembrane residues, the mechanism by which Mg^{2+} *influx* occurs is not known, nor is it clear whether Mg^{2+} is a primary or secondary substrate. With respect to CorA, extensive mutagenesis has clearly defined those intramembrane residues necessary for transport, but that information has only opened more questions since the movement of the charge dense Mg^{2+} ion through the bilayer without benefit of electrostatic interactions is a mechanistic

mystery. Moreover, our recent discovery that CorA is involved in virulence has shown that Mg^{2+} homeostasis is part of a complex network of cellular controls having widespread effects on cell function. It is abundantly clear that much is left to be discovered in the areas of Mg^{2+} transport and homeostasis.

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References

Basolo F, Pearson RG. 1967 *Mechanisms of Inorganic Reactions: A study of metal complexes in solution*, 2 ed. New York: John Wiley.

Black CB, Cowan JA. 1997 Inert chromium and cobalt complexes as probes of magnesium-dependent enzymes. Evaluation of the mechanistic role of the essential metal cofactor in *Escherichia coli* exonuclease III. *Eur J Biochem* **243**, 684–689.

Blanc-Potard AB, Groisman EA. 1997 The *Salmonella selC* locus contains a pathogenicity island mediating intramacrophage survival. *EMBO J* **16**, 5376–5385.

Blanc-Potard AB, Solomon F, Kayser J, Groisman EA. 1999 The SPI-3 pathogenicity island of *Salmonella enterica. J Bacteriol* **181**, 998–1004.

Buchmeier N, Blanc-Potard A, Ehrt S, Piddington D, Riley L, Groisman EA. 2000 A parallel intraphagosomal survival strategy shared by *Mycobacterium tuberculosis* and *Salmonella enterica. Mol Microbiol* **35**, 1375–1382.

Bui DM, Gregan J, Jarosch E, Ragnini A, Schweyen RJ. 1999 The Bacterial Magnesium Transporter CorA Can Functionally Substitute for Its Putative Homologue Mrs2p in the Yeast Inner Mitochondrial Membrane. *J Biol Chem* **274**, 20438–20443.

Carafoli E, Brini M. 2000 Calcium pumps: Structural basis for and mechanism of calcium transmembrane transport. *Curr Opin Chem Biol* **4**, 152–161.

Clarke DM, Loo TW, Inesi G, MacLennan DH. 1989 Location of high affinity Ca^{2+}-binding sites within the predicted transmembrane domain of the sarcoplasmic reticulum Ca^{2+}-ATPase. *Nature* **339**, 476–478.

Cowan JA. 1991 Metallobiochemistry of Magnesium. Coordination Complexes with Biological Substrates: Site Specificity, Kinetics and Thermodynamics of Binding, and Implications for Activity. *Inorgan Chem* **30**, 2740–2747.

Cowan JA. 1993 Metallobiochemistry of RNA. $Co(NH_3)_6^{3+}$ as a probe for Mg^{2+}(aq) binding sites. *J Inorg Biochem* **49**, 171–175.

Cowan JA. 1998 Metal activation of enzymes in nucleic acid biochemistry. *Chem Rev* **98**, 1067–1087.

Diebler H, Eigen M, Ilgenfritz G, Maass G, Winkler R. 1969 Kinetics and mechanism of reactions of main group metal ions with biological carriers. *Pure Appl Chem* **20**, 93–115.

Faguy DM, Doolittle RF. 2000 Horizontal transfer of catalase-peroxidase genes between Archaea and pathogenic bacteria. *Trends Genet* **16**, 196–197.

Gibson MM, Bagga DA, Miller CG, Maguire ME. 1991 Magnesium transport in *Salmonella typhimurium*: The influence of new mutations conferring Co^{2+} resistance on the CorA Mg^{2+} transport system. *Mol Microbiol* **5**, 2753–2762.

Groisman EA, Chiao E, Lipps CJ, Heffron F. 1989 *Salmonella typhimurium phoP* virulence gene is a transcriptional regulator. *Proc Natl Acad Sci USA* **86**, 7077–7081.

Grubbs RD, Maguire ME. 1987 Magnesium as a regulatory cation: Criteria and evaluation. *Magnesium* **6**, 113–127.

Hmiel SP, Snavely MD, Florer JB, Maguire ME, Miller CG. 1989 Magnesium transport in Salmonella typhimurium: Genetic characterization and cloning of three magnesium transport loci. *J Bacteriol* **171**, 4742–4751.

Hmiel SP, Snavely MD, Miller CG, Maguire ME. 1986 Magnesium transport in *Salmonella typhimurium*: Characterization of magnesium influx and cloning of a transport gene. *J Bacteriol* **168**, 1444–1450.

Huang H-W, Cowan JA. 1994 Metallobiochemistry of the magnesium ion–Characterization of the essential metal-binding site in *Escherichia coli* ribonuclease. *Eur J Biochem* **219**, 253–260.

Jones WJ, Leigh JA, Mayer F, Woese CR, Wolfe RS. 1983 *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. *Arch Microbiol* **136**, 254–261.

Jou R, Cowan JA. 1991 Ribonuclease H activation by inert transition-metal complexes. Mechanistic probes for metallocofactors: Insights on the metallobiochemistry of divalent magnesium ion. *J Am Chem Soc* **113**, 6685–6686.

Kehres DG, Lawyer CH, Maguire ME. 1998 The CorA magnesium transporter gene family. *Microb Compar Genomics* **43**, 151–169.

Kucharski LM, Lubbe WJ, Maguire ME. 2000 Cation hexaammines are selective and potent inhibitors of the CorA magnesium transport system. *J Biol Chem* **275**, 16767–16773.

MacDiarmid CW, Gardner RC. 1998 Overexpression of the *Saccharomyces cerevisiae* Magnesium Transport System Confers Resistance to Aluminum Ion. *J Biol Chem* **273**, 1727–1732.

MacLennan DH, Rice WJ, Green NM. 1997 The mechanism of Ca^{2+} transport by sarco(endo)plasmic reticulum Ca^{2+}-ATPases. *J Biol Chem* **272**, 28815–28818.

Maguire ME. 1990 Magnesium: A regulated and regulatory cation. *Metal Ions Biol* **26**, 135–153.

Maguire ME, Snavely MD, Leizman JB, Gura S, Bagga D, Tao T, Smith DL. 1992 Mg^{2+} transporting P-type ATPases of *Salmonella typhimurium*. Wrong way, wrong place enzymes. *Ann NY Acad Sci* **671**, 244–256.

Martin RB. 1990 Bioinorganic Chemistry of Magnesium. *Metal Ions Biol* **26**, 1–13.

Meek DW, Ibers JA. 1970 The Crystal Structure of Hexaamminecobalt(III) tetrachlorozincate(II) chloride, $[Co(NH_3)_6][ZnCl_4]Cl$. *Inorgan Chem* **9**, 465–470.

Merino S, Gavin R, Altarriba M, Izquierdo L, Maguire ME, Tomas JM. 2001 The MgtE Mg^{2+} transport protein is involved in *Aeromonas hydrophila* adherence. *FEMS Microbiol Lett* **198**, 189–195.

Miller SI. 1991 PhoP/PhoQ: Macrophage-specific modulators of *Salmonella* virulence. *Mol Microbiol* **5**, 2073–2078.

Miller SI, Kukral AM, Mekalanos JJ. 1989 A two-component regulatory system (*phoP phoQ*) controls *Salmonella typhimurium* virulence. *Proc Natl Acad Sci USA* **86**, 5054–5058.

Moncrief MBC, Maguire ME. 1998 Magnesium and the role of *mgtC* in *Salmonella typhimurium. Infect Immun* **66**, 3802–3809.

Romani A, Scarpa A. 2000 Regulation of cellular magnesium. *Front Biosci* **5**, D720–D734.

Scarborough GA. 1999 Structure and function of the P-type ATPases. *Curr Opin Cell Biol* **11**, 517–522.

Silver S. 1969 Active transport of magnesium in *Escherichia coli. Proc Natl Acad Sci USA* **62**, 764–771.

Smith DL, Tao T, Maguire ME. 1993a Membrane topology of a P-type ATPase: The MgtB Mg^{2+} transport protein of *Salmonella typhimurium. J Biol Chem* **268**, 22469–22479.

Smith RL, Banks JL, Snavely MD, Maguire ME. 1993b Sequence and topology of the CorA magnesium transport systems of *Salmonella typhimurium* and *Escherichia coli*. Identification of a new class of transport protein. *J Biol Chem* **268**, 14071–14080.

Smith RL, Gottlieb E, Kucharski LM, Maguire ME. 1998a Functional similarity between Archaeal and Bacterial CorA magnesium transporters. *J Bacteriol* **180**, 2788–2791.

Smith RL, Kaczmarek ML, Kucharski LM, Maguire ME. 1998b Magnesium transport in *Salmonella typhimurium*: Induction of MgtA and MgtCB expression during invasion of epithelial and macrophage cells. *Microbiology* **144**, 1835–1843.

Smith RL, Maguire ME. 1995a Distribution of the CorA Mg^{2+} transport system in Gram-negative bacteria. *J Bacteriol* **177**, 1638–1640.

Smith RL, Maguire ME. 1995b Genetics and molecular biology of magnesium transport systems. In: Cowan JA, ed. *The biological chemistry of magnesium*. London: VCH Publishing Co.; 211–234.

Smith RL, Maguire ME. 1998 Microbial magnesium transport: Unusual transporters searching for identity. *Mol Microbiol* **28**, 217–226.

Smith RL, Szegedy MA, Walker C, Wiet RM, Redpath A, Kaczmarek ML, Kucharski LM, Maguire ME. 1998c The CorA magnesium transport protein of *Salmonella typhimurium*: Mutagenesis of conserved residues in the third transmembrane segment identifies part of a Mg^{2+} pore. *J Biol Chem* **273**, 28663–28669.

Smith RL, Thompson LJ, Maguire ME. 1995 Cloning and characterization of *mgtE*, a putative new class of Mg^{2+} transporter from *Bacillus firmus* OF4. *J Bacteriol* **177**, 1233–1238.

Snavely MD, Florer JB, Miller CG, Maguire ME. 1989 Magnesium transport in *Salmonella typhimurium*: $^{28}Mg^{2+}$ transport by the CorA, MgtA, and MgtB systems. *J Bacteriol* **171**, 4761–4766.

Snavely MD, Gravina SA, Cheung TT, Miller CG, Maguire ME. 1991a Magnesium transport in *Salmonella typhimurium*: Regulation of *mgtA* and *mgtB* expression. *J Biol Chem* **266**, 824–829.

Snavely MD, Miller CG, Maguire ME. 1991b The *mgtB* Mg^{2+} transport locus of *Salmonella typhimurium* encodes a P-type ATPase. *J Biol Chem* **266**, 815–823.

Suga H, Cowan JA, Szostak JW. 1998 Unusual metal ion catalysis in an acyl-transferase ribozyme. *Biochemistry* **37**, 10118–10125.

Szegedy MA, Maguire ME. 1999 The CorA Mg^{2+} Transport Protein of *Salmonella typhimurium*: Mutagenesis of Conserved Residues in the Second Membrane Domain. *J Biol Chem* **274**, 36973–36979.

Tao T, Grulich PF, Kucharski LM, Smith RL, Maguire ME. 1998 Magnesium transport in *Salmonella typhimurium*: Biphasic time and magnesium dependence of the transcription of the *mgtA* and *mgtCB* loci. *Microbiology* **144**, 655–664.

Tao T, Snavely MD, Farr SG, Maguire ME. 1995 Magnesium transport in *Salmonella typhimurium: mgtA* encodes a P-type ATPase and is regulated by Mg^{2+} in a manner similar to that of the *mgtB* P-type ATPase. *J Bacteriol* **177**, 2654–2662.

Townsend DE, Esenwine AJ, George J, III, Bross D, Maguire ME, Smith RL. 1995 Cloning of the *mgtE* Mg^{2+} transporter from *Providencia stuartii* and the distribution of *mgtE* in the eubacteria. *J Bacteriol* **177**, 5350–5354.

Toyoshima C, Nakasako M, Nomura H, Ogawa H. 2000 Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 Å resolution. *Nature* **405**, 647–655.

Zhang P, Toyoshima C, Yonekura K, Green NM, Stokes DL. 1998 Structure of the calcium pump from sarcoplasmic reticulum at 8 Å resolution. *Nature* **392**, 835–839.