Magnesium

Mg$^{2+}$ is the most abundant divalent cation in living cells. It is present at a total cellular concentration of 15–25 mM in both prokaryotic and mammalian cells (39, 53, 55). In the cytosol, the majority of Mg$^{2+}$ is bound to ATP and other phosphonucleotides and to multiple enzymes. In all cells, Mg$^{2+}$ serves as an essential structural element for ribosomes and membranes and as a required cofactor for ATP in the catalytic pocket of a multitude of enzymes. In prokaryotes, Mg$^{2+}$ has also been identified as an important regulatory signal essential for virulence (10, 70).

The chemistry of divalent magnesium is unique among the biologically important cations. The hydrated radius of Mg$^{2+}$ is ~400 times larger than the dehydrated radius, a much larger difference than that seen with Na$^+$ and Ca$^{2+}$ (~25-fold) or K$^+$ (fourfold). Of all biological cations, Mg$^{2+}$ is the most charge dense, holding the waters within its hydration shell tighter by a factor of 10$^3$–10$^4$ than do Ca$^{2+}$, K$^+$, and Na$^+$ (39). In addition, the hydrated Mg$^{2+}$ cation is more rigid than other cations, always hexacoordinate, and almost always prefers to coordinate with oxygen. Proteins that transport Mg$^{2+}$ must be able to recognize the very large hydrated cation, strip the tightly bound hydration shell from the cation, and only then transport the dehydrated form. These chemical properties of Mg$^{2+}$ thus predict that proteins that recognize and transport Mg$^{2+}$ will be unique (19, 36).

Mg$^{2+}$ Transport Proteins

The most thoroughly characterized Mg$^{2+}$ transport proteins to date are from prokaryotic sources. Although several genes associated with Mg$^{2+}$ transport in eukaryotic systems have been recently identified (Table 1), this review will focus on the only two divalent cation channels to be crystallized: CorA and MgtE. The first prokaryotic Mg$^{2+}$ transport system identified and cloned was termed corA for the Co$^{2+}$ resistance screen by which it was discovered in Escherichia coli and Salmonella enterica serovar Typhimurium (22, 23, 44, 49, 61). A locus termed mgt was also found to be associated with Mg$^{2+}$ transport and eventually shown to encode a P-type ATPase that mediates Mg$^{2+}$ influx with rather than against its electrochemical gradient (22, 66–69, 72, 73). Additional work uncovered another widespread Mg$^{2+}$ influx system in prokaryotes encoded by mgtE (65, 75). Work from this laboratory has subsequently characterized Mg$^{2+}$ flux mediated by each of these systems.

The Unique Nature of Mg$^{2+}$ Channels

Considering the biological abundance and importance of Mg$^{2+}$, there is a surprising lack of information regarding the proteins that transport Mg$^{2+}$, the mechanisms by which they do so, and their physiological roles within the cell. The best characterized Mg$^{2+}$ channel to date is the bacterial protein CorA, present in a wide range of bacterial species. The CorA homolog Mxs2 forms the mitochondrial Mg$^{2+}$ channel in all eukaryotes. Physiologically, CorA is involved in bacterial pathogenesis, and the Mxs2 eukaryotic homolog is essential for cell survival. A second Mg$^{2+}$ channel widespread in bacteria is MgtE. Its eukaryotic homologs are the SLC41 family of carriers. Physiological roles for Mg$^{2+}$ and its homologs have not been established. Recently, the crystal structures for the bacterial CorA and MgtE Mg$^{2+}$ channels were solved, the first structures of any divalent cation channel. As befits the unique biology of Mg$^{2+}$, both structures are unique, unlike that of any other channel or transporter. Although structurally quite different, both CorA and MgtE appear to be gated in a similar manner through multiple Mg$^{2+}$ binding sites in the cytosolic domain of the channels. These sites essentially serve as Mg$^{2+}$ "sensors" of cytosolic Mg$^{2+}$ concentration. Many questions about these channels remain, however, including the molecular basis of Mg$^{2+}$ selectivity and the physiological role(s) of their eukaryotic homologs.
CosA is an ion channel present in approximately half of the microbial genomes currently sequenced. It mediates the influx of Mg\(^{2+}\), Ca\(^{2+}\), and Ni\(^{2+}\); it does not transport Mn\(^{2+}\), Zn\(^{2+}\), or Fe\(^{2+}\) (45, 66). The eukaryotic homolog of CosA is Mxs2, the Mg\(^{2+}\) channel of the inner mitochondrial membrane (3, 56).

Mgt\(_{\text{E}}\), like CosA, is the primary Mg\(^{2+}\) transport system in about half of bacterial genomes sequenced to date. A minority of organisms carry both CosA and Mgt\(_{\text{E}}\). Mgt\(_{\text{E}}\) is able to mediate the flux of Mg\(^{2+}\) and Co\(^{2+}\). Ni\(^{2+}\) is an inhibitor of Mgt\(_{\text{E}}\), but, unlike CosA, Ni\(^{2+}\) is not transported by Mgt\(_{\text{E}}\) (65, 75). The eukaryotic homologs of Mgt\(_{\text{E}}\) are the SLC41 family of solute carriers (77).

**The CorA Mg\(^{2+}\) Channel**

Three independent crystal structures of *Thermotoga maritima* CorA have recently been published with resolutions from 2.9 to 3.9 Å. All three structures give essentially the same picture of an apparent closed form of the channel (9, 33, 51). CorA is a homopentamer with two transmembrane (TM) segments per monomer (FIGURE 1). Both the amino and carboxytermini are positioned in the cytosol. The large NH\(_{2}\)-terminal cytoplasmic domain structure is formed from a new protein fold, consisting of a seven stranded parallel/anti-parallel β-sheet sandwiched between two sets of α-helices (α\(_{1}\)α\(_{2}\)α\(_{3}\)) and (α\(_{4}\)α\(_{5}\)α\(_{6}\)). A stalk helix (α\(_{7}\)) links this cytosolic domain to TM1. The NH\(_{2}\)-terminal regions of the stalk helix with part of the α\(_{7}\) helix form a funnel-like structure opening into the cytosol. The stalk helix extends ~100 Å from the cytoplasm into the membrane, forming the inner wall of the funnel and TM1. At the membrane-cytoplasm interface, the funnel is relatively narrow in diameter at 5 Å, whereas its cytoplasmic mouth is much wider at 20 Å. Many of the α\(_{7}\) and α\(_{8}\) stalk helix residues facing the interior of the funnel are negatively charged or bear hydroxyl groups (FIGURE 2). Since CorA is a homopentamer, this arrangement provides a succession of concentric, negatively charged or polar residues that putatively would interact with Mg\(^{2+}\) as it exits the membrane pore.

The membrane domain of CorA is formed by two TM segments per monomer, giving 10 TM segments in total.

**Table 1. Prokaryotic and eukaryotic Mg\(^{2+}\) transporters**

<table>
<thead>
<tr>
<th>Superfamily</th>
<th>Identified Members</th>
<th>Apparent Km for Mg(^{2+})</th>
<th>Type of Transporter</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CorA</td>
<td>CosA, ALR1/ALR2,</td>
<td>~15 μM</td>
<td>Channel, Channel</td>
<td>22, 23, 66, 67</td>
</tr>
<tr>
<td></td>
<td>Mxs2/AlmMxs2,</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Lpx10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MgtE</td>
<td>MgtE, SLC41A1,</td>
<td>0.7 mM, 0.3 mM</td>
<td>Channel, Carriers</td>
<td>65, 75, 13, 14, 54</td>
</tr>
<tr>
<td></td>
<td>SLC41A2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRPM</td>
<td>TRPM6</td>
<td></td>
<td>Channel/kinasin</td>
<td>1, 5, 57, 58, 76, 42, 43, 59</td>
</tr>
<tr>
<td></td>
<td>TRPM7 (LTRPC7,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TRP-LPK)</td>
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<tr>
<td>Claudins</td>
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<td></td>
<td>Claudins</td>
<td>62</td>
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<tr>
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<td>(Paracellin-1)</td>
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<td></td>
<td>Claudin-19</td>
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<td>ACDP</td>
<td>ACDP2 (CNNM2)</td>
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<td>Putative channel</td>
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<tr>
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<td>Putative channel</td>
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<tr>
<td>MgtT</td>
<td>MgtA, MgtB</td>
<td>10 μM</td>
<td>Putative channel</td>
<td>64, 66, 69</td>
</tr>
<tr>
<td>NIPA1</td>
<td>NIPA1 (SPG6)</td>
<td>0.7 mM</td>
<td>P-type ATPases</td>
<td>12</td>
</tr>
<tr>
<td>MMgtT</td>
<td>MMgtT1, MMgtT2</td>
<td>1.5 mM, 0.6 mM</td>
<td>Putative channels</td>
<td>16</td>
</tr>
</tbody>
</table>

Text in **bold** denotes prokaryotic origin. Text in regular font denotes eukaryotic origin.

---

**FIGURE 1.** CorA Mg\(^{2+}\) channel

A: CorA Mg\(^{2+}\) channel. The homopentamer is shown from the side with each of the five monomers in a different color. The extracellular space (periplasm) is at top; B: a single monomer is shown with TM2/α\(_{7}\) in gray, TM1/α\(_{2}\) in blue, the yellow helices (α\(_{2}\) and α\(_{3}\)) in purple, the β-sheet (β\(_{1}\)β\(_{2}\)) in yellow, and the remaining helices (α\(_{4}\)α\(_{5}\)α\(_{6}\)) in red.

**FIGURE 2.** Cytoplasmic mouth of the CorA Mg\(^{2+}\) channel. A: cartoon view. B: water map of the channel. The helices of CorA proteins contain a funnel shaped cavity that contains a series of universally conserved negatively charged residues. Both CorA proteins and their eukaryotic homologs mediate Mg\(^{2+}\) transport Mn\(^{2+}\), Ca\(^{2+}\), Zn\(^{2+}\), or Fe\(^{2+}\) (65, 66, 69, 76, 42, 43, 59).

The helices of CorA proteins form a funnel, which is essentially the apparent closed form of the channel. The funnel is relatively narrow in diameter at 5 Å, whereas its cytoplasmic mouth is much wider at 20 Å. Many of the α\(_{7}\) and α\(_{8}\) stalk helix residues facing the interior of the funnel are negatively charged or bear hydroxyl groups (FIGURE 2). Since CorA is a homopentamer, this arrangement provides a succession of concentric, negatively charged or polar residues that putatively would interact with Mg\(^{2+}\) as it exits the membrane pore.
The helices of TM2 form a ring around the outside of the pore, which is composed solely of TM1 helices in the apparent closed form of the channel (FIGURE 3). A proline and a glycine cause TM1 to kink and taper the pore near the periplasmic surface. Distortion by proline and glycine residues in similar positions is a common property of ion channels (74). The distal portion of TM1 near the periplasm contains the signature sequence of all CorA proteins, YGMNF. A BLAST search of this five-amino acid string returns no hits except CorA. This universally conserved feature of CorA is a short six-amino acid cytosolic domain of multiple arginines that CorA initially interacts with the fully hydrated Mg$^{2+}$ cation (30). In addition, the ability of Co(III) and Ru(III) hexaammines to inhibit CorA indicates that this initial interaction is not based strictly on charge since both cations are charge neutral. An obvious candidate for site of initial interaction of the channel with a Mg$^{2+}$ cation is the only part of CorA exposed on the periplasmic face of the membrane, the nine-amino acid loop connecting TM1 and TM2 (33). This loop always contains a significant negative charge blocked by two TM helices in total.

**FIGURE 2.** Cytosolic charged domains of CorA. A: Cartoon view of charged residues in the CorA cytosolic domain. The CorA Mg$^{2+}$ channel of T. maritima is shown in cartoon form in green with two monomers removed. The five-amino acid loop connecting TM1 and TM2 (33) is shown in green. One of the five monomers has been removed for clarity to allow better visualization. The charged residues that line the inner wall of the funnel formed by $\alpha_2$ and $\alpha_5$ are represented as spheres with alternate magenta, red, and yellow colors. An alternative view is also shown in FIGURE 4. B: Cartoon view of charged residues lining the interior face of the funnel of CorA. The cytosolic portions of most of the $\alpha_2$ and $\alpha_5$ stalk helices of the T. maritima CorA are shown in green. Two of the five monomers have been removed for clarity to allow better visualization. The charged residues that line the inner wall of the funnel formed by $\alpha_2$ and $\alpha_5$ are represented as spheres with alternate magenta, red, and yellow colors. An alternative view is also shown in FIGURE 4.
as well as the highly conserved MPEL motif. This suggests that the periplasmic loop may act as a selectivity filter. In contrast, Payandeh and Pai’s crystal structure (51) modeled this unresolved loop by lengthening TM2 and reducing the loop to seven amino acids. The results of their modeling suggested that the MPEL loop lies roughly parallel to the membrane and thus would be poorly positioned to interact with Mg$^{2+}$ and act as a selectivity filter. Further experiments will be required to determine whether Mg$^{2+}$ initially binds to CorA. The CorA pore is ~40 Å in length. It is noteworthy that none of the almost 800 currently known CorA homologs contains a charged amino acid in either TM1 or TM2. Thus electrostatic interactions are not involved in movement of the most charge dense of all biological cations through the membrane. Indeed, mutational analysis of the S. typhimurium CorA indicates that it is the backbone carbonyls within the pore that primarily interact with Mg$^{2+}$ during its passage (71). In addition to the constriction formed by the ring of five asparagine residues from the YGMNF motif, two additional sites of pore constriction are apparent, creating a maximum width of 6 Å narrowing to 2.5 Å. The smallest of these constrictions is formed by bulky hydrophobic residues at the cytosol-membrane interface (Leu294 and Met291 in the T. maritima CorA). A large hydrophobic residue is conserved at the equivalent of position 294 in CorA homologs. Adjacent to this constriction but outside the pore is the basic sphincter, the lysine/arginine ring formed from the COOH terminus. The combination of positive potential from this ring and pore constriction would appear to provide a formidable barrier to passage of a positive cation. How does Mg$^{2+}$ pass through all of these barriers? The relative positioning of the positively charged basic sphincter and the negatively charged willow helices suggests an answer (9, 33, 51). The electrostatic attraction generated between the basic sphincter and the willow helices could induce movement of the basic sphincter outward and/or upward further into the cytosol, away from the pore and the block formed by Leu294 and Met291. This would necessitate movement of the stalk helix since part of the lysine/arginine ring is formed from residues of TM2 near the cytosolic end of the pore. The 100 Å length of the stalk helix provides a very long relatively rigid “lever” through which movements elsewhere in the homopentamer could be transmitted to the pore, thereby opening it. Recent mutational analysis of T. maritima CorA by Payandeh et al. (50) supports such a gating role for the willow helices. FIGURE 3. CorA membrane domain A. CorA as seen from the periplasm. The CorA pentamer is shown from outside the cell looking down the pore, which is formed by TM1 in this closed form. Each monomer is colored differently. The five TM2 helices lie outside this pore, approximately 15 Å from the adjacent TM1 and 23 Å from another TM2. B. CorA membrane domain as cylinders. TM1 and TM2 are shown as cylinders with each monomer colored differently. The five TM2 helices lie outside this pore, approximately 15 Å from the adjacent TM1 and 23 Å from another TM2. The view is from the periplasm. Each TM2 is approximately perpendicular to the membrane, whereas each TM1 is angled slightly with respect to TM2 and TM9 near the cytosolic end of TM2 are indicated on two monomers each. Cysteine substitution at either of these residues results in a pentamer containing five cysteines within the membrane. It is of interest that Y292C can spontaneously cross-link to another Y292C residue. CorA carrying the M299C substitution does not spontaneously cross-link via a disulfide bond, but it can be induced to do so with copper-phenanthroline oxidation (71). Since each TM2 is ~10.2 Å from the adjacent TM2, these data strongly suggest that the TM2 helices must move in toward the apparent TM1 pore at some point during flux of Mg$^{2+}$. FIGURE 4. Im A. a view of the CorA pentamer in the closed form. The yellow residues are the willow helices helping to hold the protein in the cytosol. The CorA heteropentamer is also shown in this closed form.
Regulation of Mg\(^{2+}\) transport

How might movement of the willow helices and basic sphincter be controlled to regulate opening and closing of the channel? The structure from Lunin et al. (33) and that from Payandeh and Pai (51) were solved in the presence of Mg\(^{2+}\) and Ca\(^{2+}\), respectively. In both, electron density was detected near residues Asp89 of the \(\alpha_3\) helix and Asp253 of the \(\alpha_7\) stalk helix consistent with cation binding (FIGURE 4). Mg\(^{2+}\) bound to this site would stabilize a closed conformation of the protein by associating the NH\(_2\)-terminal \(\alpha_7\) domain of one monomer with the \(\alpha_7\) stalk helix of the adjacent monomer. Mg\(^{2+}\) dissociation from these sites could elicit rotation of the \(\alpha_7\) domain of one monomer away from the \(\alpha_7\) helix of the funnel wall (9, 33, 51). The movement of the willow helices as part of the \(\alpha_7\) domain would presumably exert an attraction on the positively charged basic sphincter residues, relieving the block at the membrane-cytosol interface and allowing the protein to open like an iris. Recent mutational data supports this hypothesis (50). It remains to be definitively demonstrated that the Mg\(^{2+}\) ions bound to the cytosolic domain have an actual function in gating. Nonetheless, available data indicate that these bound ions act essentially as sensors of cytosolic Mg\(^{2+}\) concentration, thus tightly regulating Mg\(^{2+}\) movement through CorA and consequently regulating Mg\(^{2+}\) homeostasis.

The CorA superfamily

The CorA protein superfamily is widespread in the Eubacteria with about 800 sequences currently known. Although phylogenetic analysis of the family suggests at least two additional subfamilies in addition to the CorA Mg\(^{2+}\) channels (26, 27), information on function is available only for the subfamily represented by ZntB, which mediates the efflux of Zn\(^{2+}\) (4, 80). Given its sequence similarity and identical secondary structure to CorA, it has been hypothesized to have a structure similar to that of CorA (38). This hypothesis has recently been confirmed with solution of the crystal structure of the soluble domain of ZntB from Vibrio parahaemolyticus (Tan et al., PDB code 3C56) and S. typhimurium (Maguire et al., unpublished observations). These structures show that ZntB is a funnel-shaped homopentamer assembled almost identically to CorA. Efflux of Zn\(^{2+}\) via ZntB would move the cation against the electrochemical gradient. This obviously requires energy, most likely in the form of a counter- or co-transported ion, although this has not been formally shown. Thus the CorA superfamily appears to contain both ion channels and transporters, much like the CIC family of carriers (35, 41).

Eukaryotic homologs of CorA include the Mrs2 and Lpe10 inner mitochondrial membrane proteins, which were originally identified as being required for mitochondrial Mg\(^{2+}\) gradients (71). Since these data were published, it has been demonstrated that the Mg\(^{2+}\) channel from the mitochondria is homologous with CorA (52), and that from the plasma membrane is homologous with ZntB (53). Thus regulation of Mg\(^{2+}\) transport in both Eubacteria and Eukaryotes appears to involve movement of the willow helices as part of the \(\alpha_7\) domain of the basic sphincter.
**Structure of MgtE from The MgtE Mg²⁺ channel**

**FIGURE 5.** Mg²⁺ ions are labeled as follows: Mg₁ within the pore is in wheat, Mg₂ each monomer are shown in different colors and labeled. The H₁b colored identically. The N domain is shown in red, the CBS domains in blue, and the connecting helices in blue. Each of the TM segments of each monomer are shown in different colors and labeled. The H₁b helices at the extracellular membrane face are shown in purple. Bound Mg²⁺ ions are labeled as follows: Mg₁ within the pore is in wheat, Mg₂ and Mg₃ are in gray, and Mg₄ and Mg₅ are in purple. One of the Mg₂ residues is obscured in this view.

The Mg²⁺ Mg³⁺ Channel

The crystal structure of the full-length bacterial *Thermus thermophilus* MgtE was recently determined by Hartori et al. (20, 21) to a resolution of 3.5 Å (FIGURE 5). In addition, the structure of the cytosolic domain was resolved to 2.3 Å in the presence of Mg²⁺ and to 3.9 Å in the absence of Mg²⁺. MgtE exists as a homodimer with five transmembrane segments per monomer, giving, like *CorA*, 10 total TM segments. The cytosolic domain is highly acidic and composed of two subdomains. The first, the N domain, is located at the NH₂-terminal region of the cytosolic domain and is a right-handed superhelix with 10 total α-helices in each domain. Pallen and Gophna noted that the N domain is structurally homologous to the soluble domain of FliG, a component motor of the flagellum (44a). Immediately following the N domain is a tandemly repeated cystathionine-β-synthase (CBS) domain, a known dimerization domain in several transporters (25).

Each monomer has five transmembrane helices. The helices are connected by five loops, designated L₀–L₄ with the L₁ and L₄ loops containing short α-helices termed H₁b and H₄b, respectively (FIGURE 5). The transmembrane domain of each monomer is linked with the cytosolic domain by a connecting helix, which interacts with the TM₅, TM₂, and H₄b helices through both dipole moments and van der Waals forces. The connecting helix of each monomer interacts with and is parallel to the connecting helix of the other monomer. Hydrophobic interactions between the TM domains appear to drive dimerization, which is stabilized by hydrogen bond interactions between highly conserved charged and/or polar residues. As is a general feature of many ion channels, several MgtE Mg²⁺ ions are directly conserved along the dimer interface, participating in hydrogen bonding, coordination, and dimerization.

**Interaction of MgtE Mg²⁺ with cytoplasmic domain**

The crystal structure of MgtE Mg²⁺ with the cytoplasmic domain was resolved to 2.3 Å (44a). The ion-binding site is largely hydrophobic and lined with charged residues that contribute to specificity for Mg²⁺. Mg²⁺ ions are directly conserved between monomers, participating in hydrogen bonding, coordination, and dimerization. The bound Mg²⁺ ions are positioned in a groove of negatively charged residues in the N domain and the CBS domain.

**Regulation of MgtE Mg²⁺ transport**

The structure of the MgtE Mg²⁺ channel was recently solved by Hattori et al. (20, 21) at a resolution of 3.5 Å (FIGURE 5). In addition, the structure of the cytosolic domain was resolved to 2.3 Å in the presence of Mg²⁺ and to 3.9 Å in the absence of Mg²⁺. MgtE exists as a homodimer with five transmembrane segments per monomer, giving, like *CorA*, 10 total TM segments. The cytosolic domain is highly acidic and composed of two subdomains. The first, the N domain, is located at the NH₂-terminal region of the cytosolic domain and is a right-handed superhelix with 10 total α-helices in each domain. Pallen and Gophna noted that the N domain is structurally homologous to the soluble domain of FliG, a component motor of the bacterial flagellum (44a). Immediately following the N domain is a tandemly repeated cystathionine-β-synthase (CBS) domain, a known dimerization domain in several transporters (25).

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Component motor Mg\(^{2+}\) transport ALR homologs their overexpression his arginine to MPEL motif in d and/or polar ps, designated by a post-translationally modified amino acid. ALR2 arrive dimerization with another monomer giving, an homodimer. Each monomer amin form an almost 800 homologs now known carries a charged residue in either TM1 or TM2.

**Interaction of MgtE with Mg\(^{2+}\)**

The full-length crystal structure of MgtE was solved in the presence of 40 mM Mg\(^{2+}\). The structure revealed five peaks of electron density on each monomer near conserved acidic residues. Bond angles and bond lengths support the interpretation of these densities as bound Mg\(^{2+}\) ions. Mg1 is in the ion-conducting pore and coordinated with the carbonyl group of the aspartate residue in TM5 and to the carboxyl group of an alanine one turn of the helix away. Mg1 coordination is clearly octahedral with short 2.0 Å bond angles near 90°, which is typical of Mg\(^{2+}\). Mg2 and Mg3 are located ~6 Å apart at the interface of the cytosolic and transmembrane domains. Mg2 is coordinated with one residue of the CBS domain and two residues of the connecting helix. Mg3 coordinates with a residue of the CBS domain, a residue of the connecting helix, and a third residue between TM4 and TM5. These locations suggest that Mg\(^{2+}\) ions are involved in positioning the connecting helix for proper interaction with TM5 and Mg4-Mg5 would thus appear to connect the N and CBS domains. These Mg\(^{2+}\) ions are positioned to fix the connecting helix in place and stabilize the interactions between the N and CBS domains. Mg2-Mg3 and/or Mg4-Mg5 would thus appear to play a role similar to the Mg\(^{2+}\) ions bound between monomers in CorA.

**Regulation of MgtE**

The structure of the N-domain and the structure of the CBS-domain in the presence of Mg\(^{2+}\) are virtually identical. However, the absence of Mg\(^{2+}\) from the Mg2-Mg3 and Mg4-Mg5 sites appears to allow the rotation of TM1 and TM2 by ~120° away from the CBS domain. The dimerization of the two tandem CBS domains within each monomer is also altered in the absence of Mg\(^{2+}\). The connecting helices unlock and rotate ~20° away from each other. Thus the absence of Mg\(^{2+}\) induces marked cytosolic domain flexibility. These movements would disrupt the interaction between the transmembrane domains and the connecting helices, allowing rearrangement of the helices that form the pore and presumably opening the pore (FIGURE 6). These observations suggest that the cytosolic domain of MgtE acts essentially as a Mg\(^{2+}\) sensor, allowing the cytosolic domain to regulate gating of the ion-conducting pore. Similarly in CorA, the Mg\(^{2+}\) ions bound between each monomer suggest that the cytosolic domain of CorA acts as a Mg\(^{2+}\) sensor. Thus, despite their markedly different structures, CorA and MgtE permeability likely is regulated identically in response to changes in cytosolic Mg\(^{2+}\) concentration. Unlike CorA, MgtE exhibits an additional level of regulation by Mg\(^{2+}\). Recently, Mg\(^{2+}\) regulated riboswitches have been demonstrated in the promoters of two different classes of Mg\(^{2+}\) transporter, the Bacillus subtilis mgtA and S. Typhimurium mgtA genes (7, 8).

**The MgtE superfamily**

Phylogenetic analysis of bacterial MgtE proteins appears to indicate a single family (77), unlike CorA, which has two and likely three subfamilies (26, 27). MgtE homologs in eukaryotes are represented by the SLC41A family of solute carriers with three members to date (SLC41A1–3). The SLC41A family is homologous to MgtE within the transmembrane domain, however, SLC41A transporters appear to be distinct from MgtE transporters.
have only a short NH2-terminal domain that lacks similarity to either the N or CBS domains (77). Furthermore, although they contain 10 TM segments like MgtE, these are the product of a single gene, indicating a tandem fusion during evolution. Both SLC41A1 and SLC41A2 have been expressed in Xenopus laevis oocytes and have been shown to generate Mg2+ currents (13, 14). More detailed characterization has been performed by Kolosiek et al. (28) after expression of SLC41A1 in both HEK293 cells and the Mg2+ transport-deficient strain of S. Typhimurium (22). Interestingly these studies revealed that SLC41A1 functions as a single efflux system in contrast to the Mg2+ influx activity of MgtE. SLC41A2 has been subsequently expressed in TRPM7–deficient DT40 cells by Sabitli et al. (54) where it appears to mediate Mg2+ influx. It remains to be determined whether SLC41A3 mediates influx or efflux.

SLC41A1 mRNA is highly expressed in heart and testis tissues and to a lesser degree in skeletal muscle, kidney, colon, pancreas, prostate, ovaries, adrenal gland, and thyroid gland (77). Expression is regulated by dietary Mg2+. Mice fed a low magnesium diet for 5 days showed a two- to fourfold increase in SLC41A1 mRNA in kidney, colon, and heart (13). In contrast, expression of SLC41A2 mRNA was not significantly changed in either mouse distal convoluted tubule cells maintained low magnesium or in kidney tissue isolated from mice kept on low magnesium diets (14). The molecular mechanism and physiological role of SLC41A1 regulation are not known.

**Remaining Questions and Future Directions**

Although the determination of the crystal structures of CorA and MgtE have provided valuable insight into the function of Mg2+ channels, there are still numerous aspects of protein function and physiology that have not been resolved. Some important outstanding questions are explored below.

First, how do CorA and MgtE recognize and differentiate between cations? Each channel is highly selective for Mg2+ (66). Other than Co2+ and Ni2+, neither channel will interact with either Cu2+ or Zn2+. S. Typhimurium CorA (28) after expression of SLC41A1 in both HEK293 cells and the Mg2+ transport-deficient strain of S. Typhimurium CorA (71). Thus, in CorA, Mg2+ appears to interact primarily if not solely with backbone carbonyls within the pore. Given the pentameric nature of the channel, this raises issues of coordination. Mg2+ strongly prefers interaction with oxygens in an octahedral conformation with all bond angles close to 90°. Hexacoordinate binding of Mg2+ with backbone carbonyls while maintaining any other close to a 90° bond angle is possible only if Mg2+ interacts with the backbone within a short time, the other sites being filled with water. This would require a very large pore diameter. Since such a large pore diameter would not be particularly selective for any cation other than Mg2+, this consideration in turn suggests either that Mg2+ coordination within the CorA pore is distorted markedly from the preferred octahedral conformation or that movement of TM1 somehow allows residues within TM2 to also interact with Mg2+. The former scenario would require a great deal of energy to form and maintain the distorted conformation. It seems more likely that the open form of the channel will involve interaction of Mg2+ with residues within TM2, even though the currently available structures of CorA suggest that the pore is formed entirely by TM1.

Like CorA, the outer half of the MgtE pore also appears to be highly hydrophobic in virtually all homologs, again suggesting Mg2+ interaction with backbone carbonyls. In contrast to CorA however, the inner half of the pore contains a single negatively charged residue, usually an aspartate. In the homodimer, this results in two carbonyl groups that interact with Mg2+, as shown in the crystal structure. Nevertheless, sequence alignments of the hundreds of MgtE homologs currently known indicate that this conserved aspartate is likely the only charged or polar residue within TM2. In CorA and MgtE however, the inner half of the pore contains a single negatively charged residue, usually an aspartate. In the homodimer, this results in two carbonyl groups that interact with Mg2+, as shown in the crystal structure. Nevertheless, sequence alignments of the hundreds of MgtE homologs currently known indicate that this conserved aspartate is likely the only charged or polar residue within the pore. In both CorA and MgtE, therefore, the primary interaction of Mg2+ within the pore is with backbone carbonyls. In MgtE, with its homodimeric structure, this would provide two cognate carbonyl groups spaced 180° apart all through the pore thus readily satisfying Mg2+’s preference for rather rigid 90° bond angles. This preference for 90° bond angles is also satisfied in the binding of Mg2+ within the MgtE pore to the carbonyl groups of the two aspartate residues and the carbonyls of the adjacent alanine residues of the dimer, leaving two positions to be filled by waters.

Gating of the CorA and MgtE Mg2+ channel is also unknown. Both structures contain Mg2+ ions bound within the cytosolic domain of the channel with a potential to regulate conformation and thus activity. The cation-free crystal structures of the soluble domains, compared with their structures in the presence of cation, clearly suggest that association and dissociation of these bound Mg2+ ions has marked effects on the structure and positioning of the cytosolic domain.

In the intact cell, Mg2+ is carried to the cytosolic side of CorA and MgtE. Although it is inferred that Mg2+ ions enter the channel, the molecular basis for Mg2+ transport is largely unknown. Another obstacle is that Mg2+ channel structures of many bacteria are not available. Nevertheless, sequences in the CorA, MgtE, and other divalent cation channel families will well be conserved.

Finally, the structures of Zn2+ channel homologs, for example, show that the channel architecture is clearly similar to CorA and MgtE. Similar proteins one or more such as ZntB are found in Zn2+ transport systems in eukaryotes requiring for example, for trafficking of the Zn2+ transporter ZnT1. It is not clear, however, how these channel families will differ from the divalent cation channel families.

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P2 of the CorA does not involve Mg2+. The Typhimurium magnesium transporter MgtE has a histidine-rich loop within the pore that closely interacts with the external Mg2+ site of the channel, this loop is thus strongly preferred for conformational-nucleotidization while main- taining a Mg2+ ion at a given site. This loop is very likely to be a Mg2+ site of the channel, which would provide an explanation for the difference in the chemistry of Mg2+ compared with Ca2+ and other divalent cations. Independent of structure, however, regulation of divalent cation channels by divalent cation bound to the cytosolic domain could well be a common feature.

Finally, the apparent function of the CorA paralog ZntB is to mediate efflux of Zn2+ rather than the influx of Mg2+ (80). The recent deposition of coordinates for the structures of the ZntB soluble domain and our own unpublished structural data on ZntB (see above) clearly show that CorA and ZntB share a quite similar architecture. However, the cation binding sites of Mg2+ on CorA and Zn2+ on ZntB must be different despite similar protein structures. ZntB has to interact first to bind to one or more sites within the cytosolic domain before passage out through the pore. Given the current CorA structures, Mg2+ must bind either to the external loop between TM1 and TM2 or to residues at the periplasmic end of TM1. In contrast, Zn2+ would not need to interact with similar residues at the external end of the pore since this would only impede flux. This is evident in the sequence of the short loop between TM1 and TM2. In CorA, this loop is always composed of large bulky residues, several of which are charged. This loop in ZntB homologs contains little or no charge and is composed largely of smaller residues including three or even four glycines. Moreover, since energy would be required for Zn2+ efflux, a counterion would be composed largely of smaller residues including three bulky residues, several of which are charged. This loop in ZntB homologs contains little or no charge and is composed largely of smaller residues including three or even four glycines. Moreover, since energy would be required for Zn2+ efflux, a counterion would be required, most likely Na+ or H+, with unknown stoichiometry. The CorA Mg2+ channel requires only a single Mg2+ ion for Zn2+ efflux, a counterion would be required for Zn2+ efflux, a counterion would be required, most likely Na+ or H+, with unknown stoichiometry. The CorA Mg2+ channel requires only a single Mg2+ ion for Zn2+ efflux, a counterion would be required, most likely Na+ or H+, with unknown stoichiometry.
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