Magnesium and Mg\textsuperscript{2+} Transport Proteins in Cells

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Magnesium is one of the most important and abundant divalent cations in living cells. Organisms must maintain physiological levels of Mg\textsuperscript{2+} because this divalent cation is critical for the stabilization of membranes and ribosomes and for the neutralization of nucleic acids. Over 300 enzymes are known to be Mg-dependent. The cellular concentration of Mg\textsuperscript{2+} is regulated by transmembrane pathways. Prokaryotes carry three classes of Mg\textsuperscript{2+} transport systems: CorA, MgtE, and MgtA. Some of eukaryotic Mg\textsuperscript{2+} transport proteins have some similarity to those found in prokaryotes. Mitochondrial RNA splicing protein 2 (MRS2) shares many of properties of the bacterial CorA protein. The Solute Carrier Family 41 Member 1 (SLC41A1) and Cyclin and CBS Domain Divalent Metal Cation Transport Mediator (CNNM) family proteins have a similarity with some regions of the bacterial MgtE and CorC proteins, respectively. Mammalian Mg\textsuperscript{2+} homeostasis is also regulated by Mg\textsuperscript{2+} transport proteins including Transient Receptor Potential Cation Channel Subfamily M, Member 6/7 (TRPM6/7), Claudin-16, Magnesium Transporter 1 (MAGT1), and Nonimprinted in Prader-Willi/Angelman Syndrome Region (NIPA) proteins that are not represented in prokaryotic genomes. These eukaryotic Mg\textsuperscript{2+} transport proteins have no obvious amino acid sequence similarities, indicating that there are many ways to transport Mg\textsuperscript{2+} across membranes.

Keywords: Magnesium, Membrane transport, Channel, Exchanger, Selectivity

1. Introduction
Magnesium (Mg) is one of the most important and abundant divalent cations in living cells, and it is required for multiple biochemical reactions. Mg exists in two forms in living cells. Some is free form and the rest is bound to substances such as ATP and RNA. Approximately 50% of
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The cellular Mg is estimated to be bound to ATP and other nucleotides [1], where Mg\textsuperscript{2+} acts as a counter-ion for the negatively charged phosphate groups. Mg\textsuperscript{2+} is an essential cofactor in hundreds of enzymes in numerous biological reactions [1]. Mg is part of the active site of DNA polymerases as well as kinases and NTPases, where it is involved in nucleotide binding, and some enzymes such as phosphoribosyl pyrophosphate synthase and the rate-limiting enzymes of nucleotide syntheses require free Mg\textsuperscript{2+} as an essential activator that acts on enzyme proteins as well as on MgATP as a substrate [2]. In all cells, Mg\textsuperscript{2+} is essential for ribosome assembly [3], and serves as an essential structural element for membranes. Mg is the central atom of the chlorophyll molecule in plants. In prokaryotes, Mg\textsuperscript{2+} has also been identified as an important regulatory signal essential for virulence [4]. The total Mg concentration in cells is in the millimolar range (15-25 mM) [5], and free Mg\textsuperscript{2+} concentration is considerably lower on the order of 0.1-0.7 mM [6]. The intracellular Mg\textsuperscript{2+} concentration is tightly adjusted and controlled. As with any cation, the cellular concentration of Mg\textsuperscript{2+} is regulated by transmembrane pathways. The aim of this review is to present the current knowledge on the structure, function, and regulation of Mg\textsuperscript{2+} channel and transporter systems.

2. Chemical properties of magnesium
The chemistry of Mg\textsuperscript{2+} is unique among the biologically important cations. The ionic radius of Mg\textsuperscript{2+} is substantially smaller and its hydrated radius is substantially larger than that of K\textsuperscript{+}, Na\textsuperscript{+}, and Ca\textsuperscript{2+}. Then, the hydrated radius of Mg\textsuperscript{2+} is approximately 400 times larger than the dehydrated radius, a much larger difference than that seen with Na\textsuperscript{+} and Ca\textsuperscript{2+} (25-fold) or K\textsuperscript{+} (fourfold) [1]. A transport protein is generally thought to interact with and recognize the hydrated cation first, then remove the hydration shell and deliver the bare ion into the transport pathway through the membrane. A Mg\textsuperscript{2+} transport protein must recognize the very large hydrated cation, strip the tightly bound hydration shell from the cation, and transport the dehydrated form. While transport proteins for other cations face the same issue, the challenge for a Mg\textsuperscript{2+} transport protein is far greater than for any other cation transport system. These chemical properties of Mg\textsuperscript{2+} predict that Mg\textsuperscript{2+} transport proteins are generally quite unusual members of the transport family, while the voltage-gated ion (K\textsuperscript{+}, Ca\textsuperscript{2+}, and Na\textsuperscript{+}) channel protein superfamily is one of the largest families of signaling proteins, and the family is likely to have evolved from an ancestor like the bacterial potassium channel [7].

3. Prokaryotic magnesium transport proteins
Three distinct classes of Mg\textsuperscript{2+} transport proteins have been identified from Bacteria and Archaea: CorA, MgtE, and MgtA (Table I). The first prokaryotic Mg\textsuperscript{2+} transport system identified and cloned was termed corA for the Co\textsuperscript{2+} resistance screen in Escherichia coli (E. coli) [10] and Salmonella enterica serovar Typhimurium (S. Typhimurium) [11]. CorA is an ion channel and it was the first divalent cation
Table I. Prokaryotic and eukaryotic Mg\textsuperscript{2+} transport proteins [8],[9]

| Superfamily | Members | TMs | Oligomeric state | Mechanism            |
|-------------|---------|-----|-----------------|----------------------|
| CorA        | CorA, ALR1/ ALR2, MRS2, LPE10 | 10  | (2TMs x 5)      | Channel              |
| MgtE        | MgtE, SLC41A1, SLC41A2, SLC41A3 | 10  | (5TMs x 2)      | Channel /Exchanger   |
| Mgt         | MgtA, MgtB | 10  |                 | P-type ATPase        |
| TRPM        | TRPM6, TRPM7 | 24  | (6TMs x 4)      | Channel/kinase (Chanzyme) |
| CNNM        | CNNM1, CNNM2, CNNM3, CNNM4 |      | (3.5TMs x 2)    | Channel /Exchanger?  |
| Claudin     | Claudin-16 (Paracellin-1), Claudin-19 | 4    | oligomer        | Channel              |
| MagT        | MAGT1, TUSC3 | 4   |                 | Channel              |
| NIPA        | NIPA1, NIPA2, NIPA3, NIPA4 | 8-9 | dimer           | Channel              |

transport protein to be crystallized [12-14]. CorA is a funnel-shaped homopentamer with two transmembrane (TM) segments per monomer (Fig. 1(A)), giving ten TM segments in total. Each monomer consists of a large N-terminal cytoplasmic domain, with no significant sequence similarity to other protein families, and two C-terminal TM domains (TM1 and TM2) connected by a short periplasmic loop. The pore is composed solely of the five TM1 helices. The distal portion of TM1 near the periplasm contains the signature sequence of all CorA proteins, YGMNF. This sequence is absolutely required for CorA function [15]. Mg\textsuperscript{2+} apparently binds as the fully hydrated cation to an external Mg\textsuperscript{2+} binding loop comprised of 7-8 amino acid residues that connect TM1 and TM2. In the homopentamer, entry of Mg\textsuperscript{2+} to the ion conduction pathway is blocked by the five Asn residues, part of the universally conserved YGMNF sequence of the CorA family [13]. A fully hydrated Mg\textsuperscript{2+} ion becomes partially dehydrated as it traverses the membrane. Putative Mg\textsuperscript{2+} binding sites are located at the top of the funnel in the cytoplasmic domain. Mg\textsuperscript{2+} ions bound to these sites are thought to regulate channel opening and closing in response to intracellular Mg\textsuperscript{2+} levels [13]. It has been proposed that loss of Mg\textsuperscript{2+} at these sites facilitates opening of the gate and import of Mg\textsuperscript{2+} [16,17].

A second Mg\textsuperscript{2+} channel widespread in bacteria is MgtE. MgtE is unrelated to CorA in both sequence and structure. It is a homodimer, in
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Fig. 1. Schematic representation of the three bacterial Mg$^{2+}$ transport protein classes. Each single monomer is shown. (A) CorA is a homopentamer in which each monomer contains a large N-terminal cytoplasmic domain and two transmembrane helices. (B) MgtE is a homodimer in which each monomer contains five transmembrane helices. (C) MgtA is a monomeric P-type ATPase whose topology is thought to be analogous to that of the sarcoplasmic reticulum Ca$^{2+}$ ATPase.

which each monomer contains five TM helices (Fig. 1(B)) [18]. The cytoplasmic portion of each monomer contains a tandemly repeated cystathionine $\beta$-synthase domain, a known dimerization domain in several transport proteins [19]. As with CorA, intracellular Mg$^{2+}$ levels are thought to regulate gating of MgtE via Mg$^{2+}$ binding sites in the cytoplasmic domain [18], suggesting that the cytoplasmic domain of MgtE acts as a Mg$^{2+}$ sensor. Thus, although structurally quite different, both CorA and MgtE appear to be gated in a similar manner through multiple Mg$^{2+}$ binding sites in the cytoplasmic domain of the channels. These sites essentially serve as Mg$^{2+}$ sensors of cytoplasmic Mg$^{2+}$ concentration.

MgtA is a monomer that has 10 TM helices (Fig. 1(C)). Members of this class of Mg$^{2+}$ transporters are P-type ATPases. The MgtA class is divided into two groups, MgtA and MgtB (Table I), which exhibit ~50% amino acid identity with each other [20]. In contrast to other P-type ATPases, which use energy derived from ATP hydrolysis to move their substances up an electrochemical gradient, the MgtA class of proteins was reported to move Mg$^{2+}$ down an electrochemical gradient. PhoP/PhoQ, a two-component regulatory system, drives transcription of the mgtA and mgtB genes [4].

Most bacterial genomes encode multiple Mg$^{2+}$ transport proteins that belong to either the same or different classes. Although an additional class of prokaryotic Mg$^{2+}$ transport protein exists, the three prokaryotic Mg$^{2+}$ transport classes likely represent the primary Mg$^{2+}$ transport systems in both Bacteria and Archaea. CorA and MgtE have a wide phylogenetic distribution, although both channels are expressed together in only few species [21]. The corresponding genes are transcribed from constitutive promoters. MgtA occurs in only a subset of bacteria and the mgtA gene is transcriptionally induced in low Mg$^{2+}$ environment [22]. S. Typhimurium has three Mg$^{2+}$ transport systems, CorA, MgtA and MgtB, mgtB is the second gene of the mgtCB operon. A strain of S. Typhimurium carrying mutations in all three genetic loci lacks detectable Mg$^{2+}$ transport under usual growth conditions and requires 10-100 mM Mg$^{2+}$ in the growth medium. Introduction of the gene for any single S. Typhimurium Mg$^{2+}$ transport protein or a gene encoding a putative Mg$^{2+}$ transporter from another organism into this Mg$^{2+}$ transport-deficient strain restores Mg$^{2+}$ transport and confers the ability to grow without Mg$^{2+}$ supplementation [23]. E. coli has CorA and MgtA, but does not have either mgtCB operon or mgtE gene. Either CorA or MgtA is necessary for normal E. coli growth in LB medium without
Mg\(^{2+}\) supplementation and YhiD plays a role in Mg\(^{2+}\) transport under high-Mg\(^{2+}\) growth conditions [24]. YhiD is an integral membrane protein with five transmembrane helices, and it belongs to the MgtC family [25].

Cyanobacteria are the largest group of oxygenic photoautotrophic prokaryotes. As is common for Gram-negative bacteria, cyanobacteria contain an outer and inner cytoplasmic membrane, but in contrast to other bacteria, cyanobacteria contain an additional internal membrane system, the thylakoid membrane. Analyses of cyanobacterial genomes show that several cyanobacteria encode only a single MgtE homolog but no CorA protein, while other cyanobacteria encode two CorA homologs but no MgtE protein. In some cyanobacteria, both, an MgtE and two CorA homologs are encoded [26]. While the observation that multiple Mg\(^{2+}\) channels are encoded in some cyanobacterial species could imply that one channel is located within the cytoplasmic membrane and the remaining in the thylakoid membrane, not much is known about protein targeting and sorting in cyanobacteria [27].

4. Eukaryotic magnesium transport proteins

Several apparently unique Mg\(^{2+}\) transport proteins have evolved within the eukaryotic domains of life. MRS2-ALR1/2 and SLC41 are the eukaryotic homologs of the prokaryotic CorA and MgtE Mg\(^{2+}\) transport proteins, respectively (Table I).

MRS2

The CorA family belongs to the 2-TM-GxN superfamily of metal ion transport proteins. The 2-TM-GxN superfamily is characterized by the only two TM helices, both located at the end of C-terminus (Fig. 2(A)), and connected by a conserved loop containing the signature motif of the family, namely the amino acid sequence of GxN, where x could be M, V, or I [28]. The CorA family members are found in both prokaryotes and eukaryotes [28], but with the exception of the GMN motif, there is almost no sequence homology between the CorA members from prokaryotes and eukaryotes. The conservation of the amino acid sequences in the CorA family is
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The eukaryotic CorA homologs have been characterized in the inner mitochondrial membrane of yeast and mammals (MRS2, LPE10), in the plasma membrane of yeast (ALR1/2, MNR), and in the plant Arabidopsis thaliana (MRS2). The first Mg\textsuperscript{2+} transport protein characterized in Metazoa is the Mitochondrial RNA splicing protein 2 (MRS2/MRS2p). MRS2 mediates a high capacity Mg\textsuperscript{2+} influx in isolated yeast mitochondria driven by the inner membrane potential, but also transports other divalent cations such as Ni\textsuperscript{2+}, Co\textsuperscript{2+}, and Cu\textsuperscript{2+}. Overexpression of MRS2 increases Mg\textsuperscript{2+} influx and deletion of the gene abolishes Mg\textsuperscript{2+} uptake. The MRS2 protein is therefore described as an essential Mg\textsuperscript{2+} transport protein in mitochondria [29]. In yeast, the essential system for maintaining Mg homeostasis includes five members of the CorA family; ALR1 and ALR2 are localized in the plasma membrane [30], MRS2 and LPE10 are on the mitochondrial inner membrane [31], and MNR2 is localized on the vacuole membrane [32]. Only single genes of the MRS2 type have been identified in yeast and animals, whereas numerous MRS2 genes have been found in plants. A family of nine genes (and two pseudo-genes) in Arabidopsis thaliana has been annotated as AtMRS2 [33] or AtMGT [34]. A family of nine MRS2 members has been identified in rice (Oryza sativa L.) [35], and ten MRS2 genes in banana (Musa acuminata) have been isolated [36]. In the characterization of the function of MRS2 channels in plant, complementation assays using the Mg\textsuperscript{2+} uptake-deficient S. Typhimurium strain MM281, the Saccharomyces cerevisiae strain CM66, and the E. coli strain TM2 show evidence for the Mg\textsuperscript{2+} transport capability of members of the plant MRS2 family [24,37]. Some members of the AtMRS2 family can also be functionally reconstituted into liposomes without any accessory proteins [38-40]. Although knowledge regarding plant MRS2 channels is increasing, their in planta functions are still uncertain.

**TRPM channels**

The genetic analysis of patients affected by hypomagnesemia with secondary hypocalcemia has resulted in the discovery of TRPM6 as a crucial component of epithelial Mg\textsuperscript{2+} transport [41,42]. This channel, a member of the melastatin subfamily of transient receptor potential (TRP) superfamily, is permeable to Mg\textsuperscript{2+} and is regulated by changes in intracellular Mg\textsuperscript{2+} or MgATP level [43]. TRPM7 channel, another member of the TRPM family, also show permeation to Ca\textsuperscript{2+} and Mg\textsuperscript{2+}. Like in other transient receptor potential (TRP) proteins, the TRPM7 channel segment comprises six transmembrane helixes (Fig. 2(B)). A short sequence located between the 5th and 6th helixes of TRPM7 forms a predicted pore helix followed by a pore-forming loop. As postulated for other TRP channels, TRPM7 functions as a tetrameric channel complex implying that the pore loops of four TRPM7 subunits contribute to a common ion selectivity filter. Similar to TRPM6, TRPM7 is also regulated by changes in intracellular Mg\textsuperscript{2+} or MgATP. This modulatory activity occurs via the kinase domain present at the C-terminus of these channels [44]. Both TRPM6 and TRPM7 share the unique feature of
an atypical α-kinase domain at their C-terminus for which they have been termed “chanzymes” (channels + enzymes) (Table I).

**SLC41**

In eukaryotes, three mgtE-like genes belonging to the SLC41 family of solute carriers have been identified. Hydropathy analysis of the SLC41A1 protein predicts to presence of 10 TMs (Fig. 2(C)), two of which are very similar (52% and 45%) to the integral membrane domains of the MgtE protein, with an overall similarity of 15% between human SLC41A1 and *Bacillus furmus* MgtE amino acid sequences [45]. Thus, the SLC41 proteins have undergone a gene duplication and fusion event from their primordial mgtE ancestor. However, the N-terminal regulatory domain of MgtE are missing in the SLC41 proteins, suggesting that these proteins have evolved alternate mechanisms of regulation. Although the three members of the SLC41 family transport a wide range of divalent cations such as Co²⁺, Cu²⁺, and Zn²⁺, they function as a transport protein with preference for physiological Mg²⁺ concentrations [46].

**CNNM Family**

The Cyclin and CBS Domain Divalent Metal Cation Transport Mediator (CNNM) family was first known as the Ancient Conserved Domain Protein (ACDP) family by the evolutionarily conserved domain from bacteria [47]. The CNNM family has some similarity to CorC of *S. Typhimurium*, which is related to the maintenance of Mg²⁺ and Co²⁺ homeostasis in bacteria [23]. However, *S. Typhimurium* CorC requires CorA to function as a cation transporter and does not possess any apparent TMs, so does not transport Mg²⁺ itself [48]. In mammals, there are four CNNM family member proteins, CNNM1-CNNM4. These proteins commonly possess a domain of unknown function 21 (DUF21) that spans the plasma membrane three and a half times (Fig. 2(D)), followed by cystathionine-β-synthase (CBS) domains in the cytoplasmic region [49]. They extrude Mg²⁺ from cells and maintain intracellular Mg²⁺ levels. CNNM proteins associate with phosphatase of regenerating liver (PRL) [50,51]. In mammals, there are 3 family member proteins, PRL1-PRL3. All family member proteins of PRL (PRL1-3) and CNNM (CNNM1-4) can bind to each other, and the interaction occurs at the CBS domain of CNNM proteins. Mg²⁺ efflux by CNNM proteins is suppressed by the binding with PRL, and the formation of the complex is dynamically regulated by cysteine phosphorylation of PRL. The crystal structures of the cytosolic fragments of CNNM2 and CNNM3 and biophysical studies show a tight correlation between MgATP binding and CBS-domain dimerization [52].

**Claudins**

Claudin-16 (paracellin-1) is a member of the claudin protein family [53], which comprehends a group of tight junction proteins with 4 TM spans, and both N- and C-termini on the cytoplasm side. Claudin-16 mediates paracellular Mg²⁺ and Ca²⁺ reabsorption throughout the nephron.

**MAGT1**

The Magnesium Transporter 1 (MAGT1) was identified as a gene upregulated in conditions of Mg²⁺ deficiency [54]. This protein has 5 TM domains in its immature form, and the mature
protein contains 4 TM spans. MAGT1 is a controversial protein that has been described as an evolutionally conserved Mg\(^{2+}\)-specific transporter found in all animals at the plasma membrane, but also as an endoplasmic reticulum localized subunit of the oligosaccharyltransferase complex involved in the posttranslational transfer of glycans onto proteins [55]. MAGT1 possesses channel-like characteristics and high selectivity of Mg\(^{2+}\). It would appear that this transporter is essential to regulate Mg\(^{2+}\) homeostasis in mammalian cells. It has been recently observed that MAGT1-dependent glycosylation is regulated by Mg\(^{2+}\) levels and that reduced Mg\(^{2+}\) impairs immune-cell function via the loss of specific glycoproteins [56].

**NIPA**

The family of Mg\(^{2+}\) transporters to be identified with microarray analysis comprises the NIPA genes designated nonimprinted in Prader-Willi/Angelman syndrome [57]. The human and mouse genomes contain four members of the NIPA family, termed NIPA1 through NIPA4, with an overall similarity of ~40%. *NIPA1* and *NIPA2* genes are in tandem on human and mouse chromosomes. NIPA1 and NIPA2 can both operate as Mg\(^{2+}\) transporters. NIPA1 can also transport Sr\(^{2+}\), Fe\(^{2+}\) or Co\(^{2+}\) [57]. In contrast, NIPA2 is highly specific for Mg\(^{2+}\) [58]. *NIPA1* has also been more directly implicated in another distinct disorder termed autosomal-dominant hereditary spastic paraplegia. The absence of NIPA2 enhances neural excitability through BK (big potassium) channels [59]. The NIPA family of proteins has been proposed to transport Mg\(^{2+}\), based on overexpression and Mg\(^{2+}\) currents in *Xenopus laevis* oocytes, but NIPA proteins have a role in bone morphogenetic protein signaling [60]. Therefore, there are claims that the role of NIPA proteins in Mg\(^{2+}\) transport should be questioned.

## 5. Conclusions and perspectives

Circadian rhythms in the concentration of intracellular magnesium have been revealed [61]. Given the role of Mg\(^{2+}\) in cells, circadian rhythms in the concentration of intracellular magnesium provide a surprisingly effective means to dynamically tune cellular biochemistry and energy consumption throughout the daily cycle. Determination of the structures of the CorA and MgtE channels has provided valuable insight into the mechanism by which Mg\(^{2+}\) moves across membranes. Nonetheless, when considering the essential function of magnesium in cells, such as the activities of estimated 600 enzymes being Mg\(^{2+}\)-dependent, it is surprising that little is known about Mg\(^{2+}\) transport. While Mg\(^{2+}\) is the most abundant divalent cation in the cell, its role in physiology is less extensively studied than that of other abundant cations such as Ca\(^{2+}\), Na\(^{+}\) or K\(^{+}\). Studies on Mg\(^{2+}\) transport proteins are expected to elucidate the role of the intriguing proteins and the biological importance of Mg\(^{2+}\) regulation.

**Conflict of interest**

None declared.

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Communicated by Shingo Fujisaki