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Magnesium Transporters: Properties, Regulation and Structure

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1. ABSTRACT

The chemistry of Mg^{2+} is unique amongst biological cations, and the properties of Mg^{2+} transport systems reflect this chemistry. Prokaryotes carry three classes of Mg^{2+} transport systems: CorA, MgtA/B and MgtE. CorA and MgtE are widely distributed in both *Eubacteria* and *Archaea*, while the MgtA/B class is found primarily in the *Eubacteria*. Eukaryotic homologs of CorA, although clearly functional as Mg^{2+} transporters, have minimal sequence homology and include the Mrs2p mitochondrial Mg^{2+} channel and the ALR proteins of fungi. MgtE homologs are more recognizable in eukaryotes as the SLC41 class of transporters. The MgtA/B Mg^{2+} transporters belong to the P-type ATPase superfamily, but mediate Mg^{2+} influx down its electrochemical gradient rather than against the gradient as with other P-type ATPases. Their physiological role is not clear. CorA is the only Mg^{2+} transporter whose structure has been solved. It is a homopentamer with two transmembrane domains per monomer, the first of which forms the ion conduction pathway. Mg^{2+} transport involves first the binding of the fully hydrated cation to an extracellular binding loop connecting the transmembrane domains. Passage through the membrane involves no electrostatic interactions, but two cytosolic domains, one carrying extremely high concentrations of positive charge and the other negative charge appear to help control Mg^{2+} flux, in concert with an intracellular Mg^{2+} bound between domains of each monomer. Neither CorA nor MgtE appear to be transcriptionally regulated, implying they are primarily "housekeeping" genes. Nonetheless, mutation of the *corA* gene in *Salmonella enterica* serovar Typhimurium leads to attenuation of virulence and other defects, even though the strain carries two additional Mg^{2+} transporters and the mutant exhibits no Mg^{2+}-dependent growth deficit.

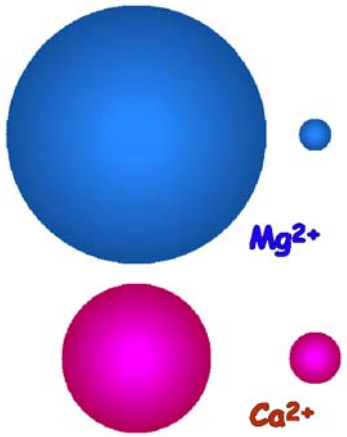

Figure 1. Atomic and hydrated radii of Mg^{2+} and Ca^{2+}. The hydrated (left) and unhydrated/atomic (right) sizes of Mg^{2+} and Ca^{2+} are illustrated to scale (18,54).

2. INTRODUCTION

The basic chemical properties of Mg^{2+} make it unique amongst biological cations (54). Its size, charge density, structure in aqueous solution and aqueous chemistry differ greatly from other monovalent or divalent cations of biological relevance. Mg^{2+} is a very hard Lewis acid. Mg^{2+} readily interacts with carboxylate and phosphate anions in solution or on proteins (4,54). Mg^{2+} binds to other molecules almost exclusively *via* oxygen (14,54) although the relatively few examples of binding *via* nitrogen are of obvious importance, *e.g.*, chlorophyll. Unlike most other cations, Mg^{2+} does not bind *via* sulfur. The ionic radius of Mg^{2+} is among the smallest of all cations while it's hydrated radius is by far the largest of all cations. Since volume is proportional to r^3, this difference is clearest when comparing the ratio of the hydrated volume to the ionic volume of each cation. Ca^{2+} and Mg^{2+} are compared in Figure 1. The hydrated Mg^{2+} cation is approximately 400X larger than its ionic, dehydrated form. In contrast, Na^+ and Ca^{2+} are only 25X larger and the hydrated K^+ cation only 4X larger than the dehydrated forms. Moreover, the exchange rate of solvent waters around the hydrated Mg^{2+} is very slow, 3-4 orders of magnitude slower than for Na^+, K^+ or Ca^{2+} and equivalent to the transition metal cations (18).

These chemical properties will be reflected in the interaction of Mg^{2+} with biological macromolecules. Cation transporters and channels are generally thought to transport a largely dehydrated cation. Thus, a Mg^{2+} transport protein must first recognize the very large hydrated cation which is about the same size as a glucose molecule. Then, the transporter must be able to remove the hydration shell and finally deliver the very small bare ion into the transport pathway through the membrane. While transporters for other cations face the same issue, the challenge for a Mg^{2+} transporter is far greater than for any other cation transport system because of the volume change and relative strength of interaction with the waters of hydration.

Other properties such as coordination number will also play a role in determining Mg^{2+}'s biological functions. Mg^{2+} is invariably hexacoordinate as is Na^+. Ca^{2+} in contrast is relatively promiscuous and can adopt bonding arrangements that are 6, 7, 8 and less often 9 coordinate . The coordination sphere, an octahedron for hexacoordinate cations, is highly flexible with Ca^{2+} but much more constrained with Mg^{2+}. With hexacoordinate Ca^{2+}, determined by crystal structure studies, the distances between the cation and the oxygen atom of proteins and small molecules vary widely, between 2.2 and 2.7 Å. For Mg^{2+} the bond lengths are much shorter, 1.95-2.2 Å. A much greater flexibility in structures formed with Ca^{2+} allows greater angular deviation from the 90° angle expected in an octahedral configuration, up to 40°. By contrast, the variation with Mg^{2+} is usually no more than 5-10%, never greater than 20% (19). The relatively rigid structure of Mg^{2+} complexes probably dictates in significant part the very different roles of Ca^{2+} and Mg^{2+} in biological systems. Ca^{2+} is generally a signaling molecule. It must bind to a variety of proteins, modulating a conformational change in the process. Thus the geometry of the Ca^{2+} binding site must exhibit some flexibility to accommodate Ca^{2+} bound to at least 2 states of the protein in question (1,7,8). In contrast, Mg^{2+} commonly binds water, ATP or another nucleotide triphosphate in the catalytic pocket of an enzyme. The purpose of Mg^{2+} binding to the phosphoryl moieties of ATP in many cases appears to be activation of the phosphate ester toward hydrolysis. When bound to ATP in the active site of an enzyme, one or more waters remain coordinated with the Mg^{2+} and help hold the ATP in a particular conformation and position. For those (non-ATP-dependent) enzymes that bind magnesium as an essential cofactor, the magnesium ion often serves to hold a water molecule in a specific position. This water then helps form a particular structure or participates directly in the enzymatic mechanism, an example of outer sphere complexation, a reaction mechanism exhibited by few metals. Most metals in enzymes or chemical reactions react *via* direct or inner sphere complexation where the metal participates directly in catalysis (4,13,15). Thus the biochemistry of Mg^{2+} is multifaceted and unusual. Because of this unique set of biochemical properties, we postulated several years ago that Mg^{2+} transport proteins would either lack homology to other known transporters or would be highly unusual members of known families (32,52). The three distinct families of prokaryotic Mg^{2+} transport proteins identified and cloned to date, MgtE, CorA, and MgtA/B, amply support this hypothesis.

While the physiology of Mg^{2+} transport processes have been extensively studied in mammalian and other eukaryotic systems, to date there are virtually no studies of putative eukaryotic Mg^{2+} transporters at the molecular level. Several recent reviews of transport studies in eukaryotes have appeared (69-71,76,100). In addition, recent work has suggested that some members of the TRPM ion channels, specifically TRPM6 and TRPM7, may be Mg^{2+} channels (62,64,73,74,95,96) as may Paracellin-1 in gap junctions (6,78). While TRPM6 and TRPM7 are clearly involved in homeostasis of Mg^{2+} as opposed to other cations, actual Mg^{2+} flux through the channels has only been inferred but not yet directly demonstrated.

Table 1. Properties of Prokaryotic Mg^{2+} Transporter Systems [1]

CorA			
Transported Cations	*Cation*	*Affinity (μM)*	*Notes*
	Mg^{2+}	10-15	
	Co^{2+}	20-30	
	Ni^{2+}	200	
Inhibitory Non-Transported Cations	Mn^{2+}	30	Not competitive
	Ca^{2+}	>5000	
Non-inhibitory Cations	Fe^{2+}		
	Fe^{3+}		
	Zn^{2+}		
	Sr^{2+}		
	Ba^{2+}		
MgtA			
Transported Cations	Mg^{2+}	30	
	Ni^{2+}	5	
Inhibitory Non-Transported Cations	Co^{2+}	40	
	Ca^{2+}	300	
	Zn^{2+}	7	
	Mn^{2+}	-	35% maximal inhibition at > 1 mM
MgtB			
Transported Cations	Mg^{2+}	6	
	Ni^{2+}	2	
Inhibitory Non-Transported Cations	Co^{2+}	8	
	Ca^{2+}	>30,000	
	Mn^{2+}	40	
Non-inhibitory Cation	Zn^{2+}		
MgtE			
Transported Cations	Mg^{2+}	50	
	Co^{2+}	80	
Inhibitory Non-Transported Cations	Ni^{2+}	>200	
	Ca^{2+}	50	
	Mn^{2+}	70	
	Zn^{2+}	20	
	Sr^{2+}	80	
Non-inhibitory Cation	Ba^{2+}		

[1] Data are primarily from *S.* Typhimurium for the CorA and MgtA/B transporters (36,84,87) and from *Bacillus firmus* OF4 for the MgtE transporter (86). More limited characterization in other bacterial systems give similar values.

In prokaryotes, the situation is different. This review will outline our current knowledge at the molecular level of the prokaryotic MgtE, CorA, and MgtA/B Mg^{2+} transporters with references to homologs and orthologs of MgtE and CorA in eukaryotes where appropriate. Although quite possibly an additional class of prokaryotic Mg^{2+} transport protein exists, given the number of microbial genomes sequenced and the widespread distribution of CorA and MgtE homologs, these three currently known prokaryotic Mg^{2+} transport classes likely represent the primary Mg^{2+} transport systems in both Bacteria and Archaea.

3. PROKARYOTIC MAGNESIUM TRANSPORTER CLASSES

Magnesium transporters that have been cloned from Bacteria and Archaea fall into three families, based on sequence conservation: CorA, MgtE, and MgtA/B (for previous reviews see 41,84,85). Their basic properties are summarized in Table 1. Only CorA and MgtA/B from *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) have been studied in any depth. In the initial stages, to identify Mg^{2+} transport loci, *S.* Typhimurium was extensively mutagenized and subsequently selected to require high levels of supplemental Mg^{2+} in the medium for growth (35,36,87,88). The resulting strains, MM77 and its derivative MM281, require 10-100 mM Mg^{2+} for growth in minimal media and have no detectable Mg^{2+} influx capacity. Introduction of the gene for any single *S.* Typhimurium Mg^{2+} transporter or of a gene encoding a putative Mg^{2+} transporter from another organism allows growth without supplemental Mg^{2+} and restores transport. Complementation of these Mg^{2+}-transport deficient strains allowed identification of the corA, *mgtA* and *mgtCB* loci of *S.* Typhimurium (32), of CorA homologs from many other species (37,49,81,83) and of the *mgtE* loci from *Providencia stuartii, Bacillus firmus* OF4 and *Aeromonas hydrophila* (58,86,92).

3.1. CorA Magnesium Transporters

A putative Mg^{2+} transporter from *Escherichia coli* was identified by Silver and colleagues in 1969 (77) and its transport properties described. The locus was

named *corA* because the phenotype of the mutant is resistance to growth inhibition by Co^{2+} (***co***balt ***r***esistance). The *corA* gene was cloned from *S.* Typhimurium and the protein initially characterized in 1985. *corA* was the first Mg^{2+} transport locus identified at the molecular level (36). In keeping with the unique biological chemistry of the Mg^{2+} cation (54), CorA has no homology to any other type of transporter or membrane protein.

3.1.1. Transport

Transport parameters have been established for CorA systems from the Bacteria *S.* Typhimurium and *E. coli* and the Archaeon *Methanococcus jannaschii*. CorA mediates the influx of Mg^{2+}, Co^{2+}, and Ni^{2+} (36,87), Mn^{2+} is a relatively poor, non-competitive inhibitor and is not transported. Ca^{2+}, Sr^{2+}, Ba^{2+}, and Zn^{2+} do not inhibit significantly. Despite inference that Fe^{2+} may be transported by CorA (11,30), direct transport assays show that Fe^{2+} is not transported and that neither Fe^{2+} nor Fe^{3+} inhibit CorA (65). The affinity ($K_{0.5}$) [1] of CorA for Mg^{2+} is 15 μM. Affinities for Co^{2+} and Ni^{2+} have been measured only in *S.* Typhimurium and *E. coli* and are 20-40 and 200-400 μM, respectively. This affinity is clearly within the toxic range for these bacteria. Thus uptake of Co^{2+} and Ni^{2+} is unlikely to be of great importance physiologically. Nonetheless, since the requirement of a cell for Co^{2+} and Ni^{2+} is small, their "leakage" through CorA might provide some or all of the cell's requirements under many environmental situations.

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. However, uptake of $^{28}Mg^{2+}$ at 37 °C is linear for only 10-15 sec. Uptake plateaus rapidly within 60 sec. In contrast, while the rate of Co^{2+} or Ni^{2+} uptake is not markedly slower than that of Mg^{2+}, the rate remains linear for 10-15 min at 37°C and up to 30 min at room temperature. These rates imply that CorA is in effect functioning as a cation channel that rapidly desensitizes during Mg^{2+} uptake. The sequence of CorA contains no recognizable ATP binding site, and transport *via* CorA is completely dependent on membrane potential. Although a Mg^{2+}/H^+ antiporter mechanism driven by the overall electrochemical gradient cannot be excluded currently, the most likely mechanism of Mg^{2+} uptake is through CorA functioning as a Mg^{2+} channel driven by the inward electrochemical Mg^{2+} potential, especially given the demonstration of channel-like properties by Schweyen's group for the homologous Mrs2p protein of yeast mitochondria (42).

3.1.2. The CorA protein

The CorA of most Bacteria is a protein of ≈316 amino acids that has a variably conserved N-terminal hydrophilic domain of about 260 amino acids followed by a fairly well conserved hydrophobic membrane domain of 55 or so amino acids. On gel electrophoresis, CorA runs slightly anomalously at 42 kDa rather than the expected 36-37 kDa. The large majority of CorA's have only a short soluble sequence of 6 amino acids at the C-terminus that always contains a total of three lysines or arginines. A few CorA homologs have a longer C-terminal tail of about 25-35 amino acids, but the positively charged residues near the membrane interface are retained.

The topology and oligomeric state of CorA have been problematic. Initial studies using fusion protein constructs with *blaM* and *lacZ* indicated 3 C-terminal transmembrane (TM) segments (80). However, subsequent studies using primarily *phoA* and *lacZ* indicated only 2 TM segments, both at the C-terminus (68,99). The reason for this discrepancy in the fusion protein data is not apparent. Solution of the crystal structure of CorA clearly shows 2 TM segments (49). CorA is thus a two-domain protein: a large N-terminal cytosolic domain and a smaller C-terminal membrane domain. CorA contains a relatively unusually high percentage of charged amino acids in its soluble domain and is predicted to have a pI of about 4. A truncated protein consisting of the entire soluble domain has been purified using a 6X His tag. The purified protein appears to retain structure, and, as predicted by various computer algorithms, is virtually 100% α-helix as measured by circular dichroism. The presence of only 2 TM domains suggests the hypothesis that a single CorA protein is insufficient for transport. Since genetic data indicate that the CorA protein alone is sufficient for transport, this suggests that CorA functions as a homo-oligomer. Crosslinking experiments with cell membranes carrying the intact protein or with purified soluble domain were interpreted to indicate that CorA was most likely tetrameric although some experiments suggested a pentamer (98). Again, solution of the crystal structure of CorA unambiguously demonstrated that CorA is a homopentamer (49).

Of additional note is the ubiquity of the core properties of CorA. For example, *M. jannaschii* is an Archaeal microbe isolated from deep sea vents (36). It contains an apparent *corA* gene, the same length as that of *S.* Typhimurium, but only 22% identical to it.. Strikingly, when this distantly related protein is expressed in *S.* Typhimurium, it exhibits transport properties virtually identical to those of *S.* Typhimurium CorA (81). 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+} (39). Yet the *M. jannaschii* CorA exhibits an identical affinity for Mg^{2+} and other divalent cations, including the hexaammines (see below), 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.

As a second example, again despite very minimal sequence homology, the basic transport parameters of the yeast mitochondrial Mg^{2+} channel, the Mrs2p protein, appear to be very similar to that of Eubacterial and Archaeal CorA transporters, and the yeast and Eubacterial proteins exhibit genetic complementation (10). Thus the basic properties of the CorA transporter seem to be preserved even in CorA homologs from different Kingdoms.

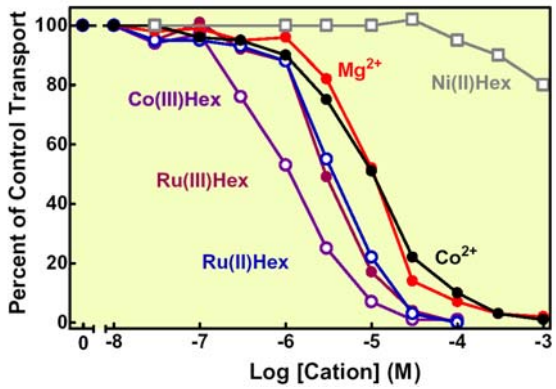

Figure 2. Inhibition of CorA by cation hexaammines. Uptake via CorA was measured in S. Typhimurium using $^{63}Ni^{2+}$ as a surrogate for the unavailable $^{28}Mg^{2+}$. Uptake was measured at the $K_{0.5}$ for Ni^{2+} of 200 μM so that the 50% inhibitory concentration corresponds to twice the actual K_i for the compound. Data are taken from ref. (43) and unpublished data.

3.1.3. Inhibition of CorA

The Mg^{2+} cation is almost invariably hexacoordinate, binding 6 waters in an octahedral conformation to form a tightly bound hydration shell of almost 5Å in diameter, far larger than for other divalent cations. Cations of cobalt, nickel and ruthenium among other transition metals also prefer a hexacoordinate liganded state. However, because of their *d* electron shell, transition metal cations 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 (trichloride) consists of a trivalent Co cation covalently bonded to six ammines (NH_3) with a geometry and size identical to a hydrated Mg^{2+} cation (2,56). Such substituted cations can mimic the action of hydrated Mg^{2+} in many enzyme active sites (3,13,37). Co(III)-, Ru(II)-, and Ru(III)-hexaammines are potent inhibitors of CorA from the Eubacterium *S.* Typhimurium or the Archeon *M. jannaschii* but are not transported (Figure 2). These compounds do not inhibit other Mg^{2+} transporters and Mg^{2+}-binding proteins (43). CorA exhibits an affinity for the hexaammines about 3-10-fold greater than for Mg^{2+} itself. Other cation hexaammines such as Ni(II)hexaammine and some related complex cations do not inhibit CorA. Structural data shows that those compounds that inhibit are all roughly 5 Å in diameter or slightly less. In contrast, Ni(II)hexaammine is significantly larger (38,56). Thus, the initial binding site for Mg^{2+} in the small periplasmic domain of CorA (see below) is not only large, around 5Å in diameter, it must bind a fully hydrated Mg^{2+} cation. This is quite unlike enzymatic binding sites for Mg^{2+} or other cations that bind a partially or even completely dehydrated Mg^{2+}. Moreover, the initial binding site does not discriminate between divalent and trivalent metals. Thus the function of the soluble periplasmic domain appears to be to bind a hydrated cation, strip its hydration shell, and present the dehydrated cation to the membrane pore or channel. It is then presumably the channel itself that determines which cations are actually transported. Further, the cation hexaammines inhibit *M. jannaschii* CorA with affinities identical to those for the *S. typhimurium* CorA. Therefore, despite minimal sequence identity the structure of at least the Mg^{2+} binding site in Archaeal and Bacterial CorA's must be similar.

3.1.4. Other properties of CorA

S. Typhimurium CorA is a constitutively expressed protein whose promoter does not respond to changes in extracellular magnesium concentration. The promoter contains no obvious binding sites for known transcription factors. The level of CorA expression does not markedly vary with growth phase or during growth on rich *versus* minimal media (82,90). Nothing is known about transcriptional regulation of CorA from other organisms. It is thus 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. Nonetheless, although CorA appears to be constitutively expressed and to function as a homo-oligomer, the actual activity of the transporter can be regulated (by unknown means). Groisman's laboratory has recently shown that the level of CorA-mediated $^{63}Ni^{2+}$ uptake is increased over 10-fold in a *phoP* strain compared to wild type without any increase in the amount of CorA protein (11).

The *S.* Typhimurium CorA can also mediate Mg^{2+} efflux but does not appear to be able to efflux Ni^{2+} or Co^{2+} nor can Ni^{2+} or Co^{2+} influx activate Mg^{2+} efflux (24,87). Efflux occurs only under conditions of relatively high (mM) extracellular Mg^{2+} concentration, two orders of magnitude above the $K_{0.5}$ for influx. These are conditions that *S.* Typhimurium is quite unlikely to encounter outside the laboratory. Since the efflux occurs only at saturating Mg^{2+} concentrations for influx, it cannot represent a simple Mg^{2+}-Mg^{2+} exchange process. 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. The significance of CorA's ability to efflux Mg^{2+} is not known.

3.1.5. The structure of CorA

Mg^{2+} is the most charge dense of the biological cations with only 10 electrons to counter 12 protons. Among the properties that result in part from this high charge density, the unhydrated Mg^{2+} ion has a small diameter of only about 0.65Å while the hydrated cation is 5.0Å in diameter. Despite this charge density however, the TM segments of CorA never contain charged residues. Of several hundred prokaryotic and eukaryotic CorA homologs whose sequence is now available, only a handful have even a single charged residue in either TM1 or TM2. The lack of negatively charged residues in particular within the membrane domain sets CorA apart from many other cation transporters which require multiple glutamate and aspartate 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 and thus without electrostatic interactions.

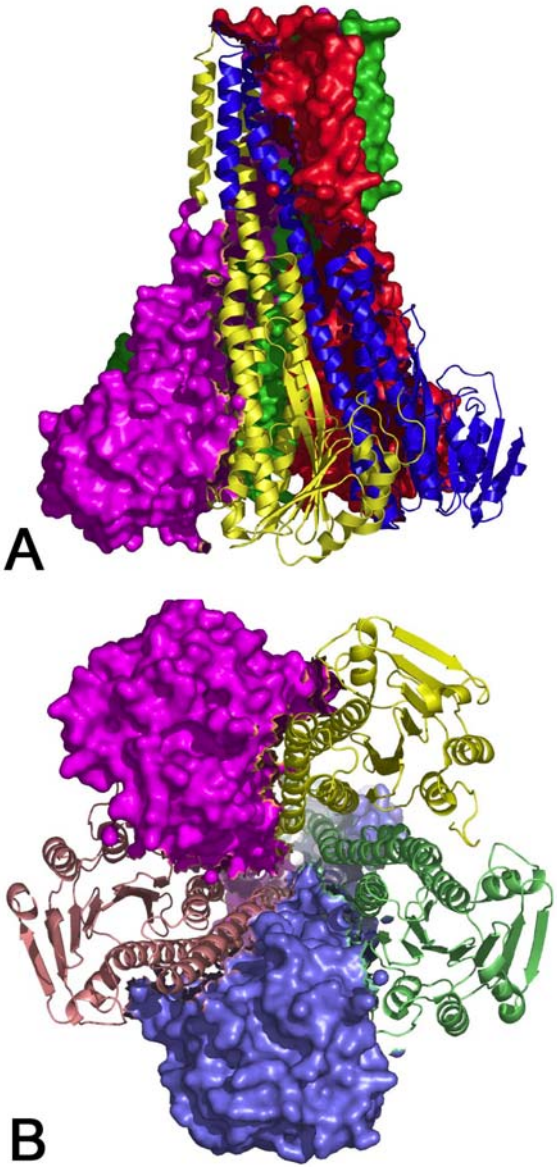

Figure 3. Structure of *T. maritima* CorA. The structure of CorA (PDB code: 2BBJ) is shown viewed from the side (A) and from the cytosol (B). The figures were drawn using PyMol (17) with some chains shown as surfaces and others as ribbon cartoons.

The crystal structure of CorA from *Thermatoga maritima* has recently been solved to a resolution of 3.9Å for the entire protein and 1.85Å for the soluble domain (49). The current structure (Figures 3A and 3B) is apparently in the closed state and is unfortunately missing two segments of importance: the apparent periplasmic Mg^{2+} binding loop between TM1 and TM2 and the C-terminal two residues, "WL", which are highly conserved. Although a 3.9Å structure does not allow unambiguous placement of all amino acid side chains in the membrane domain, the presence of several methionines in the membrane, coupled with use of selenomethionine derivatives for phasing has allowed good placement of numerous important amino acid side chains within the membrane sequence, providing potential insight into the structure of the ion conduction pathway and the mechanism of Mg^{2+} flux.

CorA is a funnel-shaped homopentamer with two transmembrane helices per monomer. The inner group of five helices form the ion conduction pathway and are comprised of the TM1 segment of each monomer. The TM2 helices lie perpendicular to the membrane outside the TM1 core. Bulky hydrophobic residues putatively gate the ion conduction pathway near the cytosolic membrane interface. The large cytoplasmic domain forms a funnel whose wide mouth points into the cell and whose walls are formed by five long α-helices that are extensions of the TM1 transmembrane helices. These are termed the "stalk" helices. The cytoplasmic neck of the pore is surrounded, on the outside of the funnel, by a ring of positive charge, termed the "basic sphincter" comprised of several highly conserved lysines at the C-terminus of the protein extending from TM2 plus 2 conserved lysines on the outer face of the long stalk helix just after it emerges from the membrane. From the cytosolic ends of the stalk helices at the top of the funnel, two α-helices extend towards the membrane like the branches of a weeping willow tree, hence the term "willow helices". More than 50% of the residues in the membrane proximal portions of the willow helices are Asp or Glu and thus are highly negatively charged. This area of negative charge abuts the basic sphincter's ring of positive charge. The willow helices are part of an α-β-α sandwich domain, comprised of 3 α-helices, a seven-stranded antiparallel β-sheet and 3 final α-helices. This structure comprises a new fold. The crystal structure shows an apparent Mg^{2+} ion binding site between Asp residues, one of which is near the end of the stalk helix and the other is in the α-β-α domain of the adjacent monomer. The location of this binding site, 5 of which are present in the intact homopentamer, suggest a mechanism to link gating of the pore to the intracellular concentration of Mg^{2+}.

The details of the structure and its proposed mechanism of transport have been presented elsewhere (49,53). Briefly, Mg^{2+} (and analogs like Co(III)hexaammine) apparently bind as the fully hydrated cation to an external Mg^{2+} binding loop comprised of 7-8 amino acids that connect TM1 and TM2. Entry to the ion conduction pathway is blocked by (using *T. maritima* numbering) Asn314, part of the universally conserved "YGMNF" sequence of the CorA family. The ring of Asn314 residues is held in place by a base-stacking interaction between Tyr311 of one monomer and Phe315 of an adjacent monomer. An additional block of the pathway appears to be formed by the side chains of the bulky Leu294 and Met291 residues near the cytosolic face in TM1. These residues are at almost the same level as the ring of positive residues forming the basic sphincter outside the pore surface just at the cytosol-membrane interface. The basic sphincter would provide a very high positive field potential at approximately the same level as the "gate" formed by Leu294/Met291, thus blocking passage of the positive Mg^{2+} cation.

Gating of the pore is proposed to be under the control of the 5 Mg^{2+} ions bound at the top of the funnel in the cytosolic domain of the protein. Mg^{2+} appears to be bound between 2 Asp residues with 4 water molecules occupying the remaining coordination sites. Such a binding site, based on studies of similar binding sites in other proteins (16), would likely have an affinity for Mg^{2+} of 0.1-0.3 mM, slightly less than the free concentration of Mg^{2+} within cells. Thus the sites would generally be mostly occupied. However, stochastically, there is a reasonable probability that all five sites could be unoccupied in any individual protein complex. Under these conditions, the α-β-α domain of one monomer could move slightly away from the stalk helix of the adjacent monomer. Since this helix is extremely long, only a small movement of a few tenths of an angstrom would be sufficient to allow the membrane proximal end of the helix (TM1) to move a much larger distance, potentially opening the ion conduction pathway and relieving the apparent block caused by Asn314 and by Leu294/Met291. At the same time, the movement of the α-β-α domain would cause the negatively charged ends of the willow helices to possibly move away from the positively charged basic sphincter ring thus relieving the positive field potential surrounding the Leu294/Met291 gate, thereby allowing easier passage of Mg^{2+}. Once Mg^{2+} enters the interior of the funnel, it is drawn into the cell by many potential liganding residues since the interior funnel surface is heavily lined with residues displaying hydroxyl or carboxyl side chains. Rebinding of Mg^{2+} to the site at the top of the funnel would bring the stalk helix of a monomer back adjacent to the α-β-α domain of the adjacent monomer, closing the ion conduction pathway. Further structural and mutagenesis studies are required to evaluate this model.

3.1.6. Genomics

CorA is very widespread within the Eubacteria (40) with sequences known in well over 200 distinct species. MgtE is almost as widespread in the Eubacteria as CorA although many species appear to contain both transporters. CorA tends to be absent from organisms with very small genomes where MgtE appears to prevail. CorA is also widespread in the Archaea.

Many if not most bacterial species possessing a CorA Mg^{2+} transporter have one or more additional CorA-like sequences (40,83). There is relatively high homology in the TM segments between the presumed CorA Mg^{2+} transporters and these additional sequences but much less though still discernible conservation the soluble domain. Phylogenetic analysis comparing characterized CorA Mg^{2+} transport proteins with these additional sequences clearly shows that there are two major branches of the CorA family which likely diverged relatively early in evolution. In contrast to CorA, these additional CorA paralogs cannot apparently mediate Mg^{2+}. This is shown clearly by genetic analysis in *S.* Typhimurium where mutation of *corA*, *mgtA*, and *mgtB* gives a strain incapable of transporting Mg^{2+} into the cell even though the CorA paralog is present. Worlock and Smith (99) determined that the *S.* Typhimurium CorA paralog mediates the efflux of Zn^{2+}, and adventitiously of Cd^{2+} and hence have named this class of proteins ZntB. They have further shown that ZntB has the same membrane topology as the CorA Mg^{2+} transporter. It remains to be seen whether all ZntB paralogs mediate only Zn^{2+} efflux or whether some or all can mediate efflux of other cations.

3.1.7. CorA homologs in eukaryotes

There are a number of eukaryotic proteins with very weak homology to CorA in yeast, plants and mammals including humans. Like the ZntB family the sequence homology to CorA is almost entirely within the membrane domain. The GMN core of the YGMNF motif at the end of TM1 is preserved. The homology is sufficiently weak that it was initially questionable whether these proteins were actually Mg^{2+} transport systems. Data however now clearly indicates that these proteins, as a class, can affect Mg^{2+} homeostasis, almost certainly by direct Mg^{2+} transport in all cases (10,23,28,29,42,46,47,75).

There is also weak homology between CorA and the yeast ALR1 and ALR2 genes responsible for aluminum resistance (20,48,50). Gardner's laboratory has shown that mutation of ALR1 and ALR2 results in a yeast strain that requires supplemental Mg^{2+}. Conversely, increased extracellular Mg^{2+}, but not several other cations, is protective against Al^{3+} toxicity. Overexpression of ALR1 reverses the phenotype concomitant with increased $^{57}Co^{2+}$ uptake similar to that seen with CorA and recent electrophysiological data demonstrates clear Mg^{2+} channel-like behavior. The ALR genes are much larger than the bacterial CorA, somewhat over 800 amino acids. The initial 500 amino acids are unique to the ALR family and presumably are involved in Al^{3+} binding whereas the C-terminal 300 amino acids bear the weak homology to CorA.

It is curious enough that evolution has allowed a Mg^{2+} transporter to apparently mediate Al^{3+} uptake, but Schweyen and colleagues have implicated two yeast mitochondrial RNA splicing factors, Mrs2p and Lpe10p, in Mg^{2+} uptake. Some though not all of the phenotypes exhibited by mutants in Mrs2p are relieved by expression of bacterial CorA targeted to the mitochondria (10,29,75). Recent work of Kolisek *et al.* (42) using the Mag-Fura dye has provided convincing evidence that expression of Mrs2p or CorA in yeast mitochondria correlates with increased Mg^{2+} uptake and increased intramitochondrial free Mg^{2+} (42). The human homolog of Mrs2p can functionally substitute for its yeast counterpart (102), and similar complementation can be shown for the *Arabidopsis* Mrs2p homolog, *atmrs2-1* (75). Recent electrophysiological experiments demonstrate, as with the ALR proteins, channel-like behavior (R. Schweyen, personal communication). Thus these homologs appear to be involved in maintaining appropriate Mg^{2+} levels in mitochondria. The relationship between Mg^{2+} transport and RNA splicing is currently a mystery, although the simplest explanation is that the transporter is not directly involved in mRNA splicing but is instead involved in maintaining very precise control of intramitochondrial Mg^{2+} levels for splicing reactions.

Arabidopsis possesses a large family of such Mrs2p homologs but only some have putative

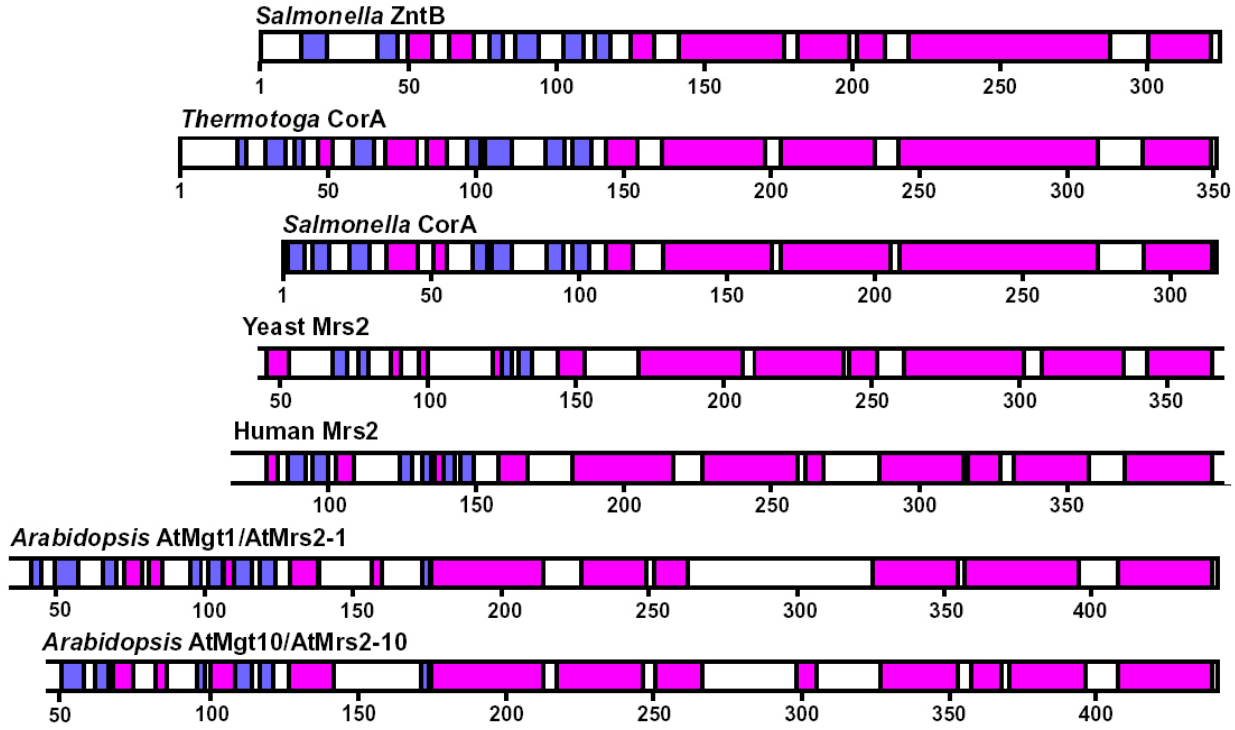

Figure 4. Secondary structure comparison of CorA homologs. The secondary structures of the indicated sequences were predicted using the www.predictprotein.org server and plotted along the linear sequence with α-helices in magenta and β-sheets in blue. The yeast, human and *Arabidopsis* proteins have additional N-terminal sequence and the yeast and human proteins have additional C-terminal sequence not homologous to CorA not shown here and indicated by the open ends of the bars.

transmembrane segments. *atmrs2-2* lacks any domains sufficiently hydrophobic to insert in a membrane and does not complement the yeast mutant as does its sibling *atmrs2-1* which does have sequence homologous to TM1 and TM2. Direct and convincing evidence for Mg^{2+} uptake by the *Arabidopsis* CorA/Mrs2p homologs has been provided by Li *et al.* (47). Genes encoding such homologs are widespread in eukaryotes.

It is of interest to note that the overall structure of CorA appears to be conserved even in these distant homologs despite the low sequence homology Figure 4 illustrates this conservation. When the known structure of the *T. maritima* CorA is compared to the predicted secondary structures of *S.* Typhimurium CorA and ZntB, yeast and human Mrs2p and *Arabidopsis* Atmrs2 proteins, it is clear that they have quite comparable secondary structures. Modeling of these proteins onto the *T. maritima* structure shows that they can fit the CorA structure extremely well in both the membrane and soluble domains (53), suggesting strongly that all CorA homologs have the same basic structure regardless of sequence diversity.

One conclusion that might be drawn from the ability of such weak homologs to transport Mg^{2+} is that evolution has found a unit protein structure (TM1 plus TM2) that can form a relatively non-specific channel for divalent cations with a high degree of sequence flexibility (except in the "YGMNF" motif). Then, in accord with the increasingly evident building block domain structures of proteins (45,66,67), the membrane motif links variants of the soluble domain structures to deliver a cation to the ion conduction pathway within the membrane from either the cytosolic or extracellular side. For example, in the CorA family of Mg^{2+} transporters, the short extracellular loop between TM1 and TM2 is always highly negatively charged in keeping with its apparent function of binding Mg^{2+}. In contrast, in the ZntB family of CorA paralogs mediating Zn^{2+} efflux, initial cation binding would occur in the cytosol, not in the extracellular space. Thus, the extracellular loop between TM1 and TM2 would not be required to bind cation; indeed, the transporter would not want to bind cation at this site since it might block transport. Correspondingly, in the ZntB family, this loop has no negative charge and is composed of a high percentage of Gly residues. It presumably serves only to connect TM1 and TM2 for proper arrangement within the membrane.

3.1.8. Physiology

Because CorA is expressed constitutively and is the bacterial 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 for routine cell metabolism. Yet this does not seem to be the case for *corA*. Mutation in the *corA* gene does not elicit any significant Mg^{2+}-dependent growth phenotype from *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+} and which can apparently supply sufficient Mg^{2+} for normal growth. Nonetheless, *corA* appears to influence a number of functions within the cell. Specifically, *S.* Typhimurium *corA* mutants are markedly defective for invasion of epithelial cells (K. Papp-Wallace and M.E. Maguire, unpublished data). Moreover, the virulence of a *corA* mutant strain is significantly attenuated in the mouse infection model (D.G. Kehres and M.E. Maguire, unpublished data). The basis for this decreased virulence is not yet known, but mutation of *corA* results in defective expression of the Type III protein secretion system encoded on *Salmonella* Pathogenicity Island 1 as well as altered regulation of the expression of a number, though not all, of 40 or so genes regulated by the Mg^{2+} receptor PhoQ and its response regulator (transcription factor) PhoP. 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. These varied phenotypes suggest 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 these alterations in gene transcription and metabolism as well as the downstream effects are under investigation.

3.2. MgtE Magnesium Transporters

In screening microbial genomic libraries for additional CorA-like transporters, the MgtE class of Mg^{2+} transporter was unexpectedly cloned from *B. firmus* OF4 (86) and *P. stuartii* (92).

3.2.1. Transport and Physiology

When expressed from multicopy plasmids in *S.* Typhimurium, the MgtE proteins of *B. firmus* OF4 and *P. stuartii* (86,92) exhibit similar $K_{0.5}$ and V_{max} values of about 70 μM and 0.50 nmol min^{-1} 10^8 $cells^{-1}$ for $^{57}Co^{2+}$ uptake, respectively (Table 1). 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 20 to 80 μM with Zn^{2+} being the most potent. It is unlikely that MgtE transports these latter cations however since overexpression of MgtE does not lead to increased toxicity from these cations. It has not been determined if any of these latter cations is transported. In contrast to CorA, Ni^{2+} is neither transported nor does it inhibit. Regulation of MgtE expression has not been studied. Likewise, virtually nothing is known of its physiological role although in *Aeromonas hydrophila*, mutation of MgtE markedly decreases adherence of the organism to mammalian cells and decreases its ability to form biofilm (58).

3.2.2. 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 (86,92). Putative helical transmembrane segments contain a few modestly conserved charged residues and several well conserved residues bearing hydroxyl side chains. Introduction of either the *P. stuartii* (Gram-negative) or the *B. firmus* OF4 (Gram-positive) MgtE into the Mg^{2+}-transport mutant MM281 of *S.* Typhimurium rescues growth and elicits robust Mg^{2+} uptake. It can be therefore be inferred that MgtE does not require another protein for transport. It is unknown however 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.

3.2.3. Genomics

Current DNA sequence data show MgtE sequences are almost as numerous as CorA in the *Eubacteria*, with many species possessing both a CorA and a MgtE. MgtE homologs are probably more numerous in the *Archaea* than CorA. Like CorA, MgtE transporters do not belong to any known class of proteins although the N-terminal third of the protein has weak homology to inosine 5'-monophosphate dehydrogenases and perhaps some inorganic pyrophosphatases.

Whereas the eukaryotic homologs of CorA have only weak sequence (though clearly good functional) homology, MgtE homologs are widespread in eukaryotes including humans. They belong to the SLC41 class of solute transporters. Quamme and colleagues have expressed SLC41A1 and SLC41A2 in *Xenopus laevis* oocytes and determined their transport properties (25-27). Uptake is voltage dependent with apparent Mg^{2+} affinities of 0.7 mM for SLC41A1 and 0.3 mM for SLC41A2. Both transport a range of divalent cations. SLC41A1 can transport Mg^{2+}, Sr^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Co^{2+}, Ba^{2+} and Cd^{2+}, but not Mn^{2+} or Ni^{2+}. SLC41A2 can transport Mg^{2+}, Ba^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+} and Mn^{2+}, but not Zn^{2+}, or Cu^{2+}. Neither can transport Ca^{2+}. SLC41A1 is expressed in many mammalian tissues, and in some expression is regulated by decreased extracellular Mg^{2+}. In contrast, the SLC41A2 transcript is expressed in many epithelial cell types but is not responsive to magnesium. It will be of interest to determine if any of the clinical disorders in Mg^{2+} homeostasis (57,72) map to any MgtE locus.

3.3. P-Type ATPase Magnesium Transporters

3.3.1. Identification

mgtA and *mgtB* were cloned from *S.* Typhimurium by complementation of the Mg^{2+} growth phenotype of MM77 and MM281 as noted above (87). Sequence data revealed that these two distinct loci each encoded unusual P-type ATPases, MgtA and MgtB. In general, the P-type class of ATPases in both prokaryotes and eukaryotes moves cations from the cytosol into the extracellular space or into an internal compartment, in each case *against* an electrochemical gradient, hence the requirement for input of energy *via* ATP. In contrast, MgtA and MgtB mediate the influx of Mg^{2+} down its large electrochemical gradient. Moreover, these two Bacterial P-type ATPases are much more similar to eukaryotic than to

other prokaryotic P-type ATPases. Both MgtA and MgtB are single subunit enzymes of just over 900 amino acids in length which compares to about 1000 amino acids for H^+-ATPases and Ca^{2+}-ATPases in eukaryotes. The catalytic subunit of most prokaryotic P-type ATPases is about 650 amino acids and homologous to the longer P-type ATPases over their initial 600 or so amino acids.

3.3.2. Transport

Both MgtA and MgtB transport Mg^{2+} with a $K_{0.5}$ of 5-20 μM, essentially equivalent to that of CorA and MgtE. Estimation of a V_{max} is not possible in intact cells because regulation of their expression occurs over a wide range (87,90,91). It would be more relevant to refer to the rate of transport as a capacity, and in this sense, either MgtA or MgtB could potentially reach a rate of movement of Mg^{2+} into the cell of perhaps 10-50% the rate of CorA. Ni^{2+} is also transported. Unlike CorA however, MgtA and MgtB cannot transport Co^{2+}, although Co^{2+} does inhibit transport (Table 1). The two proteins exhibit significant differences in their interactions with other cations. Zn^{2+} inhibits MgtA potently with a K_i of 7 μM but cannot inhibit uptake *via* MgtB at all. Likewise, Ca^{2+} inhibits MgtA with a K_i of 300 μM but cannot inhibit MgtB mediated transport. Conversely, Mn^{2+} is a good competitive inhibitor of MgtB transport with a K_i of 40 μM, but inhibits MgtA to a maximal extent of 30-40% even at mM concentrations. There are also significant differences in temperature dependence. For example, MgtB is extremely temperature sensitive. In intact cells, it is fully active at 37°C but completely inactive (in terms of transport) at 20°C. MgtA in contrast exhibits a typical response to changes in temperature, being slightly active even at 4°C. No physiological basis for these differences is yet apparent.

3.3.3. Genomics

The MgtA/B class of Mg^{2+} transporter belong to the superfamily of P-type ATPases (44). Homologs are widely distributed in the *Eubacteria* but only a few are present in the *Archaea*. Interestingly, while many apparent P-type Ca^{2+} ATPases are also present in *Eubacteria*, MgtA/B are more similar to the yeast H^+-ATPases and the mammalian Ca^{2+}- ATPases of the sarco(endo)plasmic reticulum than to the prokaryotic Ca^{2+} P-type ATPases.

mgtA is the endogenous *Salmonella* P-type ATPase Mg^{2+} transporter; however, *mgtB* is a foreign gene, the second gene of the *mgtCB* operon (89,91), 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 (5). Although codon usage for *mgtB* translation is not markedly abnormal for *S.* Typhimurium as would be expected for an acquired gene, MgtB is only about 50% identical to MgtA. By comparison, MgtA from *E. coli* is about 98% identical to *S.* Typhimurium MgtA, precluding the possibility that MgtB arose by gene duplication. MgtA/B homologs are abundant in both Bacteria and the Archaea. By comparison, BLAST searches for MgtC show that it is also widespread among the *Eubacteria*, but only a few *Archaea* appear to have an MgtC, these mostly in *Methanothermobacter* and *Methanosarcina* sp. Moreover, while some Bacteria appear to have both *mgtB* and *mgtC*, many more species have only one of either *mgtB* or *mgtC*. This inference agrees with the conclusions previously made by Blanc-Potard and Groisman (5) from hybridization studies with probes selective for each gene. They showed that of 9 Enterobacterial species tested, all carried *mgtA*, only a subset carried either or both of *mgtB* and *mgtC*. Species carrying *mgtB* did not always carry *mgtC*. Even when found in the same species, association of *mgtB* and *mgtC* in an operon is quite uncommon.

Is the association of *mgtC* and *mgtB* in an operon functionally relevant? The question cannot be currently answered because the function of MgtC is not known. However, since many P-type ATPases have β-subunits and occasionally even additional subunits, an obvious hypothesis regarding the *S.* Typhimurium *mgtCB* operon is that MgtC could be functioning as a β-subunit for the catalytic MgtB. Deletion of *mgtC* has no effect on the MgtB-mediated Mg^{2+} transport, and MgtC protein does not seem to be expressed at the same time as MgtB even though it is transcribed with *mgtB* (63). Thus clearly the MgtC protein is not required for MgtB activity, but the question of whether it could function as a β-subunit has not been definitively answered.

mgtC is not required for invasion of macrophage or epithelial cells nor is it required for initial survival and proliferation. However, Blanc-Potard and Groisman (5) showed that *mgtC* is important for long term survival after invasion of a macrophage and that mutation of *mgtC* attenuates virulence after intraperitoneal injection into mice. The *mgtC* homolog of *Mycobacterium tuberculosis* has a similar phenotype (9). They suggested that MgtC may be a fourth Mg^{2+} transporter in *S. typhimurium*. 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, does not alter cellular Mg^{2+} content, and does not relieve the requirement for Mg^{2+} supplementation of the growth medium (63). 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 (63). 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.

3.3.4. Structure

MgtB was the first P-type ATPase, bacterial or eukaryotic, whose complete membrane topology was unequivocally determined (79). As had been predicted for the majority of eukaryotic P-type ATPases, MgtB has ten TM domains with both termini in the cytoplasm. Six of these TM domains were towards the N-terminus of the protein and matched the predicted distribution of membrane segments in other, shorter, prokaryotic P-type ATPases. Where MgtB (and MgtA) differ with many other prokaryotic P-type ATPases is in their possession of 4 additional TM domains towards their C-terminus, in

agreement with predictions for the position of the last four TM domains in eukaryotic P-type ATPases. Other prokaryotic P-type ATPases do not possess the requisite sequence, being about 300 amino acids shorter and thus have only 6 TM domains. The topology determined for MgtB in a prokaryote has now been confirmed by the crystal structure of the sarco(endo)plasmic Ca^{2+}-ATPase (93,101).

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 (12,51). These residues are reasonably conserved in MgtA and MgtB; however, mutagenesis of *S.* Typhimurium MgtB suggests some significant differences. 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, submitted for publication) and one of those two residues is uncharged. This would make MgtA/B much more similar to CorA than to other P-type ATPases in that Mg^{2+} movement through the membrane involves interactions primarily with uncharged amino acids.

3.3.5. Transport properties

MgtA and MgtB both transport Mg^{2+} with $K_{0.5}$ values of 5-20 μM, essentially equivalent to that of CorA. Their true V_{max} values cannot be measured currently because of their regulation (87,90,91). The apparent V_{max} of each transporter is lower than the "channel"-like throughput of CorA, but since they can each be induced enormously, the Mg^{2+} transport capacity provided to the cell by MgtA or MgtB when maximally induced is probably a substantial fraction of that provided by CorA.

3.3.6. 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; both the CorA and MgtE classes are constitutively expressed. *S.* Typhimurium MgtA and MgtB are repressed as the concentration of Mg^{2+} in the growth medium increases such that at any medium concentration above 100 μM, they are either not expressed or are expressed at a very low, essentially undetectable level. Conversely, both are induced to an enormous extent (at least 1000-fold) upon Mg^{2+} deprivation (90,91). This regulation is mediated by the PhoPQ two-component regulatory system (31,59,60). PhoPQ regulated genes are induced (technically, derepressed) upon invasion of macrophage or epithelial cells (59,61) or infection of the mouse (34,55). MgtA and MgtB are among those genes induced upon *S.* Typhimurium invasion of a mammalian host cell (22,34,55). Most PhoPQ-regulated genes reside on pathogenicity islands in *S.* Typhimurium and most are important for virulence. Several PhoPQ-regulated genes as well as *phoPQ* itself are essential for pathogenesis.

Intriguingly, Groisman and colleagues (21,30,94) have shown that the ligand or "hormone" that controls PhoPQ activity is Mg^{2+} itself. When sufficient Mg^{2+} is present in the medium, it can be bound by PhoQ, a membrane sensor-kinase. In this bound state, PhoQ does not autophosphorylate and then transactivate PhoP. However, when the level of Mg^{2+} in the medium drops, by simple mass action considerations, Mg^{2+} will dissociate from PhoQ. In this unliganded state, PhoQ autophosphorylates and then, acting as a kinase, transfers the phosphate to PhoP, activating PhoP as a transcription factor. A significant puzzle however, is that despite regulation by an important pathway involved in virulence, despite control of this pathway by its substrate and despite induction during invasion of the host organism, neither MgtA nor MgtB appear to have a major role in *S. typhimurium* pathogenesis (5). One point that should be made however is that experiments to date have tested only single mutations, that is, deletion of *mgtA* or *mgtB* alone. Since their transport properties and their regulation by PhoPQ are very similar, there could be a greater or lesser degree of redundancy in *S.* Typhimurium resulting from possession of two transporters mediating the same function. Thus, it is possible that the double *mgtA mgtB* mutant will prove to be attenuated for virulence.

In general, prokaryotes often have only a single enzyme or transporter for a particular task. Thus it is unusual for an organism to have three Mg^{2+} transporters. When multiple transporters are present for the same substrate, it is often the case that one transporter has a moderate affinity for the substrate while the other has a very high affinity for the substrate. In this situation, the second system can be considered a "scavenger" transporter, used by the cell to acquire the substrate when its concentration is extremely low in the environment. Thus, for example, in *E. coli*, two K^+ transporters operate to maintain high intracellular K^+. The Trk system is constitutively expressed and exhibits an affinity for K^+ of 1-2 mM. In contrast, as extracellular potassium concentrations fall below about 1 mM, the Kdp-ATPase transport system is increasingly induced. It exhibits an affinity for K^+ about 1000-fold greater, around 1-2 μM. Thus it functions to "scavenge" K^+ in potassium depleted environments (97). The relevance to MgtA and MgtB is that they do not fit into this paradigm. Both Mg^{2+} P-type ATPases have the same affinity for Mg^{2+} as the constitutively expressed CorA transporter. Thus, the physiological function of MgtA (and MgtB in those organisms that possess it) is not to scavenge Mg^{2+} but must be to acquire Mg^{2+} under some other particular environmental condition.

3.3.7. Why is ATP involved?

There is no obvious reason why ATP is required for Mg^{2+} influx. The electrochemical gradient across the bacterial membrane is highly negative inside. This should provide far more potential energy than is necessary to drive Mg^{2+} influx at even micromolar extracellular Mg^{2+} concentrations. The normal role of P-type ATPases and a requirement for ATP is to mediate the efflux of a cation *against* its electrochemical gradient. MgtA and MgtB clearly do not mediate such a process. Indeed, it is often assumed that for ions such as Mg^{2+}, there must exist an efflux activity to prevent accumulation of the ion driven by the constant electrochemical gradient. For example, the

equilibrium potential for Mg^{2+} in a bacterium would generate a free Mg^{2+} concentration of at least 50 mM, far higher than the apparent 0.5-1 mM free Mg^{2+} that is present. This is clearly not the function of MgtA and MgtB. In this context, one might assume that CorA fills the presumed requirement for efflux. However, if *corA* is mutated, there is no apparent Mg^{2+} efflux activity at all, even after several generations of growth in high Mg^{2+} concentrations (24). Thus the usual contention that a cation efflux activity is required to maintain an appropriate intracellular cation concentration does not appear to apply in the case of Mg^{2+}.

3.3.8. Do MgtA/B mediate a function other than Mg^{2+} transport?

Genomic sequence data currently available indicates a P-type ATPase is never the sole Mg^{2+} transporter of a cell. This suggests that most cells have no compelling physiological need for this class of transport system. Further, since microbes generally do not carry genes they do not need, it suggests that those cells that possess a Mg^{2+} transporting P-type ATPase have a specific need, a physiological niche that such an enzyme helps it fill. The use of ATP for Mg^{2+} influx suggests that *S.* Typhimurium and other bacteria utilizing Mg^{2+}-transporting P-type ATPases can, outside the laboratory, find themselves in an environment where the electrochemical gradient is insufficient and the additional energy of ATP is required, but such an environment is difficult to envisage. An alternative explanation for the role of ATP is that MgtA and MgtB transport of Mg^{2+} is merely incidental to the transport of some other substance, a substance that requires a very large amount of energy to be translocated across the membrane. Using the electrochemical gradient of Mg^{2+} plus the energy of hydrolysis of ATP would provide such a large amount of energy. One substrate that could require this level of energy input is phospholipid translocation across the membrane bilayer, *i.e.,* MgtA and MgtB might be phospholipid flippases. This is only one speculative example however, to make the point that the physiological function of MgtA and MgtB might not actually be Mg^{2+} influx.

A hint that *S. typhimurium* MgtB may perform some additional function comes from the phenotype of the E337A mutant of MgtB. 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+}. Thus the E337A mutation could be compromising the export half of some bi-directional transport cycle or some other transport function.

4. 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 (32,52).

Properties of prokaryotic Mg^{2+} transporters are clearly less than optimally 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 many 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. The possibility that CorA and even MgtA/B are 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.

5. ACKNOWLEDGEMENT

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Footnote: [1] Since transport is measured in intact cells, the term $K_{0.5}$ is used rather than K_m or K_a.

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